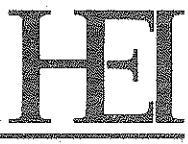
The Potential Health Effects of Oxygenates Added to Gasoline

A Review of the Current Literature

A Special Report of the Institute's Oxygenates Evaluation Committee



Health Effects Institute

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HEI HEALTH EFFECTS INSTITUTE

The Health Effects Institute, established in 1980, is an independent and impartial source of information on the health effects of motor vehicle emissions. HEI supports research on all major pollutants, including regulated pollutants (such as carbon monoxide, ozone, nitrogen dioxide, and particulate matter) and unregulated pollutants (such as diesel engine exhaust, methanol, and aldehydes). To date, HEI has supported more than 150 research projects at institutions in North America and Europe. Consistent with its mission to serve as an independent source of information on the health effects of motor vehicle pollutants, the Institute also engages in special review and evaluation activities.

Typically, HEI receives half its funds from the U.S. Environmental Protection Agency and half from 28 manufacturers and marketers of motor vehicles and engines in the United States. Occasionally, funds from other public or private organizations either support special projects or provide resources for a portion of an HEI study. However, in all cases HEI exercises complete autonomy in setting its research priorities and in reaching its conclusions. An independent Board of Directors governs the Institute. The Research and Review Committees serve complementary scientific purposes and draw distinguished scientists as members. The results of HEI-funded research and evaluations have been used in public and private decision-making.

Statement from the HEI Board of Directors

The 1990 Amendments to the Clean Air Act required that new vehicle fuels be introduced in a number of communities in 1992 to reduce wintertime vehicle emissions of carbon monoxide, and in additional communities in 1995 to reduce year-round vehicle emissions of ozone and other pollutants. The introduction of these fuels was met in some areas with public concern about health symptoms associated with using the fuels, and about the fuels' effects on price, engine performance, and fuel economy (miles per gallon).

In response to the health questions raised about these fuels, the U.S. Environmental Protection Agency and the Centers for Disease Control and Prevention asked the Health Effects Institute to review the existing science on the health effects of oxygenates (e.g., methyl *tert*-butyl ether [MTBE] and ethanol) that have been added to fuels to reduce vehicle emissions. In addition, HEI was asked to evaluate these health effects in the context of the health effects potentially caused by other components of gasoline and vehicle emissions. The HEI effort will form the core of the health effects portion of a broader review of oxygenated fuels being conducted by the White House Office of Science and Technology Policy, which is also considering questions of engine performance, fuel economy, and cost.

We appointed the HEI Oxygenates Evaluation Committee, a group of distinguished scientists, to work with the HEI scientific staff to carry out this project. We have reviewed their report, and believe it presents a thorough and responsible synthesis and evaluation of the current state of knowledge about the potential health effects of the oxygenates themselves; and a thoughtful, qualitative comparison of these health effects with (1) the health effects of other components of gasoline and (2) the health benefits and risks that may result from changes in vehicle emissions when oxygenates are used in fuel.

Despite limitations in the available data, and the difficulty of estimating both the health effects and health benefits from using these substances in fuel, the Committee was able to draw these conclusions:

- Adding oxygenates to gasoline reduces the emission of carbon monoxide and benzene from motor vehicles, and thereby potentially lowers certain risks for members of the population. At the same time, using oxygenates increases exposure to aldehydes and to the oxygenates themselves.
- Adding oxygenates to fuel is unlikely to substantially increase the health risks associated with fuel used in motor vehicles; hence, the potential health risks of oxygenates are not sufficient to warrant an immediate reduction in oxygenate use.
- However, given that observations in some experiments suggested potential health risks from these substances, a number of important questions should be answered if these substances are to continue in widespread use over the long term.

The Committee has identified the highest priority research needs in its report. The Health Effects Institute is working to implement a targeted program of research to address some of these questions, in concert with related efforts by government and industry scientists.

Beyond the questions of the health effects of these substances themselves, we concur with the Committee's finding that any future widespread introduction of such substances should be preceded by an adequate research effort into the health effects of the substance and be accompanied by rigorous monitoring of public exposure and health. It is not uncommon to introduce a new chemical to address a particular environmental or health problem, only to find that the new chemical engenders questions about its own health effects. We should learn from the experience with oxygenates that, although we can never have full knowledge about a new substance, we would be well served to anticipate better and plan for such health questions before they occur.

In addition to thanking the entire HEI Oxygenates Evaluation Committee, and the members of the Committee's Advisory Panel, we would particularly like to thank Dr. Arthur Upton, Chair of the Oxygenates Evaluation Committee, and Drs. Jane Warren and Maria Costantini, who served as HEI's scientific Project Leaders for this review.

The Potential Health Effects of Oxygenates Added to Gasoline

A Review of the Current Literature

A Special Report of the Institute's Oxygenates Evaluation Committee

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Various sections of this report were submitted for outside peer review. HEI appreciates the thoughtful critiques provided by the reviewers listed above. However, the views expressed in this report are those of the Oxygenates Evaluation Committee and no endorsement by the external reviewers should be inferred.

Although this document was produced with partial funding by the United States Environmental Protection Agency under Assistance Agreement 816285 to the Health Effects Institute, it has not been subjected to the Agency's peer and administrative review and therefore may not necessarily reflect the views of the Agency, and no official endorsement should be inferred. The contents of this document also may not reflect the views and policies of other public- or private-sector sponsors of HEI, and no endorsement by them should be inferred.

Executive Summary

INTRODUCTION

Since the passage of the Clean Air Act in 1970, the United States has endeavored to reduce human exposure to a number of air pollutants that pose threats to public health, including several—carbon monoxide, certain air toxics, and ground-level ozone—that come in significant proportion from motor vehicle fuels and exhaust emissions. A number of strategies have been implemented to reduce motor vehicle emissions, including increasingly more stringent exhaust emission standards for new vehicles, improved inspection and maintenance of all vehicles, and more recently, reformulation of motor vehicle fuels. Although many areas of the United States still face serious air pollution problems, these strategies have substantially reduced emissions from motor vehicles, and levels of general air pollution as well. However, this reduction in emissions has been offset substantially by continuous and rapid increases in the number of vehicles and the number of miles driven. Thus, the country continues to be faced with the task of both further reducing pollution in some areas and maintaining the cleaner air in those areas where it has been improved.

By enacting the Clean Air Act Amendments of 1990, the Congress took further steps toward reducing emissions from motor vehicles, including mandating, for the first time, requirements to change the formulation of gasoline. Specifically, to reduce carbon monoxide emissions, the Amendments required that areas in which the carbon monoxide standard was exceeded must, in 1992, begin to use oxygenated fuel (usually referred to as oxyfuel), which would contain at least 2.7% oxygen by weight. This oxygen content has been achieved by adding, most commonly, 15% (by volume) of methyl *tert*-butyl ether (MTBE) or 7.8% (by volume) of ethanol. To reduce ground-level ozone in the nine areas most out of compliance with the ozone standard (and in other areas that chose to be involved in the program), the Amendments required that, beginning in 1995, reformulated gasoline be used; this is a significantly changed form of conventional gasoline that contains at least 2% oxygen by weight, has a reduced content of benzene and other aromatic compounds, and produces limited emissions of total air toxics.

THE HEALTH EFFECTS INSTITUTE'S OXYGENATES REVIEW

The introduction of fuels containing oxygenates elicited concerns from workers and the general public in some areas, including reports of unpleasant odors, headaches, or other symptoms attributed to the fuels, and questions about their effects on the cost of gasoline, the performance of engines, and the economy of fuel usage (miles per gallon). In response to continuing health questions, the U.S. Environmental Protection Agency (EPA) and the Centers for Disease Control and Prevention (CDC) supported an effort by the Health Effects Institute to conduct an intensive review of (1) the existing science of the health effects of oxygenates, (2) the risk evaluations done by the EPA in 1993 and 1994, and (3) in a qualitative way, the health effects of exposure to the new additives as they relate to the health effects of other pollutants whose levels in emissions change when fuels containing oxygenates are used. In addition, the White House Office of Science and Technology Policy is conducting a broader review of the oxygenated fuels program, which will consider air quality benefits, engine performance, fuel economy, and the costs of these new fuels. This HEI review will form the core of the health effects portion of the White House effort.

In June 1995, the HEI Board of Directors appointed distinguished scientific experts to the Oxygenates Evaluation Committee to work with the HEI scientific staff to conduct this review. The Health Effects Institute also sought out a diverse Advisory Panel of individuals from federal government agencies, state health departments, industry, and labor and environmental organizations, who were asked to provide input at the planning stage of this review and on a draft of this report. Each section of the report also was reviewed carefully by experts in the appropriate scientific disciplines.

The results of the review by the Oxygenates Evaluation Committee are summarized below.

ASSESSMENT OF EXPOSURE TO OXYGENATES

The HEI Oxygenates Evaluation Committee noted that about 70 million people live in areas where oxygenates (mainly MTBE, but a substantial portion of ethanol) have been added to fuels. Although MTBE is widely used, infor-

mation on personal exposure to it is limited. However, the Committee was able to identify ranges and approximate levels of exposure for the general public in several situations, for service station attendants and mechanics, and for workers who transport and handle MTBE and fuels. Information on exposure to ethanol is much more limited, and even less is available on ethers other than MTBE.

Both the general public and service station attendants have been exposed during refueling in two ways: to inhalation levels of MTBE typically between 0.2 and 1.5 parts per million (ppm), with some peaks above 10 ppm, and to occasional skin contact by spillage. Members of the general public could be expected to be exposed to these levels very briefly while refueling, whereas service station attendants face similar levels but for longer periods (e.g., a work shift). Limited data on ethanol exposure during refueling suggest exposure levels below 1 ppm (the minimal level of detection in the studies), with some unusual peak recordings as high as 46 ppm.

The highest exposures to MTBE were noted among workers who manufacture or transport it. Those involved in the transport of neat MTBE experience median short-term (e.g., 30-minute) exposures of 13.8 ppm, with a wide range up to 1,050 ppm. For those who transport MTBE in fuel mixtures, the median was 2.4 ppm with peaks in excess of 100 ppm.

The Oxygenates Evaluation Committee also reviewed the information on groundwater contamination by MTBE. It concluded that MTBE and other oxygenates can move into underground water from contaminated soil (for example, as a result of leaks from underground storage tanks). Although the measurements of MTBE in shallow wells were limited, they support the possibility that underground water could become contaminated. Thus, both ingestion and absorption through skin contact with oxygenates are potential routes of exposure.

HEALTH EFFECTS OF OXYGENATES

The HEI Oxygenates Evaluation Committee reviewed a number of health endpoints for potential effects of MTBE, and identified two areas in which the most significant questions arose.

- *Short-Term Effects* Symptoms such as nausea, headaches, and sensory irritation were reported in some communities after oxyfuel and reformulated gasoline containing MTBE had been introduced. Based on the community and human exposure studies conducted, it appears that most people do not experience unusual symptoms or significant acute medical consequences in response to short-term exposure to gasoline containing MTBE. However, exposure to MTBE may cause acute symptoms in some individuals. In addition, on the basis of effects on motor activity observed in rats exposed to relatively high levels of MTBE (800 ppm), the Oxygenates Evaluation Committee concluded that MTBE, at these high levels, is toxic to the nervous system. Because 800 ppm MTBE was the lowest exposure concentration at which motor activity was evaluated, and because complex central nervous system functions that may be more sensitive to MTBE were not tested, MTBE's neurotoxicity at the lower levels to which most people are exposed is unknown.
- *Cancer Effects* Tumors have been observed at multiple sites in rats and mice after exposure to high levels of MTBE. The Oxygenates Evaluation Committee considered these findings to be cause for concern. At the same time, the Committee noted that the mechanisms that caused these tumors and the likelihood that these or other tumors will occur in humans exposed at substantially lower levels are both unknown.

In addition to examining the health effects of MTBE, the Committee investigated the health effects of ethanol. Extensive evidence indicates that ingesting ethanol at moderate to high levels affects the nervous system and prenatal development processes, and that ingestion over long periods of time increases the risk of certain cancers. It is unlikely that these effects would result from the very low exposure levels (by inhalation) in refueling situations, because the preexisting levels of ethanol in the blood from normal metabolic processes would not be significantly affected.

Few studies have been conducted on the health effects of other oxygenates such as ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME), or diisopropyl ether (DIPE). They deserve substantial investigation if they are likely to be placed in widespread use.

Another important question about oxyfuel and reformulated gasoline is whether their health effects actually differ from those of conventional gasoline, and if so, in what ways. The short-term effects reported after exposure to MTBE are not unlike effects reported in some individuals after exposure to gasoline vapors and motor vehicle emissions. Also, some constituents of conventional gasoline and motor vehicle emissions have been shown to cause tumors in animals, although as with MTBE, the mechanisms by which the tumors developed and whether humans are also at risk are both uncertain.

ASSESSMENT OF EXPOSURE TO GASOLINE AND MOTOR VEHICLE EMISSIONS AND THEIR POTENTIAL HEALTH EFFECTS

Oxygenates have been added to gasoline to reduce the emission of certain air pollutants. Because the oxygenates

change the mixture of both evaporative and tail pipe emissions, they also may change the health effects of exposure to the emissions. This factor must be taken into consideration when evaluating the impact of oxygenates in fuels. The primary motor vehicle emissions that are potentially affected by adding oxygenates are carbon monoxide (CO) and certain air toxics. (Although motor vehicle emissions also include precursors of ozone, and reformulated gasoline is designed to reduce these precursors, the primary means to accomplish this is by reducing other components of gasoline and not by adding oxygenates.)

Extensive testing of motor vehicle emissions with and without oxygenates demonstrates that oxygenates consistently reduce tail pipe emissions of CO by 10% to 25%. In addition, the number of days on which the ambient CO standard is exceeded has decreased in many areas since oxyfuel was introduced. However, ambient pollutant levels are affected by several factors, including changes in weather patterns and in the number and types of vehicles driven and miles traveled. Thus, assessing the trends in pollutant levels, and estimating the contribution that oxygenates have made to those trends would require multiyear analyses and was beyond the scope of this review.

Reducing the levels of CO is of particular interest. When it enters the body, CO combines with hemoglobin in blood, which reduces the ability of the blood to deliver oxygen to the tissues. People with coronary artery disease are particularly sensitive to this effect because they have impaired ability to increase coronary blood flow. This becomes a problem during exercise when the heart needs more oxygen. When blood flow through the heart is not sufficient to meet the oxygen demand, the heart becomes ischemic, resulting in chest pain (angina pectoris), or electrocardiographic changes, or both. Even relatively low CO levels may bring on ischemia more quickly for some individuals with coronary artery disease when they are exercising moderately. Although the evidence of effects of CO in human controlled-exposure studies is strong, little information has been gathered on precisely how many people in this sensitive group are actually exposed to CO at levels above the standard.

Adding oxygenates also appears to cause an overall reduction in air toxics emissions, but different air toxics are affected differently; benzene emissions are reduced, formaldehyde and acetaldehyde emissions are increased in some cases, and 1,3-butadiene emissions are either reduced slightly or not changed. All four of these air toxics emitted from motor vehicles—benzene, 1,3-butadiene, formaldehyde, and acetaldehyde—are classified as either known or probable human carcinogens. However, uncertainties in estimating personal exposure to each of the air toxics, and in projecting the carcinogenic risk from each for humans, are too great to allow a meaningful assessment of the overall impact of the changes in the emission of air toxics on the public's risk from using fuels containing oxygenates.

OVERALL ASSESSMENT

Based on its review of existing evidence on the exposure to and health effects of oxygenates used in gasoline, the HEI Oxygenates Evaluation Committee drew the following conclusions about the oxygenates themselves.

- Introducing oxygenates into gasoline to reduce CO emissions has increased exposure to MTBE for the general public during brief higher-level exposures while refueling and during more sustained but lower-level exposures while driving, and for service station employees during higher-level exposures over entire work shifts. These exposures can occur by both inhalation and skin contact. Workers who handle or transport neat MTBE can experience significantly higher average inhalation exposure levels than people in other situations. Few data on exposure to other oxygenates have been gathered.
- MTBE has been measured in some underground water; its presence in water may result in exposure by ingestion or skin contact should water supplies become contaminated.
- The potential health effects from exposure to gasoline containing MTBE include (1) headache, nausea, and sensory irritation in some (possibly sensitive) individuals, based on reports after exposure to oxygenates; (2) acute, reversible neurotoxic effects, based on changes in motor activity in rats at high exposure levels; and (3) cancer, based on increases in the frequency of tumors at multiple organ sites in rats and mice at high exposure levels. Although questions persist about how to interpret each of these observed effects, they nevertheless point to a potential human health risk.
- The health effects from exposure to ethanol by ingesting moderate to large quantities have been extensively investigated. Under these conditions, ethanol can increase the risks of certain cancers, adversely affect the developing embryo, produce neurotoxicity, and cause various other types of damage. However, it is unlikely that such effects would occur at the very low ambient levels to which most people are exposed by inhalation.
- Potential health effects from exposure to other oxygenates are not known and require investigation if their use in fuels is to be widespread.

In addition to these conclusions about the oxygenates themselves, after qualitatively assessing the health effects of gasoline and motor vehicle emissions with and without oxygenates, the Oxygenates Evaluation Committee has come to the following conclusions about gasoline containing oxygenates.

- The potential health effects of exposure to components of conventional gasoline (without oxygenates) include short-term and cancer effects similar to those that could result from exposure to gasoline containing oxygenates.
- Adding oxygenates to gasoline can reduce the emission of CO and benzene from motor vehicles, and thereby potentially lower certain risks to members of the population. At the same time, using oxygenates increases exposure to aldehydes, which are carcinogenic in animals, and to the oxygenates themselves.
- Adding oxygenates is unlikely to substantially increase the health risks associated with fuel used in motor vehicles; hence, the potential health risks of oxygenates are not sufficient to warrant an immediate reduction in oxygenate use at this time. However, a number of important questions need to be answered if these substances are to continue in widespread use over the long term.

In addition to its conclusions about possible health effects, the Oxygenates Evaluation Committee noted a general lesson to be learned from introducing oxygenates to the general public. Although it is not possible to have complete information about a substance before it is used, the diverse experiences after introducing oxygenated fuels argue strongly that any future new use of a substance should (1) be preceded by a sufficiently comprehensive research and testing program (including mechanistic and human studies), and (2) be accompanied by rigorous exposure assessment and epidemiologic studies.

RESEARCH NEEDS

The HEI Oxygenates Evaluation Committee has identified a number of research needs to reduce uncertainties about the health effects of oxygenates by themselves and as parts of gasoline mixtures. These are summarized in detail in the section, Research Priorities for Oxygenates. To some extent, a number of these needs are being addressed by ongoing or planned research programs at several organizations. Specifically, the Oxygenates Evaluation Committee identified these highest priority research needs:

- A comprehensive set of studies to assess personal exposure to oxygenates in public and occupational settings;
- Human environmental chamber studies to evaluate metabolism, symptoms, and neurotoxic effects in potentially sensitive individuals after exposure to MTBE and MTBE-gasoline mixtures;
- Epidemiologic and animal studies to improve our understanding of the potential risk of human cancer from exposure to MTBE alone and in association with gasoline vapors and vehicle exhaust; and
- Comprehensive assessments of other ethers (e.g., ETBE, TAME, DIPE) if they are to be placed in widespread use.

Preface

The Clean Air Act Amendments of 1990 led to increased public exposure to oxygenates, predominantly methyl *tert*-butyl ether (MTBE), because they required that oxygenated gasoline (referred to as oxyfuel) be used in areas with high carbon monoxide levels starting in 1992, and that reformulated gasoline containing oxygenates be used in areas with high ozone levels starting in 1995. Although MTBE had already been used as an octane booster in gasoline at lower concentrations, and had already been introduced in oxyfuel in some areas, there was heightened public concern in 1992 when oxyfuel was more widely introduced. In several states, many people reported symptoms such as headaches, dizziness, nausea, and eye irritation. To address the public's concern, several field studies and controlled human exposure studies were undertaken to investigate the acute effects of exposure to MTBE, but they did not resolve all the uncertainties. In addition, reports that MTBE was carcinogenic in animal studies caused further concern. The U.S. Environmental Protection Agency (EPA) reviewed the scientific literature and produced two assessments of the health effects of MTBE and oxyfuel (*Assessment of Potential Health Risks of Gasoline Oxygenated with Methyl Tertiary Butyl Ether* [MTBE] [1993a] and *Health Risk Perspectives on Fuel Oxygenates* [1994b]).

When reformulated gasoline was introduced in 1995, complaints were registered about MTBE in other states. In response to continued public concern, in the spring of 1995 the EPA and the Centers for Disease Control and Prevention (CDC) asked the Health Effects Institute to conduct an independent review of the risks posed by adding oxygenates to fuels. They requested that HEI focus on the EPA's two major reports on oxygenates in gasoline and include any other reports and more recent information relevant to assessing health risks.

The Health Effects Institute assembled a committee of scientific experts in the spring of 1995 to work with HEI's scientific staff to develop this report. The Oxygenates Evaluation Committee was chaired by Dr. Arthur Upton, who is also Chair of HEI's Health Review Committee. Members of the committee provided expertise in exposure assessment, biochemistry, toxicology, neurobehavior, epidemiology, pathology, carcinogenesis, statistics, and public health. The Oxygenates Evaluation Committee first met in late June 1995 to discuss the scope of the project, and again in September and November to discuss drafts of this report. Subgroups of the committee met in the intervening months to discuss specific sections of the report.

In order to access the full spectrum of existing views and concerns about using oxygenates in fuels, HEI also assembled a broad Advisory Panel of people from federal government agencies (EPA, CDC, and the National Institute of Occupational Safety and Health), state health departments, industry (chemical, motor vehicle, and oil), and labor and environmental organizations. The individuals on this Panel provided information on reported health effects from exposures to oxygenates and advice as this report was being developed. This group was invited to a workshop in July 1995 to discuss new information from human studies and to provide input on the scope and organization of HEI's review and later was asked to comment on a draft of this report.

Through discussions with the members of the Oxygenates Evaluation Committee and the Advisory Panel, HEI identified these goals for the review:

1. Extend the EPA's assessments of the health effects of oxygenates to include information on studies completed since the EPA's 1993 and 1994 reviews were published, and to the extent possible given the limited information, evaluate the potential health effects of exposure to other oxygenates whose use as additives in gasoline may increase, including additional ethers (ethyl *tert*-butyl ether [ETBE], *tert*-amyl methyl ether [TAME], and diisopropyl ether [DIPE]) and ethanol;
2. Review the EPA's assessments of the health effects of MTBE and fuels containing oxygenates in light of new information;
3. In a qualitative way, consider the health effects of exposure to the new additives as they relate to the health effects of other pollutants whose levels in emissions change when fuels containing oxygenates are used; and
4. Define research areas critical to clarifying the remaining uncertainties about the health effects of oxygenates added to gasoline.

In conducting its assessment of the health effects of oxygenates, the Oxygenates Evaluation Committee reviewed published and unpublished reports and bulletins from contract laboratories and state agencies as well as

peer-reviewed literature. At times, the Committee also relied on personal communications with scientists who had conducted some of the studies cited. During the course of the review, the Committee received letters and phone calls from individuals who reported having developed illnesses as a result of their exposure to oxyfuel or reformulated gasoline. A methodical evaluation of these anecdotal reports was beyond the scope of this project, which was to review existing studies. As identified in the section on Research Priorities for Oxygenates, these and other individuals need to be studied to determine whether they are particularly sensitive to fuel containing oxygenates and to learn more about the possible mechanisms of the health effects they reported.

Introduction

The Clean Air Act of 1970 initiated a commitment by the U.S. government to reduce the public's exposure to air pollutants that may adversely affect health. Motor vehicle emissions contribute significantly to many of these pollutants, including carbon monoxide (CO)*, ozone, particles, oxides of nitrogen (NO_x), and some air toxics. Approaches to reduce emissions from motor vehicles have included increasingly more stringent exhaust emission standards for new vehicles to meet, and in-use inspection and improved maintenance of vehicles. Although these measures have resulted in large decreases in the amount of emissions of many pollutants per mile traveled over the past couple of decades, the increase in miles driven has offset this improvement to a considerable extent. In the late 1980s when Congress considered amending the Clean Air Act, two major problems of public health concern that remained were wintertime exceedances in some areas of the National Ambient Air Quality Standard (NAAQS) for CO and frequent violations of the NAAQS for ozone in several regions. The Clean Air Act Amendments (CAAA) of 1990 mandated, for the first time, that fuels be reformulated to address these air pollution problems.

USING OXYGENATES IN FUELS

The CAAA of 1990 mandated two types of fuel reformulation: oxygenated gasoline (also known as oxyfuel) and reformulated gasoline (RFG). The main purpose of using oxyfuel is to reduce CO emissions from motor vehicles; RFG is intended to decrease the concentration of ozone-forming hydrocarbons and the total mass of air toxics in motor vehicle emissions.

Emission of CO results from incomplete combustion of fuel, particularly in cold weather. One way to increase fuel combustion and decrease the emission of CO is to increase the oxygen content of fuel. Section 211 (m) of the CAAA of 1990 mandated that, beginning on November 1, 1992, oxyfuel be used to reduce emission of CO in areas of the United States where the NAAQS was being exceeded. Oxyfuel is conventional gasoline to which a minimum of 2.7% oxygen by weight has been added. Using oxyfuel was initially mandated for at least four months of the year (winter) during which an area is prone to high ambient concentrations of CO. Section 211 allows the Administrator of the U.S. Environmental Protection Protection Agency (EPA) to waive portions of the oxyfuel requirements if states demonstrate that a possibly resulting increase in NO_x emissions would exacerbate other air quality problems.

Section 211 (k) of the CAAA of 1990 required that RFG be introduced into the nine areas with the worst ozone levels starting in January 1995. These areas have "severe" or "serious" levels of ozone and populations over 250,000. In addition, some other areas with less severe ozone problems have decided to participate in the RFG program. The following list provides the requirements for RFG related to both its composition and the emissions from its use. RFG must

- Contain at least 2.0% oxygen by weight;
- Contain not more than 1.0% benzene by volume and not more than 25% aromatic compounds by volume;
- Contain no heavy metals (this requirement can be waived by the EPA Administrator if adding the heavy metal will not increase, on the basis of aggregate mass or cancer risk, the toxic air pollutants emitted from motor vehicles);
- Reduce the aggregate emissions of ozone-forming volatile compounds by 15% compared with emissions from vehicles using conventional gasoline, and reduce them by 25% but not less than 20% by the year 2000 (with this adjustment in reduction allowed by the EPA Administrator based on the technological feasibility and cost of meeting the emissions criterion);
- Reduce the aggregate emissions of toxic air pollutants by 15% compared with emissions from vehicles using conventional gasoline, and reduce them by 25% but not less than 20% by the year 2000 (with this adjustment in reduction allowed by the EPA Administrator based on technological feasibility and cost of meeting the emissions criterion); and
- Not increase emissions of NO_x.

Oxygenates used in oxyfuel and RFG include ethers such as methyl *tert*-butyl ether (MTBE) and alcohols such as ethanol. Because the oxygenates vary in molecular weight, a different amount of each oxygenate is needed to meet the

* A list of abbreviations appears at the end of this document for your reference.

oxygen requirements. For example, 15% MTBE (by volume) or 7.8% ethanol (by volume) would be required to provide 2.7% oxygen (by weight), as required in oxyfuel. Other ethers that can be used include ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME), and diisopropyl ether (DIPE).

MTBE, the predominant oxygenate currently being used in oxyfuel and RFG, was added to gasoline before 1992. Since the late 1970s, MTBE has been used as an octane booster in gasoline in concentrations up to 9% by volume, but usually at much lower concentrations. In the late 1980s, MTBE was introduced in concentrations up to 12% by volume in areas that exceeded the CO standard (e.g., Colorado) to reduce CO emissions in winter months. Eight metropolitan areas in western states started oxyfuel programs before 1992. On November 1, 1992, in response to the requirements in the CAAA of 1990, new oxyfuel programs began in 20 metropolitan areas outside California and in eight areas within California, where a waiver had been obtained that reduced the required level of oxygen content from 2.7% to between 1.8% and 2.2%. The number of regions using oxygenates increased in January 1995, when RFG use was initiated in areas with high ozone levels. The number of people living in areas using oxyfuel is approximately 70 million and the number living in areas using RFG is approximately 57 million, with some overlap between the areas using oxyfuel in the winter months and RFG for the rest of the year (SRA Technologies 1995).

EVALUATING THE HEALTH EFFECTS OF OXYGENATES

In the late 1980s in response to a designation by the Interagency Testing Committee formed under the Toxic Substances Control Act, the fuel industry and U.S. EPA started testing the toxicity of MTBE to assess the potential health effects from its use in fuels. The widespread introduction of oxyfuel in 1992 was followed by an increase in the number of health complaints in several states, including Alaska, New Jersey, and North Carolina. The most frequently reported symptoms were headache, dizziness, eye irritation, and burning of the nose and throat. To address public concerns raised by these reports, field studies and controlled human exposure studies were conducted in the winter and spring of 1993 to investigate the acute human responses after exposure to MTBE. When RFG was introduced in 1995, complaints of symptoms came from some other states, including Wisconsin. In addition, concerns have been raised about the carcinogenicity of MTBE on the basis of results from studies of animals exposed to high concentrations of MTBE.

Using oxygenates is intended to decrease exposure to some pollutants of public health concern, but it also results in exposure either to a higher level of a previously used oxygenate (such as MTBE) and its metabolites, or to another ether or alcohol. In addition, using oxygenates results in increased exposure to aldehydes, whose toxic and potential carcinogenic effects are of concern. In evaluating the health effects of exposure to oxyfuel and RFG, the potential health effects of the total pollutant exposure from these fuels needs to be compared with the health effects of exposure to the total pollutants from conventional gasoline.

The U.S. EPA has reviewed the information available on the health effects of MTBE in two documents: *Assessment of Potential Health Risks of Gasoline Oxygenated with Methyl-Tertiary Butyl Ether* (November 1993), and *Health Risk Perspectives on Fuel Oxygenates* (December 1994). More recently, the White House Office of Science and Technology Policy (OSTP) has undertaken a broader review of oxyfuel and RFG, including their effects on air quality, engine performance, fuel economy, and groundwater, as well as public health. This HEI document reviewing the potential health effects of oxygenates is intended to serve as the core of the health effects assessment of the OSTP project. The central question asked by the HEI Oxygenates Evaluation Committee is: What are the potential health effects of using oxygenates in gasoline? To address this question, the review evaluates information on exposure to, and toxicity of, MTBE alone and in combination with gasoline. Furthermore, the review discusses the EPA's earlier conclusions about oxygenates in light of more recent information. In addition to MTBE, the review evaluates information on other oxygenates that are being used or may be used in gasoline, including other ethers and ethanol. Because much less information is available on ethers other than MTBE, an assessment of potential health effects from exposure to them cannot be made.

To provide some perspective on the health effects of oxygenates, this review also considers how using oxygenates in gasoline changes emissions from motor vehicles, and summarizes the current understanding of the health effects of exposure to those pollutants whose levels in emissions change when oxygenates are added to fuel.

The report also identifies and prioritizes research needed to decrease the uncertainty in projecting risks to the public from exposure to oxygenates in gasoline.

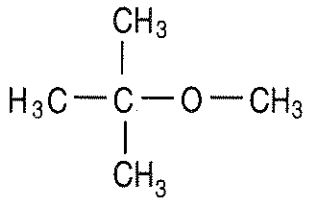
General Properties of Oxygenates

In preparing to examine the possible impact on human health of oxygenates added to gasoline, it is helpful to understand the chemical class of these compounds and the physical-chemical properties that govern their behavior. Oxygenates are compounds containing one oxygen atom within a chain of carbon and hydrogen atoms. The two classes of chemicals used as oxygenates in fuel are ethers and alcohols. Alcohols are derivatives of hydrocarbons (which are made of carbon and hydrogen) in which one or more of the hydrogen atoms have been replaced by a hydroxyl group (OH). Ethers are derived from alcohols by replacing one hydrogen atom in the hydroxyl group with an alkyl group (a chain of one or more carbon atoms with associated hydrogen atoms). Therefore, in ethers an oxygen atom is connected to two carbon atoms (C—O—C). In general, ethers are not very reactive with other compounds because the carbon-oxygen bond is not readily cleaved.

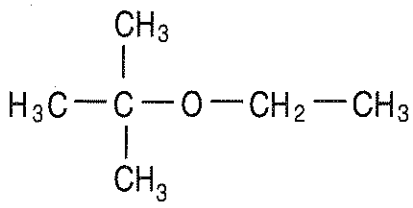
Figure 1 shows the chemical structures of the oxygenates likely to be used as gasoline additives: MTBE, ETBE, TAME, DIPE, and ethanol. Of these, the most commonly used are MTBE, ethanol, and ETBE. MTBE generally is produced by causing isobutylene to react with methanol via a catalyst. Ethanol can be obtained by fermenting carbohydrates. It is used widely as an industrial solvent and an antiseptic, and is present in alcoholic beverages. Ethanol is also the substrate for ETBE.

Table 1 summarizes some of the chemical and physical parameters of the oxygenates, including molecular weight, density, water solubility, octanol-water partition coefficient, octane number, vapor pressure, boiling point, and the percentage by volume of each chemical necessary to achieve an oxygen content by weight of 2% (as in RFG) or 2.7% (as in oxyfuel) when mixed with gasoline. For purposes of comparison, the data for benzene (a well-characterized component of gasoline) are also listed.

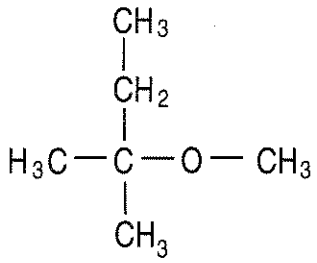
Ethanol and the ethers discussed in this report are all colorless, flammable liquids. Like *tert*-butyl alcohol (TBA) and other alcohols, ethanol is substantially less odorous than ethers. Its odor detection threshold is 49 parts per million (ppm), while the odor detection thresholds of the ethers range between 13 and 53 parts per billion (ppb) (Table 2). The differences in the oxygenates' odor recognition thresholds are comparable to the differences in their odor detection thresholds. Among the ethers, ETBE is the



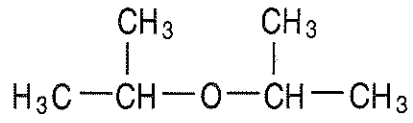
Methyl *tert*-butyl ether (MTBE)



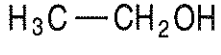
Ethyl *tert*-butyl ether (ETBE)



***tert*-Amyl methyl ether (TAME)**



Diisopropyl ether (DIPE)



Ethanol

Figure 1. Chemical structures of oxygenates likely to be used as gasoline additives.

most odorous, followed by TAME and then MTBE. Ethers also have a very low taste threshold when mixed with water. MTBE and ETBE can be detected at concentrations of approximately 50 ppb.

Ethanol is completely soluble in water, whereas ethers are only partially soluble. Among the four ethers listed in Table 1 (MTBE, ETBE, TAME, and DIPE), MTBE has the greatest water solubility (4.8 g/100 g of water) and DIPE has the lowest water solubility (0.2 g/100 g). The octanol-water partition coefficient represents the tendency of a chemical to move between an organic phase and an aqueous phase. It is defined as the equilibrium ratio between the chemical's concentration in the octanol phase and its concentration in the aqueous phase. The smaller the coefficient, the greater the tendency of the compound to mix with water rather than with octanol. In the order of most polar to least polar, the octanol-water partition coefficients are for ethanol 0.50, for MTBE 16, and for benzene 135 (see Table 1) (Weston Managers and Designers Consultants 1987). The considerable differences in these values have implications for how ethers and ethanol may move into underground water should soil become contaminated with gasoline (see Exposure Assessment section).

The octane number indicates a chemical's ability to prevent engine knocking, which is caused by fuel combusting before the piston reaches the top of its stroke. When this happens the engine pushes against the crankshaft rather than with it, which reduces the engine's power and can damage mechanical parts. To prevent this, chemicals with antiknocking properties are added to gasoline. Isooctane is generally used as a reference gasoline to determine the antiknocking properties of gasoline blends. The octane number indicates the percentage of isooctane by volume in a mixture of isooctane and normal heptane that matches the given gasoline in antiknock characteristics (Lefler 1985). Ethanol has the highest octane number (115) and TAME and DIPE have the lowest (105).

The vapor pressure is a measure of a compound's volatility. It is defined as the pressure exerted by the vapor above its own liquid when the liquid and vapor are at equilibrium at a given temperature. The boiling point is the temperature at which the vapor pressure of a liquid is equal to 1 atmosphere. A compound with a low boiling point has a high vapor pressure and is more volatile. For fuel formulation purposes, the vapor pressure is measured at 100°F in pounds per square inch (psi), and is referred to as Reid vapor pressure (RVP). Among the ethers used in gasoline formulation, TAME has the lowest vapor pressure, indicating that it is the least volatile, and MTBE has the highest. Ethanol has a lower vapor pressure than TAME. However, when mixed with gasoline, ethanol forms an azeotropic solution and tends to vaporize more easily than ethers. It also tends to make other hydrocarbons with the same boiling point more volatile. The overall result of these effects is that the RVP of ethanol in a gasoline blend is much higher than the RVP of ethanol alone (see Table 1). An oxygenate with a higher RVP will be present in evaporative emissions at higher concentrations than one with a lower RVP.

Table 1. Physical and Chemical Properties of Oxygenates and Benzene

Property	MTBE	ETBE	TAME	DIPE	Ethanol	Benzene
Molecular weight[a]	88	102	102	102	46	78
Density[a] (g/L)	0.74	0.74	0.81	0.77	0.79	0.87
Water solubility[a,b] (g/100 g water)	4.8	1.2	1.1	0.2	Infinite	0.18
Octanol-water partition coefficient[c]	16	NA	NA	NA	49	135
Octane number[d]	110	112	105	105	115	85
Vapor pressure						
torr at 25°C[e,f]	245	130	75	159	54	73
psi (RVP) at 100°F[d]	7.8	4	2.5	4.9	2.3	
Blending RVP[d]	8	4	2.5	5	18	
Boiling point[a,e] (°C)	55.2	72.7	86.2	68.5	78.5	80
Volume% for						
2% oxygen by weight[d]	11	12.8	12.8	12.5	5.7	
2.7% oxygen by weight[d]	15	17	17.4	17.4	7.8	

[a] Data taken from the Merck Index, 11th ed., 1989.

[b] Data taken from Stephenson 1992.

[c] Data taken from Piel 1989. NA = Not available.

[d] Data taken from Piel 1994.

[e] Data taken from Krahenbuhl and Gmehling 1994.

[f] Data taken from CRC Handbook of Chemistry and Physics, 67th ed., 1986–1987.

Table 2. Odor and Taste Thresholds of Some Oxygenates at 75°F

Oxygenate	Odor Detection Threshold (ppm)	Odor Recognition Threshold (ppm)
MTBE (97% purity)[a] MTBE (99% purity)[b]	0.053 0.053	0.125 0.08
ETBE (99% purity)[b]	0.013	0.024
TAME (94% purity)[c]	0.027	0.047
Ethanol[d]	49.00	101.00
TBA[e]	21.00	41.00
Taste Threshold (ppm)		
MTBE in water (97% purity)[a] MTBE in water (99% purity)[b]	0.039 0.134	
ETBE in water (99% purity)[b]	0.047	
TAME in water (94% purity)[c]	0.128	

[a] Data taken from American Petroleum Institute 1994.

[b] Data taken from TRC Environmental Corporation 1993.

[c] Data taken from American Petroleum Institute 1993b.

[d] Data taken from May 1966.

[e] Data taken from K. Vetrano (personal communication, 1995).

Exposure Assessment

INTRODUCTION

Identifying potential health risks for any environmental pollutant, assessing the magnitude of those risks, and developing cost-effective mitigation strategies require information on the nature and extent of exposures of the populations at risk. Exposures to MTBE and other fuel oxygenates can occur at any point in their manufacture, distribution, and use. Once these compounds are blended with gasoline, individual or population exposures to them take place within a background of emissions from gasoline and gasoline combustion by-products—truly complex mixtures. These exposures result in human doses that might ultimately have effects on health. In assessing exposures to MTBE and other fuel oxygenates, many factors must be considered: the fate of these compounds in the environment, routes of exposure, extent of exposure (frequency, magnitude, and duration), and populations at risk. The resulting dose to an individual (the amount actually absorbed by the body) is affected by factors related to the uptake, metabolism, and elimination of the compounds.

Although considerable concern has been expressed about health effects associated with exposure to oxygenates, no sensitive subpopulations have yet been characterized. The nonoccupational groups at risk comprise people living in areas of the country where fuels containing oxygenates are used. They may be exposed to oxygenates through inhalation, ingestion, or contact with the skin. The occupational groups at risk are those handling the neat compounds or fuels containing oxygenates, such as manufacturing and distribution workers and service station attendants, and mechanics or others associated with the repair and maintenance of motor vehicles. Occupational exposure can occur by inhalation and through contact with the skin.

The major route of exposure for both nonoccupational and occupational groups is via inhalation, and these exposures can vary considerably. Integrated personal exposures can be assessed through use of personal monitors or by summing the products of the concentrations in different environments and the time spent in those environments. Spillage during fueling or leakage from storage tanks can result in the transport of oxygenates in the groundwater and potentially lead to exposure through ingestion of contaminated drinking water. Although skin contact is known to occur in both nonoccupational and occupational settings, little or no information exists on the relative importance or extent of dermal exposures for either group.

This section considers the available data on the nature and extent of exposures to fuel oxygenates. Oxygenates in the atmosphere are derived from evaporative emissions from gasoline and to a lesser extent from gasoline combustion. The major points of evaporation from an automobile are the gas cap, the carbon canister vent for evaporative emission control, the gas tank when the fuel is excessively hot, and leaking fuel lines (R. Gorse, personal communication, 1996). The focus is largely on MTBE because few or no data exist on environmental exposures to other fuel oxygenates. Estimates for the populations at risk are derived from current use patterns in the United States; the available data on measured oxygenates in air in both nonoccupational and occupational settings are reviewed and summarized by microenvironments in which the concentrations were measured, and likely length of exposures in those microenvironments. The potential for groundwater contamination with oxygenates and ingestion of contaminated drinking water is considered. Finally, data relating air exposures to biological indicators of dose are presented.

The addition of oxygenates to fuel impacts the emissions of other air pollutants from conventional fuel mixtures, resulting in some beneficial and some potentially detrimental changes. Exposure to oxygenates has to be considered within the broader context of their impact on total emissions. Although outside the primary focus of this review, some discussion of the possible impact of adding oxygenates to fuel on other emission components (including CO, air toxics, and ozone precursors) is provided.

OXYGENATE USE IN THE UNITED STATES

Since the 1970s, MTBE has been added to fuels as an octane booster, in concentrations of less than 1% by volume in regular gasoline and 2% to 9% by volume in premium gasoline (U.S. Environmental Protection Agency 1993a). The CAAA of 1990 mandated the use of fuels with higher oxygen content (at least 2.7% by weight) during the winter months, beginning in November 1992, to reduce CO emissions from motor vehicles in areas of the United States

where the 8-hour NAAQS for CO was being exceeded. In California, the oxygen content of oxyfuel is limited to 1.8% to 2% to avoid any increase in NO_x emissions, which could exacerbate the ozone problem.

Colorado was the first state to initiate an oxyfuel program. This began in January 1988 with MTBE at a concentration of 8% by volume as the predominant oxidant in fuel. For the next oxyfuel season (starting November 1, 1988), this was increased to 11% MTBE by volume (2.0% oxygen by weight), and it was subsequently raised to 2.7% oxygen (by weight). In all, eight metropolitan areas in western states had started oxyfuel programs prior to 1992 (U.S. Environmental Protection Agency 1993d). On November 1, 1992, in response to the mandate in the CAAA of 1990, new oxyfuel programs began in 20 metropolitan areas outside California and in 8 areas within California. In the winter of 1994/1995, oxyfuel was being used in 31 areas of the United States (Table 3).

The CAAA of 1990 also required use of reformulated gasoline (RFG), starting in January 1995, in areas with the worst exceedances of the ozone NAAQS. Reformulated gasoline must contain at least 2.0% oxygen by weight, the concentrations of benzene and aromatic compounds are limited, and the emission levels of ozone-forming volatile organic compounds and total air toxics are specifically regulated. Nine areas of the United States were required to participate in the RFG program in 1995; other regions voluntarily decided to use RFG (Table 4).

MTBE is the most widely used oxygenate, followed by ethanol. Other oxygenates that are sometimes used or may be used in the future include ETBE, TAME, and DIPE. Different amounts of these oxygenates are needed to meet the oxygen requirements; for example, 15% MTBE or 7.8% ethanol (by volume) would provide 2.7% oxygen by weight, and 11% MTBE or 5.7% ethanol (by volume) would provide 2.0% oxygen (by weight). In Colorado, the amount of ethanol in oxyfuel increased from 6% of the market share in 1988 to 80% in the 1994/1995 wintertime oxyfuel season, with MTBE being used in the rest of the oxyfuel (S. Erdal and S.M. Ayres, personal communication, 1996). When RFG was introduced in the Milwaukee area in 1994/1995, about half the fuel contained MTBE, and the remainder contained either ethanol or ETBE. Table 3 presents information on the proportion of oxyfuel containing MTBE compared with that containing ethanol. The states with areas using primarily ethanol were Alaska, New Mexico, Arizona, Minnesota, Montana, Colorado, Texas, Washington, and Oregon.

The areas that do not attain the standards for both CO and ozone use RFG year-round, and increase the oxygen content in the fuel from 2.0% to 2.7% in the winter months. Approximately 70 million people live in areas where oxyfuel is used, and 57 million where RFG is used (SRA Technologies 1995). In 1991, approximately 55 million people lived in areas violating the 8-hour CO standard (U.S. Environmental Protection Agency 1992b). Personal exposure levels of individuals in these areas may, of course, be higher or lower than those recorded by ambient air monitors that are used to determine whether an area violates the standard.

METHODS FOR ANALYZING MTBE IN THE ENVIRONMENT

Several procedures have been developed to facilitate chemical analysis of MTBE. For any analytical technique to perform according to specifications, it is necessary to pay particular attention to quality control in obtaining, storing, and analyzing samples; to reproducibility of the analyses obtained with replicate samples; and to verification of the instrument calibration by including known standards over a range of concentrations.

Like other organic compounds, MTBE is collected either by trapping from an air sample passed through an appropriate sorbent (charcoal or carboxen 569), or by direct collection of an air sample in an evacuated, stainless steel (SUMMA) canister. MTBE either is removed from the sorbent by thermal treatment or solvent extraction, or is concentrated from the collected air sample by cryogenic condensation of the organic compounds present. Separation and quantitation of MTBE is characteristically performed by gas chromatography with flame ionization detection based on retention time on silica capillary columns. (The original method is referred to as NIOSH 1615.) However, without verification by mass spectrometry, there is a possibility that interference from coeluting compounds will cause MTBE values to be higher than actual concentrations (American Petroleum Institute 1993a). In some of the studies reported, gas chromatography with mass spectrometry was used for the analysis.

In a comparative study, Lioy and coworkers (1994) thoroughly documented appropriate procedures and precautions to be taken in MTBE analyses when air samples are collected by adsorption on carboxen or by direct sampling in evacuated containers. They used gas chromatography only to separate the organic compounds in the air sample into fractions for further analysis by low-resolution electron impact spectrometry. Under these conditions the limit of detectability of MTBE in the mass spectrometer was below 1 ng, and interferences in the MTBE analysis were minimized because of the selectivity of the mass spectrometer and the limited number of compounds that were volatilized. The data quality objectives of the study were consid-

ered to be met because paired analyses of adsorbent and canister samples performed in two laboratories agreed to within a factor of 2. Furthermore, except for a single sample at the lowest MTBE concentration, MTBE values in the canister samples analyzed by the two laboratories were within ± 50%, and MTBE values of the EPA Quality Assurance samples were within ± 25%.

EXPOSURES TO OXYGENATED COMPOUNDS

NONOCCUPATIONAL EXPOSURES

Air Exposures

In response to concerns about the potential adverse health effects of exposure to the oxygenates in gasoline, several studies have attempted to assess human exposure to these compounds. Concentrations of MTBE, and occasionally ETBE, TAME, and ethanol, were measured in urban air, at service stations and their perimeters, and in the cabins of motor vehicles. The data for MTBE are summarized in Table 5 and the data for ETBE and ethanol in Table 6. When possible, the median value is given because it is less sensitive to extreme values. Figure 2 illustrates the ranges in MTBE concentrations to which consumers are exposed for a variety of situations in the context of the duration of such exposures. Figure 3 illustrates the cumulative distribution of individual MTBE measurements for three different sampling conditions at service stations in Phoenix, AZ. The range of measurements extended over 3 orders of magnitude for both 1- to 2-minute and 4-hour personal breathing zone samples, indicating a wide variation in the MTBE levels. The range of measurements was much narrower for 12-hour service station perimeter samples.

Community Air MTBE and ETBE measurements were made for 24 hours on 8 separate days in January through March 1995 on the campus of the University of Wisconsin in Milwaukee. From analyses of gasoline samples taken at some service stations, Allen and Grande (1995) estimated that approximately 50% of the RFG sold contained MTBE and the remainder contained either ETBE or ethanol. Concentrations of MTBE (Table 5) and ETBE (Table 6) ranged from the minimal detectable concentration (MDC) of 0.025 ppb in nearly one-half of the samples to a maximum of 4.1 ppb for MTBE and 1.7 ppb for ETBE; median concentrations were 0.13 ppb for MTBE and 0.04 ppb for ETBE. The higher median concentration for MTBE makes sense because it was used in more of the fuel. Ethanol concentrations were not measured.

In this study, 2-hour air samples also were taken, starting at 8 a.m., at several sites within Milwaukee to measure exposure during periods of heavy traffic. Samples were taken at busy intersections, freeway interchanges, and parking garage ramps. Mean concentrations of MTBE and ETBE were 2.5 to 5 times higher than in the 24-hour samples, but most samples remained in the sub–parts per billion range (Tables 5 and 6). In this sample set, the highest concentrations were found at the parking garage ramps (mean of 2 ppb for MTBE and 1 ppb for ETBE). In general, concentrations of the other gasoline components examined (e.g., benzene and toluene) were proportionate to the concentrations of MTBE and ETBE.

Automobile Cabin During Commuting A study of commuter exposure to MTBE was performed by Lioy and coworkers (1994). MTBE levels were measured during 1-hour commutes in heavy traffic in New Jersey and Connecticut.

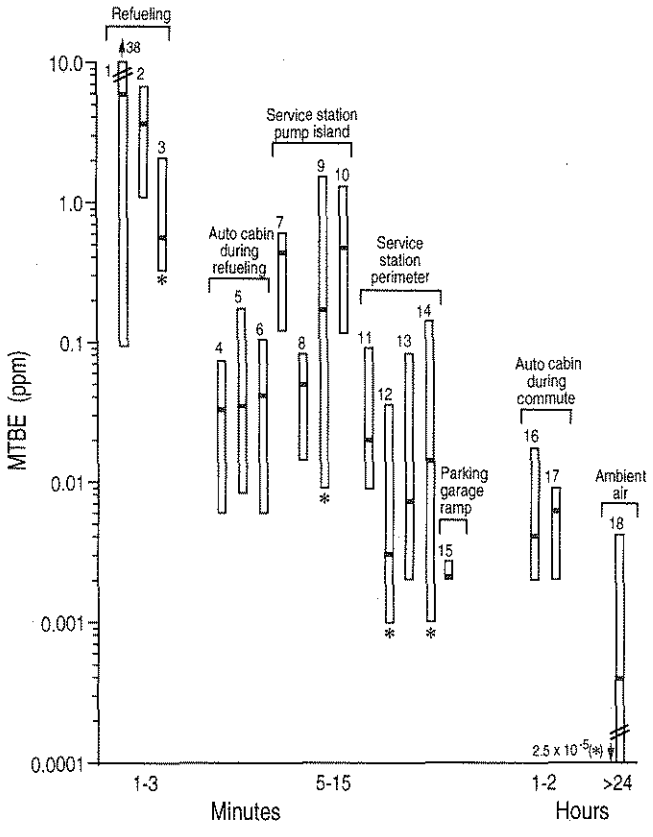


Figure 2. Time-weighted average exposures of the general public to MTBE. Only studies that provided ranges of exposure levels are included. The solid lines across the bars indicate median values. The numbers at the tops of the bars correspond to the numbers in parentheses in the "Sampling Site" column of Table 5, where the same data are expressed. An asterisk (*) denotes the minimal detectable concentration.

Each measurement consisted of an exterior hood sample (roadway air) and an interior cabin sample; MTBE concentrations ranged from 2 to 17 ppb (Table 5). Unexpectedly, one of the four vehicles used in the study had abnormally high evaporative emissions that resulted in interior cabin MTBE concentrations significantly higher than the roadway air samples. Thus, individual commuters could be subjected to different levels of MTBE depending upon the condition of the vehicle used.

Service Station During Refueling The potential for exposure of customers to MTBE or other oxygenated compounds in oxyfuel or RFG is high during refueling at service stations. Accordingly, a number of studies measured concentrations of MTBE, and occasionally other oxygenates, in a variety of service stations that differed in types of fuels dispensed, whether they provided full service, or self-service, or both, and whether they were equipped with Stage II vapor recovery systems. During refueling, samples were taken from the customer's breathing zone, the pump island, and from the automobile cabin.

Breathing zone samples were collected during the refueling process from service stations with gasoline containing MTBE in Phoenix, AZ, and Los Angeles, CA (American Petroleum Institute 1993a) (Table 5). These were composite samples representing 8 to 10 refuelings. As shown in Table 5, the samples from Phoenix, where there was no vapor recovery system, had a median MTBE concentration of 5.8 ppm, and a maximum of 38 ppm. In Los Angeles, where there was a vapor recovery system, the levels were somewhat lower: the samples had a median concentration of 3.6 ppm and a maximum of 6.5 ppm.

Lioy and coworkers (1994) also measured customer breathing zone exposures during the refueling period at service stations in the New Jersey–New York–Connecticut area in April 1993. Unlike the samples from Phoenix and Los Angeles (American Petroleum Institute 1993a), which were collected only during the actual refueling period, these samples were collected over a 5-minute interval that included times before, during, and after refueling. Breathing zone samples were collected at fuel pumps that either did or did not have vapor recovery systems. As expected, the MTBE concentrations were lower in these samples than in samples taken only during the refueling period. Median MTBE exposure levels were 0.57 ppm in the absence of the vapor recovery system and 0.37 ppm in its presence (Table 5). The maximum MTBE concentration measured was 4.1 ppm.

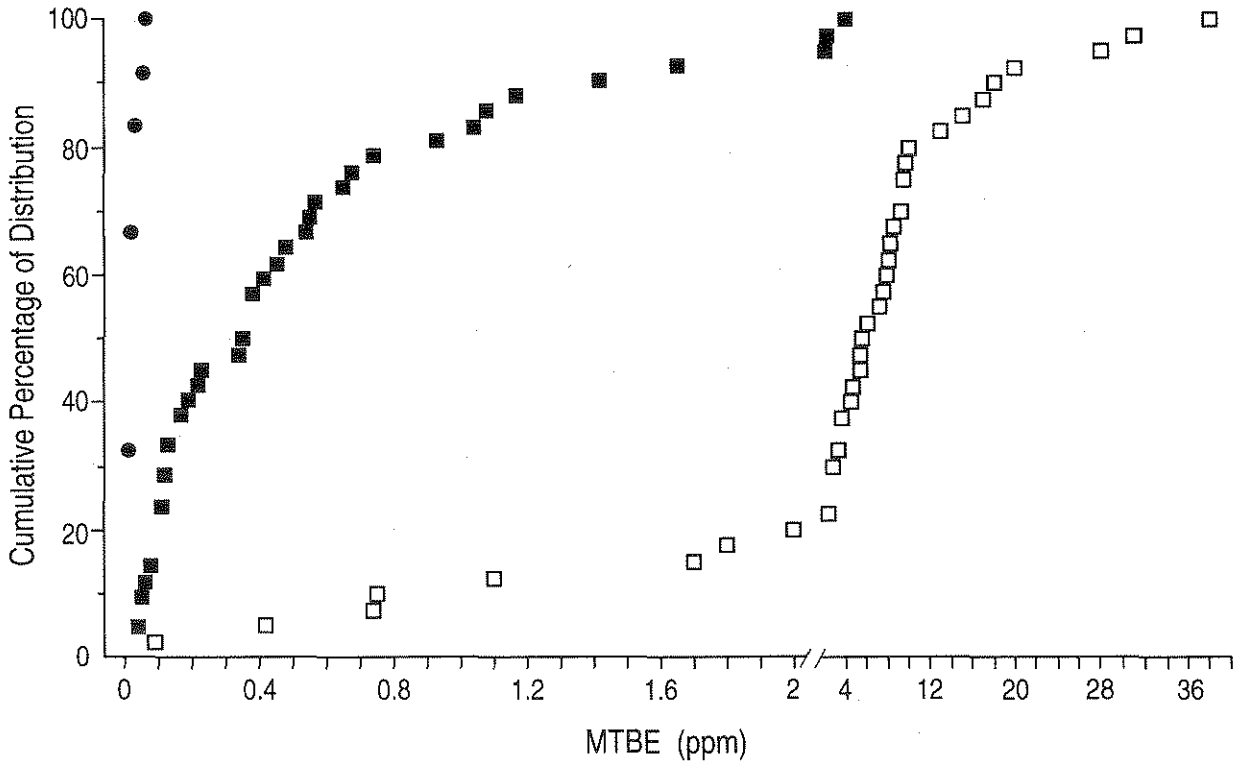


Figure 3. Cumulative percentage of distribution for selected exposures to MTBE. Solid circles (●) indicate 12-hour samples from the perimeter of a Phoenix service station; solid squares (■) indicate 4-hour personal breathing-zone samples from service station attendants; and open squares (□) indicate 1- to 2-minute personal breathing-zone samples during refueling. Data are from Hartle and coworkers (1993) and American Petroleum Institute (1993a).

Simultaneous 5-minute samples also were taken on the passenger side of the automobile interior (passenger-side window closed; driver-side window open) at the service stations in the New Jersey–New York–Connecticut area (Lioy et al. 1994). The automobile interior samples were approximately 95% lower than the corresponding 5-minute samples from the customer breathing zone. The median levels were about 0.03 ppm at stations with vapor recovery systems. The MTBE levels were similar at the stations lacking vapor recovery systems, and were comparable between full-service and self-service stations.

Breathing-zone samples also were collected during refueling for periods of 15 minutes at two service stations in Milwaukee where RFG containing MTBE was dispensed in midgrade and premium gasoline (Allen and Grande 1995). The average level was 0.39 ppm MTBE for the station with a vapor recovery system and 2.93 ppm MTBE for the station with no vapor recovery system. These values are lower than those reported by API (1993a), but the amount of MTBE in gasoline also was lower.

Table 6 presents information on ETBE concentrations in samples taken at a service station in Milwaukee where all grades of gasoline contained ETBE (11% to 12% by volume) in January through March 1995 (Allen and Grande 1995). Fifteen-minute breathing-zone samples contained a mean concentration of 0.1 ppm ETBE.

Table 6 also presents information on ethanol breathing-zone samples collected in February, March, and April 1994 during refueling at service stations in Minnesota, Arizona, and Oregon (American Petroleum Institute 1995c). Short-term (15- to 20-minute) samples ranged from less than the MDC of about 1 ppm (41/44 samples) to values of 2.4, 10, and 46 ppm. Long-term (> 6-hour) samples were below the MDC (30/31 samples), with the exception of one sample (9 ppm). These results suggest that the MDC in these samples was quite high relative to ambient levels. The two highest short-term values and the one long-term value above the MDC were questionable because of unusual behavior of the attendants. These data suggest that ethanol exposures during refueling are generally low, but that refueling has the potential to result in high exposures to gasoline (and its components).

Service Station Pump Island Another type of measurement that integrates refueling and interim periods with lower exposures is one taken at the pump island. Four-hour samples were taken during the morning and afternoon at pump islands of 10 service stations in the New Jersey–New York–Connecticut area in April 1995, when gasoline containing MTBE was being used (American Petroleum Institute 1995a). Considerable variation in the median levels was noted among different classes of stations. Two full-service stations in New Jersey using a vapor recovery system had a median MTBE concentration of 0.44 ppm, and three self-service stations in New York, also using a vapor recovery system, had a median concentration about 10-fold lower (0.05 ppm). Five self-service stations in Connecticut without a vapor recovery system had a lower median concentration (0.17 ppm) than the full-service station with a vapor recovery system mentioned above.

Service Station Perimeter MTBE concentrations were measured in 12-hour air samples from several locations around the perimeters of service stations during the autumn of 1990 in Phoenix, where oxyfuel containing MTBE was used (Table 5) (American Petroleum Institute 1993a). MTBE concentrations in samples taken at the Phoenix service stations had a median level of 20 ppb and a maximum of 90 ppb.

Samples also were taken at the perimeters of 10 service stations in the New Jersey–New York–Connecticut area using gasoline containing MTBE in April 1995 (American Petroleum Institute 1995a). The MTBE levels in these 4-hour samples were quite low, ranging from a median of 3 ppb to a median of 14 ppb. The stations with the highest median concentration (the five stations in Connecticut) did not have a vapor recovery system, while the two stations in New Jersey and three in New York did. These samples, taken at a distance from the pump islands, reflect levels of MTBE that people might be exposed to for a few minutes as they walked by a service station. Mean levels of MTBE in 2-hour samples taken at a service station in Milwaukee during January through March 1995, where MTBE (9% to 10% by volume) was used only in the higher grades of gasoline and ethanol was used in the lower grades, were also low (2 ppb).

Two-hour samples were taken at the perimeter of a service station (15 meters downwind) in Milwaukee, where all grades of gasoline contained ETBE (11% to 12% by volume), in January through March 1995 (Table 6) (Allen and Grande 1995). The mean concentration (in two samples) was 3.6 ppb. For comparison, a service station in Milwaukee with RFG (containing MTBE 9% to 10% by volume) only in the higher grades of gasoline had a level of 2.4 ppb (average of two samples), and another service station in Milwaukee without a vapor recovery system and with conventional gasoline containing MTBE (2% to 9% by volume) had a level of 4.6 ppb (based on one sample) (Table 5).

Summary of Air Exposures The general public experiences the highest levels of exposure to the oxygenated components of gasoline during vehicle refueling, with concentrations generally higher at service stations that do not have vapor recovery systems. These concentrations usually decrease from the personal breathing zone of the customer

or attendant to the pump island, and then decrease further at the station perimeter (Table 5).

Many of the exposure studies reported a small number of measurements that reflected relatively high concentrations of MTBE. Factors associated with high MTBE values for samples from the customer breathing zone and station perimeter included the occurrence of fuel spills or overflows, high dispensing rates of gasoline (> 325 gallons/hour) or high numbers of vehicles serviced, wind speed less than 3 mph, and heavy street traffic (American Petroleum Institute 1993a).

The range of measurable MTBE levels shown in Table 5 extends over more than 5 orders of magnitude, from 0.0001 ppm (ambient air in Milwaukee) to 38 ppm (a 1- to 2-minute sample taken only during refueling in Phoenix). Figure 2 presents the MTBE exposure ranges in categories of likely duration of exposure for the public. The shortest exposures tended to be the highest in concentration, while longer exposures were progressively lower in concentration. Median exposures during refueling in the customer breathing zone were typically in the range of 0.3 to 6 ppm and occasionally higher. Lower levels in the hundredths-ppm range were obtained in the vehicle interior within this time frame, reflecting potential passenger exposure. Midrange time exposures (1 to 2 hours) are represented by automobile interior cabin measurements during commuting hours in suburban traffic, with substantially lower median exposures in the thousandths-ppm (ppb) range. Finally, longer sampling time exposures of 4 to 24 hours are represented by service station perimeter measurements. These exposure values are comparable to those experienced by commuters in automobiles and could also be considered as measures of exposures experienced by those living or working close to service stations. Ambient air levels, as represented by measurements in Milwaukee, are even lower.

Groundwater Exposures

With the growing use of oxygenates in gasoline, attention needs to be paid to potential contamination of groundwater resulting either from leaking underground storage tanks or from wet deposition. MTBE, present in gasoline for many years as an octane enhancer, has often served as a marker of gasoline contamination in soil.

The diffusion of gasoline components through the soil depends on several factors. Laboratory studies were conducted to determine the effect of soil composition on absorption and retention of selected components of gasoline, using soils from two sites with histories of gasoline contamination, one from Traverse City, MI, and one from Granger, IN (Thomas et al. 1988). The investigators ran individual chemicals in aqueous solution through a glass column packed with soil. The Traverse City soil, which was low in organic carbon content, did not cause retardation of any of the organic compounds tested (benzene, toluene, *p*-xylene, ethylbenzene, MTBE, and TBA). The Granger soil with higher organic carbon content did cause retardation of the less-soluble components, but not of the more-soluble components (MTBE and TBA). In this study, microbial degradation of the hydrocarbon contaminants also was studied. The microflora present in the soil samples were capable of degrading some hydrocarbons. Soil samples from uncontaminated areas were less efficient in degrading hydrocarbons. In summary, MTBE appeared to diffuse freely through soil independent of its composition.

A modeling study (Weston Managers and Designers Consultants 1987) estimated the rate of movement through the soil of various gasoline components considering the octanol-water partition coefficient of each component, soils with different porosity, and the rate of groundwater flow. The major conclusion of the modeling is that oxygenates are not readily absorbed into soil and migrate at rates essentially similar to water. Benzene, toluene, and meta-xylene, which are less soluble in water than oxygenates, are more readily absorbed by the soil and migrate at a slower rate than water.

Piel (1989) examined tertiary mixtures of benzene, water, and an oxygenate to calculate the effects of the oxygenate on benzene solubility in water. Alcohols such as ethanol and methanol, which have a very high water-benzene solubility ratio, were shown to increase the solubility of benzene in water. MTBE, which has a lower solubility ratio than ethanol, did not seem to affect the solubility of benzene in the experimental system. The other ethers are less soluble in water than MTBE and, therefore, are not expected to increase the benzene content in water.

In summary, the fate of an oxygenate in soil depends on the physical and chemical properties of the soil, the amount of organic matter in the soil, the partition coefficient of the compound of interest, and the composition of the soil microflora. In general, ethers and alcohols are expected to migrate fairly easily from gasoline into groundwater. Although ethers are not expected to increase the concentration of less-soluble organic compounds, such as benzene, in water, ethanol may increase the concentration of less-soluble chemicals.

Levels of Oxygenates in Groundwater As part of its National Water Quality Assessment Program, the U.S. Geological Survey (1995) measured concentrations of volatile organic compounds in water samples taken from 211 wells in 8 urban areas and 524 shallow wells in 20 agricultural areas of the United States. Chloroform and MTBE were the two most frequently detected compounds. MTBE was de-

tected in 27% of urban wells and in 1.3% of agricultural wells. Although concentrations ranged from less than the MDC of 0.0002 mg/L to as high as 23 mg/L, only 3% of the wells sampled in urban areas had concentrations that exceeded 0.02 mg/L. MTBE was most prevalent in urban areas in Colorado (primarily Denver) and in New England (Connecticut, Massachusetts, and Vermont), but it was also detected in several other urban areas across the United States. The areas with the highest levels of MTBE were Denver, CO (up to 23 mg/L), and Reno, NV (up to 0.2 mg/L). Because of the widespread presence of MTBE in the samples collected, one can speculate that contamination could have occurred not only from gasoline spills and leakage from underground tanks, but also from deposition of MTBE mixed with precipitation.

The investigators commented that the wells sampled were not used for drinking water and were shallower than most wells that are used for drinking water. However, MTBE and other oxygenates tend to move into groundwater, and there is evidence that, if present in surface water or soil, they will penetrate deeper into the aquifer. Although many questions remain about the fate of oxygenates in soil, including the role of biodegradation and evaporation, these findings support the conclusion that ethers used in gasoline may eventually contaminate the water supply in some situations.

An instance of severe groundwater contamination in Orange County, NY, was described by the New York State Department of Health (1995). In December 1994 two wells supplying drinking water to a mobile home park were found to contain elevated levels of MTBE. The levels were 1.3 and 2.9 mg/L. Concentrations in tap water ranged from 0.9 to 1.4 mg/L. Levels of benzene were also very high. The source of contamination was attributed to a gasoline tank that was removed from a nearby service station in December 1994. Contamination of the service station's tap water had been reported a year earlier, in December 1993. That problem had been corrected by installing a water filter at the service station. However, water samples from wells in two nearby residences collected in March and April 1994 were found to contain MTBE levels of 1.7 and 5.9 mg/L. Contamination of wells unrelated to the ones just described was detected in other counties of the state of New York.

This brief summary of groundwater MTBE levels measured in areas of the United States suggests that oxygenate contamination of drinking water represents a possible exposure scenario. Therefore, to prevent protracted exposures of the public, drinking water samples should periodically be tested to monitor the presence of the specific oxygenates used in the area. The EPA is in the process of revising the drinking water health advisory for MTBE, which is the maximum concentration in drinking water that is not expected to cause any adverse effects over a lifetime of exposure, with a margin of safety. The 1992 draft proposed that the level should fall between 0.02 and 0.2 mg/L (U.S. Environmental Protection Agency 1992a).

OCCUPATIONAL EXPOSURES

Workers whose occupations involve handling oxygenates, alone or in combination with gasoline, are exposed to higher levels of oxygenates than the general public. Table 7 summarizes MTBE exposure information for workers in nonindustrial occupations (service station attendants and mechanics); and Table 8 summarizes industrial exposures to MTBE. Table 6 provides a small amount of information on occupational exposure to ETBE and ethanol.

Service Station Attendants

The data summarized in Table 5 concerning consumer exposures to MTBE are also applicable as measurements of exposure for service station attendants. Table 7 repeats those short-term exposure data and includes longer-term breathing zone measurements, ranging from 4 to 6 hours. The very-short-term measurements, taken for 1 to 2 minutes during refueling, reflect the highest MTBE exposure levels (medians of 5.8 ppm in Phoenix and 3.6 ppm in Los Angeles), as would be expected. Most of the median concentrations in the samples of intermediate length (5 to 30 minutes) fall in a range similar to that for the 4- to 6-hour sampling time (about 0.2 to 1 ppm), suggesting that the intermediate samples capture the average concentration in the refueling area fairly well. One exception to this conclusion is in a study of workers in various U.S. locations by the American Petroleum Institute (API) (1995b), in which the median value for sampling times of less than 30 minutes was 2.8 ppm MTBE. Of course, considerable variation has been recorded among stations. This variation seems to be related, to some extent, to the use of vapor recovery systems; stations that use them generally have lower MTBE concentrations than stations that do not. This variation is also affected by the amount of fuel dispensed in a given time period and by the meteorologic conditions.

A comprehensive survey of occupational exposures (personal breathing zone samples) to oxygenate components of gasoline was conducted with service station attendants and motor vehicle mechanics in CO-nonattainment areas in New York, Arizona, Minnesota, and Oregon (American Petroleum Institute 1995c). Short-term samples to measure task-related peak exposures and long-term samples to measure an average 8-hour full-shift exposure were collected in the winter when oxyfuel was in use and in the summer

when oxyfuel was not in use and MTBE was present in fuel at lower levels as an octane enhancer. The data summarized in Table 7 show that, for service station attendant exposures in the Northeast and Southwest, long-term winter exposures (averaged over 8 hours) were no more than 0.5 ppm MTBE. Long-term summer exposures were predictably lower (0.07 ppm MTBE) than winter exposures (not shown in Table 7). In addition to measurements of MTBE, exposures were measured when TAME or ethanol was present in the gasoline dispensed.

At service stations in New York in which TAME was present in the fuel (1% to 4% by volume), none was detected in any of the 44 samples from attendants despite the low MDCs (< 0.02 to < 0.08 ppm) in the long-term exposure samples from the same study (data not shown) (American Petroleum Institute 1995c). This negative finding reflects the low concentration of TAME in the fuel. Ethanol was found in only 4 of the 75 long-term and short-term winter samples for attendants at stations dispensing fuel containing ethanol (5% to 8% by volume). However, the MDCs for ethanol in this series of samples were quite high (< 1 to < 2.4 ppm). In summary, although the analytic methods were not sufficiently sensitive to provide quantitative information on ethanol exposure concentrations, these are usually 1 ppm or lower.

Motor Vehicle Mechanics

Motor vehicle mechanics constitute another group with significant occupational exposure to MTBE or other oxygenates used in gasoline in the workplace. Other individuals working in motor vehicle repair facilities such as parts and service managers may be exposed as well. Though working in the same general area as the mechanics, these individuals perform different jobs and generally do not directly repair vehicles. Available data on exposures of mechanics and other vehicle-related occupations are summarized in Table 7.

As can be seen in Table 7, measurements of MTBE in the breathing zone of mechanics have a broad range. For example, in a study in the Northwest and Southwest (Connecticut, New Jersey, and Arizona), where fuel containing 10% to 17% MTBE was being used (American Petroleum Institute 1995c), only 4 of 13 short-term (15- to 20-minute) winter samples were above the MDC of approximately 0.3 ppm, and the median level was below the MDC, but the highest exposure level was 32 ppm. In the same study, a higher percentage of long-term (6-hour) samples (17/20) had detectable MTBE levels, as would be expected because the MDC was 10-fold lower. The median exposure level was 0.09 ppm in the long-term samples. This value is similar to the median level of 8-hour samples of 0.11 ppm for mechanics in Stamford, CT (White et al. 1995), and 0.10 ppm for mechanics in Fairbanks, AK (Moolenaar et al. 1994). A much greater range of exposures was noted in the Stamford study (< 0.03 to 12.04 ppm) than in the Fairbanks study (0.01 to 0.81 ppm), but the Stamford study used personal breathing zone samples and the Fairbanks study used ambient air samples. Workers at service stations and garages in northern New Jersey all had fairly high exposure levels covering a narrower range (0.3 to 6.1 ppm) (Mohr et al. 1994).

Measurements of ethanol were made in the personal breathing zone of mechanics in the Midwest, Southwest, and Northwest (American Petroleum Institute 1995c) (Table 6). Only one concentration was above the MDC (which was approximately 1 to 1.5 ppm) in 31 short-term (15- to 20-minute) samples, and three were above the MDC in 30 long-term (> 6-hour) samples. The maximum measurable short-term sample had 6.5 ppm ethanol, and the maximum long-term sample had 2.1 ppm ethanol.

Manufacturing and Distribution Workers

A comprehensive survey spanning more than 10 years (1982 through 1993) of occupational exposure to MTBE in the petroleum industry provides information on exposures of manufacturing and distribution workers (American Petroleum Institute 1995b). According to survey questionnaires sent to 17 member companies of the API, measurements were obtained for 1,833 personal breathing zone and ambient air samples taken from areas with the potential for occupational exposures to MTBE. Sufficient information to permit classification according to duration of exposure was available for 1,157 (63%) of the samples. The largest proportion of samples (32%) were taken during July and August, and the remainder were distributed evenly among the other months. These data are presented in Table 8, in which exposures are divided according to industry categories and four durations: less than 30 minutes, between 30 minutes and 6 hours, between 6 and 9 hours, and longer than 9 hours. The published survey data did not distinguish between personal and area exposure measurements or between different methods of collection and analysis. However, of the total number of survey measurements, most (73%) were for personal breathing zone samples, 20% were for area samples, and the remainder were categorized as "mixed" or "unknown."

As expected, the highest median values occurred in samples from short-term exposures and the lowest were associated with 8-hour time-weighted-average (full-shift) exposures. The frequency distribution of exposure concentrations is positively skewed because most measurements showed low concentrations of MTBE. Nearly 90% of MTBE

concentrations were below 10 ppm and 3% exceeded 100 ppm. At least one sample from a short-term exposure (< 30 minutes) had more than 1,000 ppm MTBE.

The highest concentrations of MTBE were measured in samples related to occupations involving transport of MTBE, both in the neat form and blended in fuel mixtures. The lowest exposures were found in samples related to routine operations in MTBE manufacturing and blending into fuel mixtures. Exposures related to refueling at retail outlets (shown in Table 7) were higher than those during distribution (at marketing terminals and in samples for trucking personnel). As would be expected, within each exposure category exposures to neat MTBE were often substantially higher than when it was already diluted by blending into fuel mixtures. For example, the median of short-term exposure measurements for samples related to the transport of neat MTBE was 13.8 ppm compared with 2.4 ppm for those related to the transport of MTBE in fuel mixtures. Likewise, the median short-term exposure of personnel involved in handling neat MTBE during fuel blending operations was 2.9 ppm, but only 0.3 ppm for personnel handling fuel mixtures.

SUMMARY OF NONOCCUPATIONAL AND OCCUPATIONAL EXPOSURES

Microenvironments of importance for nonoccupational exposures are service stations, automobile cabins in traffic, and garages. Activities related to the fueling of vehicles are associated with exposure to the highest air concentrations of oxygenates. Several factors (percentage of oxygenate in the fuel, length of time fueling, existence of vapor recovery systems, and micrometeorologic conditions) have an impact on the actual level of exposure. The length of exposure might be several hours per day for several days per week for service station attendants and only a few minutes per week for most consumers. As shown in Figure 2, 1- to 2-minute breathing zone samples during refueling have a median of 3.6 or 5.8 ppm (in the absence of vapor recovery systems) and individual exposures range over 2 orders of magnitude with occasional peaks reaching 38 ppm. Breathing zone samples for longer periods of time (4 hours) are within a narrower range.

Service station attendants and mechanics had long-term (half- or full-shift) exposure levels of approximately 1 ppm (median range from 0.2 to 1.5 ppm). MTBE levels decrease as distance from the pump increases, with the lowest concentrations generally being present in ambient air. As expected, average commuter exposures to MTBE were lower than the short-term exposures related to vehicle refueling.

Among the occupations that involve handling or contact with MTBE fuels, the workers with the highest levels of exposure are those involved in the transport of neat MTBE (short-term peak exposure > 10 ppm; half-shift median exposure 2 ppm); the next-highest exposure levels are experienced by workers in the blending operations of neat MTBE (short-term peak exposure 2.9 ppm; half-shift median exposure 1 ppm). Manufacturing and distribution workers were exposed to lower levels (median < 1 ppm for both peak and longer-term exposures). These values represent medians of many measurements from different oil refineries. The highest values were often much higher; in some manufacturing and transport operations they exceeded 100 ppm, even averaged over a work shift.

INDICATORS OF DOSE

The studies discussed above indicate that exposure concentrations, especially in the personal breathing zone, are highly variable. Thus, ambient sampling may not represent accurately the magnitude of individual exposures. Accurate measurements of exposures are essential in any epidemiologic study. Besides ambient air and personal breathing zone sample measurements, biological markers (for example, the level of the compound in blood or urine), which may indicate the dose that a subject actually received during the exposure period, also can be used.

Community studies that were conducted to assess the potential health effects of oxyfuel and RFG relied on a variety of methods to determine exposure. These are discussed in detail in the Short-Term Effects section. For this discussion we comment on two studies in which, in addition to measurements of MTBE in the air, biomarkers were measured in blood samples obtained from occupationally exposed workers and commuters (Moolenaar et al. 1994; White et al. 1995). Blood levels of MTBE, TBA (a major metabolite of MTBE), and other pollutants present in gasoline were determined at the end of or shortly after the end of the work shift or a 1-hour commute. Great variability was found in the levels of MTBE in the blood of individuals who shared the same occupation or even the same workspace (White et al. 1995). Despite this variability, blood levels of MTBE in samples taken at the end of the exposure period correlated with levels in personal breathing zone samples (Figure 4A) (White et al. 1995) and workplace air samples (Figure 4B) (Moolenaar et al. 1994).

Controlled human exposure studies involving exposure to MTBE also have provided data on the relation between MTBE exposure and blood levels of MTBE (Prah et al. 1994; Johanson et al. 1995; Cain et al. 1996). MTBE levels inside the exposure chamber from one study in which human subjects were exposed to increasing concentrations of MTBE also correlated with the mean blood levels of the subjects as shown in Figure 4C (Johanson et al. 1995).

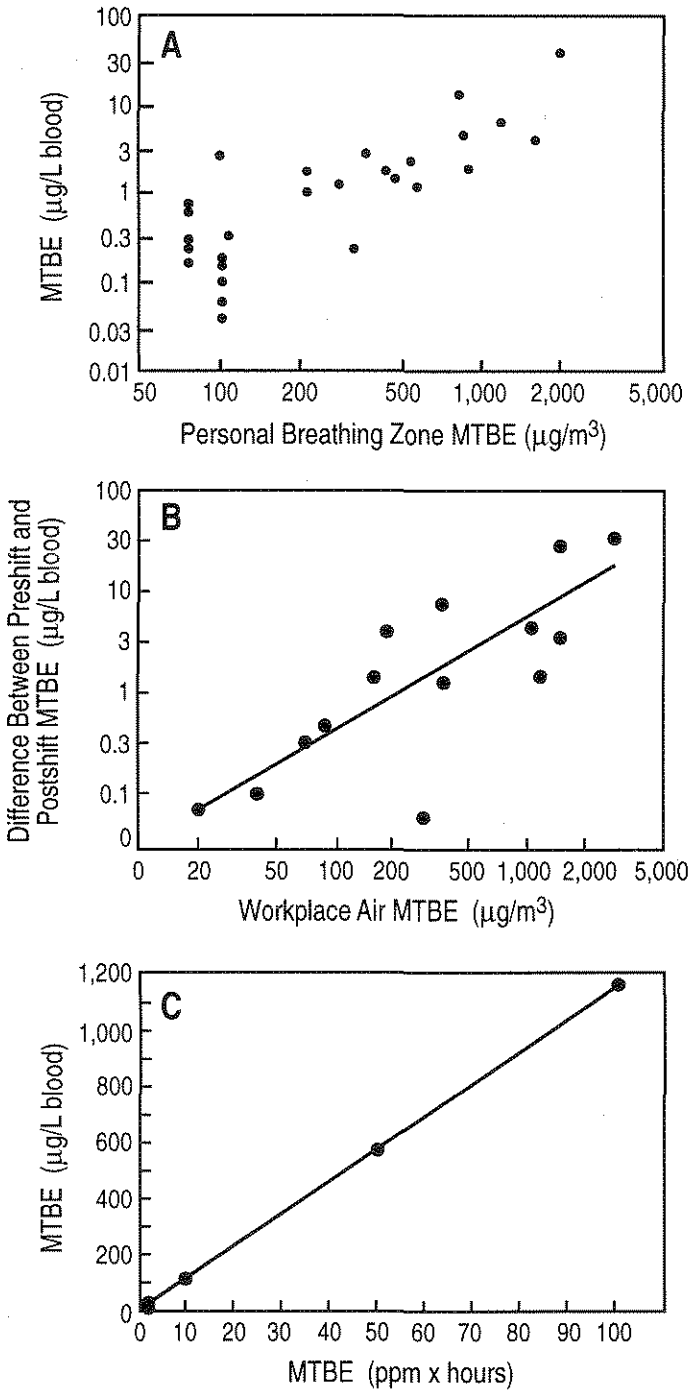


Figure 4. Relation between MTBE concentrations in air samples and individual blood levels at the end of the exposure period. (A) Personal breathing zone samples, Fairbanks, AK. Adapted with permission from Moolenaar et al. (1994). (B) Workplace air samples, Stamford, CT. For the data point at 2,000 µg/m^3, $n = 2$. Three negative values of the difference between preshift and postshift blood MTBE concentrations are not shown. Adapted with permission from White et al. (1995). (C) Data are plotted from the controlled human exposure studies of Prah et al. (1994), Johanson et al. (1995), and Cain et al. (1996).

The results of these studies suggest that MTBE levels in blood can be used as biomarkers of exposure and internal dose, even though the exact relationship between personal breathing zone exposure and blood levels needs to be further defined. TBA has also been suggested as a potential biomarker of MTBE exposure because it has a longer half-life than MTBE (see Metabolism and Disposition section). However, further work is necessary to establish the relationship between exposure and subsequent blood levels.

IMPACT ON EXPOSURE TO OTHER AIR POLLUTANTS

Although the focus of this review is on the potential health effects of the oxygenates themselves, a complete assessment of the health effects from oxygenates must also consider changes in exposure to other pollutants that may result from reformulation of gasoline. These include both potentially beneficial effects such as reduced exposures to CO, benzene, and ozone, which were goals of the oxyfuel and RFG programs, and potentially adverse effects such as increased exposures to partial-combustion products or atmospheric transformation products of the oxygenates. The goal of this summary is to draw attention to new pollutants that may be of concern and to possible changes in exposure to pollutants commonly associated with operating motor vehicles. It does not try to assess changes in the public's exposure to pollutants other than the oxygenates themselves, but rather to indicate the directions in which exposure to important pollutants are likely to change.

ATMOSPHERIC TRANSFORMATION PRODUCTS

Oxygenates are released into the atmosphere during the manufacture and distribution of oxyfuel and RFG, in the vehicle-refueling process, and from evaporative and, to a lesser extent, exhaust emissions from motor vehicles. The main atmospheric fate of ethers is likely to be reaction with OH radicals because other possible reactions are expected to be very slow (Wallington et al. 1993a). Several studies have investigated the kinetics and mechanisms of reactions between OH radicals and ethers or ethanol. A number of degradation products have been identified, which are listed in Table 9. For MTBE, the primary products are *tert*-butyl formate, formaldehyde, methyl acetate, and acetone (Tuazon et al. 1991). For ethanol, formaldehyde appears to be the main atmospheric transformation product (Atkinson 1989). There is little or no information on the levels of some of these compounds in the atmosphere; determining their levels and considering their potential health effects would

be an important component of a comprehensive assessment of oxygenates.

CHANGES IN VEHICLE EMISSIONS FROM ADDING OXYGENATES TO FUEL

Appendix A summarizes several dynamometer studies conducted to develop information on the effects on evaporative and exhaust emissions of motor vehicles caused by adding oxygenates to fuel. Several studies conducted as part of the Auto/Oil Air Quality Improvement Research Program (AQIRP) systematically investigated the effects on emissions of several changes to fuel: adding MTBE and other oxygenates, reducing the aromatic content, changing the concentration of olefins, and lowering the temperature at which 90% of the fuel is distilled (I_{90}). Other studies have compared federal RFG or California RFG (which has less aromatics, olefins, and sulfur than federal RFG) to the industry average gasoline. Taken together, these studies provide comparative information on different oxygenates and some insight into the extent to which variations in engine and emission control technologies can affect some emissions. In addition to the dynamometer studies, a recent tunnel study in San Francisco provides valuable information from an actual in-use fleet, which includes high-emitting vehicles not generally represented in the dynamometer studies.

The overall picture about emissions that emerges from looking at this literature is complex; it is summarized in a qualitative manner below.

Emissions of Carbon Monoxide

In a variety of dynamometer studies, various oxygenates added to gasoline consistently reduced CO emissions by at least 10% and up to more than 20% (Stump et al. 1990; Hochhauser et al. 1991; Reuter et al. 1992; Koehl et al. 1993; Noorman 1993; Auto/Oil AQIRP 1995). Appendix A summarizes data that demonstrate these effects with MTBE, TAME, ETBE, DIPE, and ethanol. There is some indication, however, that the beneficial effects of oxygenates on emission of CO disappear at low temperatures (+20 to −20°F), at which CO emissions from conventional gasoline are much higher than those at more moderate temperatures (U.S. Environmental Protection Agency 1993b; Prakash 1995). A recent study in California provides information on emissions from in-use vehicles. Vehicle emissions were measured at the Caldecott tunnel in August and October 1994, before and after introduction of oxyfuel in the San Francisco Bay area, where on-road vehicles contribute 75% of all CO emissions (Kirchstetter et al. 1996). The average oxygen content of gasoline increased from 0.3% to 2.0% during the interval between the two sets of measurements of tunnel exhaust. Results are from predominantly light-duty vehicles operating in a hot, stabilized mode. Emissions of CO were decreased by 21% during the period when oxyfuel was used compared with the period without oxyfuel.

Emissions of Air Toxics

Total Air Toxics One goal of the RFG program was to decrease emissions of mobile-source air toxics, which are of concern at low levels mainly because of their carcinogenic properties. Even in gasoline that does not meet RFG standards, oxygenates may affect the levels of air toxics emitted. Reducing aromatics as in RFG seems to have an even greater effect on air toxics than adding oxygenates when oxygenates and aromatics are evaluated separately, as in the Auto/Oil AQIRP dynamometer studies using 16 fuel formulations (Gorse et al. 1991). Total air toxics emissions from gasoline-fueled vehicles are dominated by benzene. In the Auto/Oil AQIRP study's current fleet of vehicles (1989 models), benzene emissions represent 65% to 80% of the emissions of air toxics, whereas in the older fleet of vehicles (1983–1985 models), which have higher total emissions of air toxics than the current fleet, formaldehyde emissions are comparable to those of benzene (Gorse et al. 1991). Gorse and coworkers proposed that decreasing fuel benzene levels may be an effective way to meet the toxic pollutant reduction required by the CAAA of 1990.

For a fleet of 1989–1991 vehicles using fuels that meet federal RFG standards and contain DIPE, MTBE, or TAME (2.7% oxygen by weight), total emission levels of air toxics were less than those with the industry average gasoline (Noorman 1993). The effect was greater in the RFG that contained MTBE (36% compared with 26% for DIPE and 28% for TAME), but this difference was not statistically significant (see Table A.3). With California RFG containing MTBE (2.0% oxygen by weight), total air toxics were reduced significantly in all vehicle fleets evaluated (older, current, and Federal Tier 1) compared with the reference industry average gasoline (Auto/Oil AQIRP 1995). In the current and Federal Tier 1 fleets, these reductions were greater than the 25% that the CAAA of 1990 required by the year 2000.

Specific Air Toxics

Formaldehyde Oxygenates that contain methanol, namely MTBE and TAME, tend to increase emission levels of formaldehyde, a partial-combustion product of methanol. However, in studies using a dynamometer, considerable

variation in results was noted with different fuels and fleets (see Tables A.1, A.2, A.3, and A.5) (Gorse et al. 1991; Reuter et al. 1992; Koehl et al. 1993; Noorman 1993; Auto/Oil AQIRP 1995); often no statistically significant effects on formaldehyde emissions were detected. Adding MTBE in the Auto/Oil AQIRP set of fuel formulations increased formaldehyde emissions by 27% in the current fleet, but did not affect formaldehyde emission levels in the older fleet (Gorse et al. 1991). In the California Caldecott tunnel study, in which emissions from on-road vehicles operating in a hot, stabilized mode were analyzed, formaldehyde emissions were increased by 13% ± 6% during the period when oxygenates were added to fuels (2.0% oxygen by weight) compared with the period when oxygenates were not added to fuels (0.3% oxygen by weight) (Kirchstetter et al. 1996). Gasoline sampling during the period when oxyfuel was being used indicated that 34 of 54 samples contained MTBE as the sole oxygenate and 5 samples contained only ethanol.

Acetaldehyde Ethanol, ETBE, and DIPE all tend to increase exhaust emissions of acetaldehyde, a partial-combustion product of ethanol (Tables A.2 and A.3) (Reuter et al. 1992; Noorman 1993). As expected, adding MTBE or TAME does not increase emissions of acetaldehyde. In the California tunnel study, acetaldehyde emissions in the period when oxygenates were added to fuels (2.0% oxygen by weight) were not significantly different from those in the period when oxygenates were not added (0.3% oxygen by weight) (Kirchstetter et al. 1996). However, this is not surprising because gasoline sampling indicated use of much more MTBE than ethanol.

Benzene Exhaust emissions of benzene are generally, but not under all conditions, reduced by adding oxygenates to fuel. In the Auto/Oil AQIRP dynamometer studies, adding MTBE did not affect emission of benzene significantly in the current fleet, but did lower it by 11% in the older fleet (Gorse et al. 1991). Ethanol and ETBE lowered benzene emissions in the current fleet by about 10% (Reuter et al. 1992). In the California tunnel study, benzene emissions were 25% ± 17% lower when fuel contained 2.0% oxygen by weight compared with the period when the oxygen content was only 0.3% (Kirchstetter et al. 1996).

1,3-Butadiene In the dynamometer studies discussed in Appendix A, adding oxygenates to fuel sometimes decreased emissions of 1,3-butadiene by a small amount and sometimes had no significant effect on them (Gorse et al. 1991; Reuter et al. 1992). In federal RFG containing MTBE, TAME, or DIPE (at 2.7% oxygen by weight), reductions of 1,3-butadiene bordered on being statistically significant; results were the same for each oxygenate (Table A.3) (Noorman 1993).

Emissions That Promote Ozone Formation

Reformulated gasoline is used in order to reduce emission of ozone-forming hydrocarbons without increasing emission of NO_x, which also contribute to ozone formation. Oxygenates (at a level of at least 2% oxygen by weight) are required in RFG, but are not the main factors in decreasing ozone-forming capacity. Because ozone is a product of atmospheric transformation, projecting ozone exposure levels from emissions data is even more complicated than projecting exposure to directly emitted compounds such as CO.

In the emissions data summarized in Appendix A, oxygenates in different fuels had different effects on the specific reactivity and ozone-forming potential of exhaust and evaporative emissions. Thus, the oxygenates by themselves seem to show no consistent overall effect in decreasing ozone-forming potential. In RFG blends, no differences were observed among DIPE, TAME, and MTBE in the ozone-forming potential of exhaust emissions (Noorman 1993). These emissions were all significantly lower in ozone-forming potential than emissions from the reference conventional gasoline that contained no oxygenates. None of these fuels resulted in a significant decrease in the ozone-forming potential of evaporative emissions compared with the reference gasoline.

Relation Between Emissions Levels and Air Levels

Although changes in ambient levels of pollutants might be expected to reflect the changes in related emissions, the extent that this would be true in any region depends on many factors, including the actual fuels used, the composition of the fleet of vehicles, the number of miles driven, the contributions of pollutants from other sources, and local atmospheric conditions such as the ratio of hydrocarbons to NO_x in the air (which affects ozone formation). Determining the actual changes in exposure to critical pollutants that can be attributed to oxyfuel or RFG involves complex modeling procedures that take all of these factors into account. The White House OSTP review of oxyfuel and RFG will address the topic of air quality in more depth.

There is evidence that CO levels have declined substantially in many areas implementing the oxyfuel program (U.S. Environmental Protection Agency 1992b), but because of the variation in weather patterns from year to year and changes in fleets that tend to reduce CO emissions, the extent of reduction in CO due to oxyfuel use will take some time to evaluate. Nevertheless, because decreasing exposure to CO at levels above the 8-hour standard was the main goal of the oxyfuel program, it is interesting to examine some data on the number of exceedances of the CO standard in several areas. In Colorado, the number of violations of

the CO standard decreased from 22 in 1987 to 4 in 1992 (Colorado State Department of Health 1993). State officials acknowledge that many factors contributed to this improvement, including fleet turnover, inspection and maintenance programs, the absence of wood burning on high-pollution days, and meteorologic changes, but feel that the oxyfuel program has contributed to the decrease. In the first year of California's oxyfuel program, there were 14 exceedances of the 8-hour CO standard, all in Los Angeles County. In the previous 7 years, the 8-hour standard was exceeded on the average 39 days per year in the South Coast Air Basin (Dolislager 1993).

In order to average out the effects of weather patterns, it may be most useful to look at composite information. Comparisons were made of peak CO concentrations recorded during October through December 1991 and October through December 1992 in sites with new oxyfuel programs, existing oxyfuel programs, and no oxyfuel programs (U.S. Environmental Protection Agency 1993d). There was a larger decrease in peak CO concentrations in areas with new oxyfuel programs than in areas with existing oxyfuel programs or no oxyfuel programs. There was a 13% decrease in CO at 96 monitoring sites in areas with new oxyfuel programs, a 5% decrease at 39 sites with existing oxyfuel programs, and a 3% decrease at 220 sites in areas not having oxyfuel programs. This analysis seems to be more informative than an analysis limited to one area, where a change in weather patterns is more likely to have affected the results in one direction. The authors suggest that the smaller changes in the areas with existing fuel programs or no fuel programs reflect variation due to changes in meteorologic conditions. Changes in the fleet, with more new vehicles on the road in all areas, may also have contributed to the decrease in CO in areas where the oxygenate content of fuel was not altered during 1991 or 1992.

CONCLUSIONS

Fuels containing oxygenates have been introduced in many areas of the country to reduce exposure of the public to air pollutants that adversely affect public health. Using these fuels results in the potential exposure of more than 70 million people to oxygenated compounds, mainly from evaporative emissions of fuel. Adding oxygenates to gasoline decreases the levels of CO and benzene in motor vehicle exhaust, but increases the levels of aldehydes that are partial-combustion products of the oxygenates.

MTBE and ethanol are the major compounds added to gasoline as fuel oxygenates. Inhalation is the major route of exposure. Air measurements are primarily for MTBE and include few data for ethanol and ETBE.

The highest concentrations of oxygenates are experienced by workers who handle neat oxygenates and fuels containing oxygenates and by mechanics or others associated with the repair or maintenance of motor vehicles. Workers involved in the transport of neat MTBE had median short-term exposure levels of MTBE of 13.8 ppm with a wide range up to 1,050 ppm; workers involved in the transport of MTBE mixed with fuel had median short-term exposure levels of 2.4 ppm with peaks in excess of 100 ppm. Air measurements integrated over an 8-hour work shift were lower.

Data gathered for service station attendants and consumers showed that median 1- to 2-minute exposure levels were highly variable, ranging from 0.3 to 6 ppm and occasionally exceeding 10 ppm. Median 6- to 8-hour exposures of service station attendants ranged from 0.2 to 1.5 ppm. Limited measurements of ethanol at service stations during refueling suggest that ethanol levels are usually less than 1 ppm (the MDC).

For mechanics, short-term exposures to MTBE ranged from 0.3 ppm to as high as 32 ppm. Long-term samples were generally less than 1 ppm. The highest short-term ethanol exposure for mechanics was 6.5 ppm, and the highest long-term exposure was 2.1 ppm; the majority of the measurements, however, were below 1.5 ppm (the MDC).

Overall, the general public is exposed to low concentrations of oxygenates. The highest concentrations are most likely encountered during vehicle refueling. These concentrations are similar to those encountered by service station attendants during refueling, but exposures will last for only a few minutes and occur once every several days. Commuters in automobiles are exposed to considerably lower MTBE concentrations, which may range up to several ppb; however, the range of exposure can vary widely depending on the individual vehicle.

One study provided some limited information on community levels of MTBE and ETBE in a region where both were used as oxygenates in gasoline. This study recorded median MTBE levels of 0.13 ppb and median ETBE levels of 0.04 ppb in 24-hour samples of ambient air. Service station perimeters, which represent a higher, more localized ambient level, generally had MTBE levels of less than 100 ppb (0.1 ppm).

Exposure levels can be highly variable even withing a given microenvironment; thus, microenvironmental sampling may not reflect the magnitude of individual exposures. Data from environmental and experimental exposures indicate that biomarkers of MTBE are measurable in blood and are related to exposure concentrations. These biomarkers

provide an indication of internal dose. The exposure-biomarker relationship, however, is not yet well defined.

Ingestion of oxygenates through consumption of contaminated water is another potential route of exposure, but the information available is not sufficient to characterize quantitatively these exposures. Data from shallow wells in various areas of the United States suggest that such contamination may be more widespread than once thought. However, levels of MTBE in drinking water have been measured only in situations of severe contamination of private wells related to documented leakages from underground storage tanks.

Another route of exposure to oxygenates is through the skin. No data exist to assess its importance during refueling or from contaminated water. The exposure measurements collected for MTBE can be used to make a rough assessment of exposure for the general population; however, the substantial uncertainties about the distribution and frequency of activities that may lead to exposure, and the extent of exposure by other routes, preclude calculation of a meaningful cumulative measure of exposure for use in risk assessment.

Adding oxygenates to gasoline decreases emission of CO and total air toxics. Effects on individual air toxics vary: benzene is decreased, 1,3-butadiene is decreased slightly or not at all, and formaldehyde or acetaldehyde tends to be increased, depending on which oxygenate is used. However, the effects that these emission changes have on ambient levels of CO and air toxics have not been evaluated.

Table 3. Areas Participating in the Wintertime Oxyfuel Program in 1994/1995[a]

Participating Area	Population (in 1,000s)	Percentage of Fuel Containing the Oxygenate	
		MTBE (%)	Ethanol (%)
New York City, Northern NJ, CT	19,384	95	5
Los Angeles, Anaheim, Riverside CA	14,818	90	5
San Francisco, Oakland, San Jose CA	6,322	90	5
Philadelphia PA, Wilmington DE, Trenton NJ, MD	5,925	95	5
Washington DC, MD, VA	4,293	85	15
Seattle, Tacoma WA	3,054	1	99
Minneapolis, St. Paul MN, WI	2,583	5	95
San Diego CA	2,549	90	5
Baltimore MD	2,414	85	15
Phoenix AZ	2,287	40	60
Denver, Boulder CO	2,034	20[b]	80[b]
Portland OR, WA	1,570	1	99
Sacramento CA	1,388	90	5
Las Vegas NV	925	30	70
Raleigh-Durham NC	883	90	5
Fresno CA	781	90	5
El Paso TX	612	15	85
Albuquerque NM	602	15	85
Stockton CA	493	90	5
Colorado Springs CO	404	50	50
Modesto CA	387	90	5
Spokane WA	374	1	99
Provo, Orem UT (program suspended)	270	20	80
Reno NV	263	95	5
Anchorage AK	226	0	100
Fort Collins, Loveland CO	106	50	50
Chico CA	72	90	5
Medford OR	62	1	99
Missoula MT	43	0	100
Klamath County OR	18	1	99
Grant's Pass OR	17	1	99

[a] Adapted from SRA Technologies 1995.

[b] Values modified according to K. Livo (personal communication, 1996).

Table 4. Areas Participating in the Reformulated Gasoline Program Beginning January 1, 1995[a]

Participating Area	Population (in 1,000s)
Areas Required by EPA to Participate	
Los Angeles, Anaheim, Riverside CA	13,000
San Diego County CA	2,498
Hartford, New Britain, Middletown, New Haven, Meriden, Waterbury CT	2,470
New York City, Northern NJ, Long Island NY, CT	17,947
Philadelphia PA, Wilmington DE, Trenton NJ, Cecil County MD	6,010
Chicago IL, Gary IN, Lake County IL, IN, WI	7,886
Baltimore MD	2,348
Houston, Galveston, Brazoria TX	3,731
Milwaukee, Racine WI	1,735
States Participating Voluntarily	
Connecticut	
Massachusetts	
Rhode Island	
States with Areas Participating Voluntarily	
Delaware	
Kentucky	
Maine	
Maryland	
New Hampshire	
New Jersey	
New York	
Texas	
Virginia	
Washington DC	
Wisconsin	

[a] Adapted from SRA Technologies 1995.

Table 5. Measurements of Nonoccupational and Consumers' Exposures to MTBE[a]

Sampling Site[b]	Oxygenate Content (vol%)	Vapor Recovery System[c]	Samples > MDC/ Total Samples	MDC (ppm)	MTBE (ppm)		Sampling Conditions	Collection and Analytic Methods[d]	Miscellaneous Information	Reference
					Range	Median or (Mean)				
COMMUNITY AIR										
Milwaukee WI (18)	RFG in use*	Some yes	6/11	0.000025	< MDC–0.00413	0.00013	Jan.–March 1995; 24-hr samples	Collected in evacuated canisters; GC (FID)	*Approx. 50% contained MTBE, remainder ETBE or ethanol	Allen and Grande 1995
			3/5	0.000025	< MDC–0.00106	0.00052	Feb.–March 1995; 2-hr samples			
PARKING GARAGE RAMP										
Milwaukee WI (15)	RFG in use*	NA	8/8		0.0023–0.0037	(0.002)	Feb.–March 1995; 2- to 3-hr samples	Collected in evacuated canisters; GC (FID)	*Approx. 50% contained MTBE, remainder ETBE or ethanol	Allen and Grande 1995
AUTOMOBILE CABIN FOR COMMUTERS										
NJ (16)	15 MTBE	NA	20/20		0.002–0.017*	0.004	April 1993; approx. 1-hr samples	Adsorbed onto carboxen 569; collected in evacuated canisters; GC/MS	*Estimated from graphed data in the original publication	Lioy et al. 1994
CT (17)			20/20		0.003–0.009*	0.006				
SERVICE STATION REFUELING										
Phoenix AZ (1)	12*	No	40/40		0.09–38	5.8	Oct.–Nov. 1990; each sample was collected during the refueling of 8 to 10 vehicles; each refueling was sampled for 1 to 2 min	Adsorbed onto charcoal; GC (FID)	*Samples taken from one station in which only premium gasoline was oxygenated	American Petroleum Institute 1993a
Los Angeles CA (2)	13	Yes	6/6		1.1–6.5	3.6				
NJ, NY, CT	10–15	Yes	4/4		Not stated	0.370*	April 1993; 5-min breathing-zone samples before, during, and after refueling	Adsorbed onto carboxen 569; GC/MS	*Estimated from graphed data in the original publication	Lioy et al. 1994
		No	4/4		Not stated–4.1	0.572*				

(Table continues next page.)

Table 5. Measurements of Nonoccupational and Consumers' Exposures to MTBE[a] *(Continued)*

Sampling Site[b]	Oxygenate Content (vol%)	Vapor Recovery System[c]	Samples > MDC/ Total Samples	MDC (ppm)	MTBE (ppm) Range	MTBE (ppm) Median or (Mean)	Sampling Conditions	Collection and Analytic Methods[d]	Miscellaneous Information	Reference
SERVICE STATION REFUELING *(Continued)*										
Milwaukee WI										
Station A	9–10*	Yes	?/6		Not stated	(0.39)	Jan.–March 1995; 15-min breathing-zone samples	Adsorbed onto charcoal; GC (FID)	RFG with MTBE used only in higher grades; *ethanol used in regular gasoline; **2% MTBE used in regular gasoline	Allen and Grande 1995
Station D	9**	No	?/2		Not stated	(2.93)				
Northeast and Southwest Areas Short-term sample (3)	10–17	Yes	8/17	< 0.32	< MDC–2.1	0.57	Feb.–April 1994; 15- to 20-min personal breathing zone samples	Adsorbed onto charcoal; GC (FID)	*Northeast = CT and NJ locations; Southwest = AZ locations	American Petroleum Institute 1995c
IN AUTOMOBILE WHILE REFUELING CT, NJ, NY Service Stations										
Self-serve (4)	10–15	Yes	4/4		0.006–0.072*	0.03*	April 1993; 5-min breathing-zone samples before, during, and after refueling	Adsorbed onto carboxen 569; GC/MS	*Estimated from graphed data in the original publication	Lioy et al. 1994
Full-serve (5)		Yes	8/8		0.008–0.172	0.034				
Self-serve (6)		No	4/4		Not stated	0.015				
Full-serve		No	4/4		0.006–0.103	0.041				
SERVICE STATION PUMP ISLAND										
NJ (7) Full-serve		Yes	4/4		0.120–1.600	0.440	April 1995; 4-hr breathing-zone samples during both refueling and not refueling	Collected in 6-L evacuated canisters; GC/MS		American Petroleum Institute 1995a
NY (8) Self-serve		Yes	6/6		0.014–0.080	0.048				
CT (9) Self-serve		No	9/10	0.09	< MDC–1.500	0.170				
NJ (10)	15	Yes	3/3		0.08–0.24	0.24	Nov.–Dec. 1994; 7- to 8-hr samples	Adsorbed onto charcoal; GC (FID)		Cook and Kovein 1994

(Table continues next page.)

Table 5. Measurements of Nonoccupational and Consumers' Exposures to MTBE[a] *(Continued)*

Sampling Site[b]	Oxygenate Content (vol%)	Vapor Recovery System[c]	Samples > MDC/ Total Samples	MDC (ppm)	MTBE (ppm)		Sampling Conditions	Collection and Analytic Methods[d]	Miscellaneous Information	Reference
					Range	Median or (Mean)				
SERVICE STATION PERIMETER										
Phoenix AZ (11)	12	No	24/24		0.009–0.09	0.02	Oct.–Nov. 1990; 12-hr samples; 4 perimeter samples plus samples upwind and downwind from the pump island for each sample set	Adsorbed onto charcoal; GC (FID)		American Petroleum Institute 1993a
NJ (12) Full-serve		Yes	15/16	0.001	< MDC–0.036	0.003	April 1995; 4-hr samples	Collected in 6-L evacuated canisters; GC/MS		American Petroleum Institute 1995a
NY (13) Full-serve		Yes	24/24		0.002–0.083	0.007				
CT (14) Self-serve		No	38/40	0.001	< MDC–0.140	0.014				
Milwaukee WI Station A	9–10*	Yes	?/2		Not stated	(0.0024)	Jan.–March 1995; 2-hr area samples	Collected in evacuated canisters; GC (FID)	*RFG, MTBE used only in higher grades; ethanol in lower grades **2% MTBE in regular gasoline	Allen and Grande 1995
Station D	2–9**	No	1/1		Not stated	(0.0046)				

[a] Asterisks (*) in any column indicate that further explanation is provided in the Miscellaneous Information column.

[b] The numbers in parentheses in this column refer to bars with the same numbers in Figure 2, where the data for that sampling site are also shown. Data for sampling sites without numbers in parentheses are not shown in Figure 2.

[c] NA = not applicable.

[d] GC/MS = gas chromatography with verification by mass spectrometry; GC (FID) = gas chromatography with flame ionization detection.

Table 6. Measurements of Nonoccupational Exposure to ETBE and Ethanol

Sampling Site	Oxygenate Content (vol%)	Vapor Recovery System	Samples > MDC/ Total Samples	MDC (ppm)	Oxygenate (ppm)		Sampling Conditions	Collection and Analytic Methods	Miscellaneous Information	Reference
					Range	Median or (Mean)				
ETBE Milwaukee WI										
Community Air	RFG in use	NA	6/8	0.000025	< MDC to 0.0017	0.00004	Jan.–March 1995; 24-hr samples	Collected in evacuated canisters; GC (FID)	Approximately 50% containing ETBE or ethanol; remainder containing MTBE	Allen and Grande 1995
			?/9		< MDC to 0.0006	0.0004	Feb.–March 1995; 2-hr samples			
Service Station Refueling Station B	11–12	Yes	5/7	?	Not stated	0.1	Jan.–March 1995; 15-min breathing-zone samples	Adsorbed onto charcoal; GC (FID)	RFG, ETBE used in all gasoline grades	
Service Station Perimeter Station B		Yes	?/2			0.0036	Jan.–March 1995; 2-hr samples			
Parking Garage Ramp	RFG in use		8/8			0.0011	Feb.–March 1995; 2- to 3-hr samples	Collected in evacuated canisters; GC (FID)	Approximately 50% containing ETBE or ethanol; remainder containing MTBE	
ETHANOL Midwest, Southwest, Northwest in Winter										
Service Station Refueling							Air samples collected in Feb.–April 1994; personal breathing-zone samples: short-term, between 15 and 20 min; long-term, > 6 hr	Adsorbed onto charcoal; GC (FID)	Midwest = MN, Southwest = AZ, Northwest = OR	American Petroleum Institute 1995c
Short-term			3/44	< 1.1	< MDC–46	(< MDC)				
Long-term			1/31	< 1	< MDC–9	(< MDC)				
Motor Vehicle Mechanics										
Short-term			1/31	< 1.5	< MDC–6.5	(< MDC)				
Long-term			3/30	< 1	< MDC–2.15	(< MDC)				

Table 7. Measurements of Nonindustrial Occupational Exposure to MTBE[a]

Occupation, Activity, Sampling Site, Sampling Period[b]	Oxygenate Content (vol%)	Vapor Recovery System[c]	Samples > MDC/Total Samples	MDC (ppm)	MTBE (ppm)		Sampling Conditions	Collection and Analytic Methods	Miscellaneous Information	Reference
					Range	Median or (Mean)				
SERVICE STATION ATTENDANTS DURING REFUELING										
Phoenix AZ 1–2 min	12	No	40/40		0.09–38	5.8	Oct.–Nov. 1990; each breathing-zone sample was collected during the refueling of 8–10 vehicles; each refueling was sampled for 1–2 min	Adsorbed onto charcoal; GC (FID)		American Petroleum Institute 1993a
Los Angeles CA 1–2 min	13	Yes	6/6		1.1–6.5	3.6				
NJ, NY, CT 5 min	10–15	Yes	4/4		Not stated	0.37*	April 1993; 5-min breathing-zone samples before, during, and after refueling	Adsorbed onto carboxen 569; GC/MS	*Estimated from graphed data in original publication	Lioy et al. 1994
		No	4/4		Not stated–4.1	0.572				
Milwaukee WI Station A 15 min	RFG 9–10	Yes	?/6		Not stated	0.31	Jan.–March 1995; 15-min breathing-zone samples during refueling	Collected in evacuated canisters	This station used MTBE in middle and premium grade gasoline, ethanol in regular gasoline	Allen and Grande 1995
Northeast and Southwest in Winter 15–20 min	10–17	Yes	8/17	0.32	< MDC–2.1	0.57	Feb.–April 1994; personal breathing-zone samples; mostly 15- to 20-min personal breathing-zone samples during refueling	Adsorbed onto charcoal; GC (FID)	Northeast = CT and NJ locations; Southwest = AZ locations	American Petroleum Institute 1995c

Table 7. Measurements of Nonindustrial Occupational Exposure to MTBE[a] (*Continued*)

Occupation, Activity, Sampling Site, Sampling Period[b]	Oxygenate Content (vol%)	Vapor Recovery System[c]	Samples > MDC/Total Samples	MDC (ppm)	MTBE (ppm)		Sampling Conditions	Collection and Analytic Methods	Miscellaneous Information	Reference
					Range	Median or (Mean)				
SERVICE STATION ATTENDANTS DURING REFUELING (*Continued*)										
Various U.S. locations										
< 30 min	Not stated	Not stated	9/11	0.16	MDC–136.1	2.8	1982–1993; data reported by American Petroleum Institute member companies; higher frequency of measurements in 1989–1993	GC (FID) and other procedures	Service station and retail outlet personnel	American Petroleum Institute 1995b
30 min–6 hr			5/5		0.01–2.7	0.34				
6–9 hr TWA			13/13		0.09–34.0	0.59				
> 9 hr			11/11		0.01–17.20	1.1				
NJ 4 hr (Full-serve)	13–16	Yes	4/4		0.084–0.52	0.245	April 1995; 4-hr breathing-zone samples, during refueling and not refueling	Collected in 6-L evacuated canisters; GC/MS		American Petroleum Institute 1995a
NY 4 hr (Self-serve)		Yes	6/6		0.077–0.78	0.205				
CT 4 hr (Self-serve)		No	10/10		0.170–2.60	1.5				
Phoenix AZ 4 hr	14	No	42/42		0.04–3.88	0.55	Oct.–Nov. 1990; 4-hr (half-shift) breathing-zone samples (avg. time 224 min)	Adsorbed onto charcoal; GC (FID)		Hartle 1993
Northeast and Southwest in Winter > 6 hr	10–17	Yes	18/21 (< 0.03)	< 0.03–< 0.11	< MDC–0.5	0.27	Feb.–April 1994; personal breathing-zone samples, most sampling times were > 6 hr	Adsorbed onto charcoal; GC (FID)	Northeast = CT and NJ locations; Southwest = AZ locations	American Petroleum Institute 1995c
NJ Full shift	15	Yes	21/21	Not reported	0.12–1.42	0.48	Nov.–Dec. 1994; breathing-zone samples for 3–8 hr	Adsorbed onto charcoal; GC (FID)		Cook and Kovein 1994

(Table continues next page.)

Table 7. Measurements of Nonindustrial Occupational Exposure to MTBE[a] *(Continued)*

Occupation, Activity, Sampling Site, Sampling Period[b]	Oxygenate Content (vol%)	Vapor Recovery System[c]	Samples > MDC/Total Samples	MDC (ppm)	MTBE (ppm)		Sampling Conditions	Collection and Analytic Methods	Miscellaneous Information	Reference
					Range	Median or (Mean)				
SERVICE STATION ATTENDANTS AT PUMP ISLAND										
NJ 4 hr (Full-serve)	13–16	Yes	4/4		0.12–1.60	0.44	April 1995; 4-hr breathing-zone samples during refueling and not refueling	Collected in 6-L evacuated canisters; GC/MS		American Petroleum Institute 1995a
NY 4 hr (Self-serve)		Yes	6/6		0.014–0.08	0.048				
CT 4 hr (Self-serve)		No	9/10	0.0005	< MDC–1.5	0.17				
NJ 8 hr	15	Yes	3/3	Not reported	0.08–0.24	0.24	Nov.–Dec. 1994; 7- to 8-hr samples	Adsorbed onto charcoal; GC (FID)		Cook and Kovein 1994
PARKING GARAGE RAMP										
Milwaukee WI 2–3 hr	RFG in use	NA	8/8		0.0023–0.0037	(0.002)	Feb.–March 1995; 2- to 3-hr samples	Collected in evacuated canisters; GC (FID)	Approx 50% contained MTBE, remainder contained ETBE or ethanol	Allen and Grande 1995
MECHANICS										
Northeast and Southwest in Winter 15 min	10–17	Yes	4/13 (< 0.26)	< 0.26–< 0.35	< MDC–32	< MDC	Feb.–April 1994; personal breathing-zone samples; most short-term sampling times were 15–20 min; most long-term sampling times were > 6 hr	Adsorbed onto charcoal; GC (FID)	Northeast = CT and NJ locations; Southwest = AZ locations	American Petroleum Institute 1995c
> 6 hr			17/20 (< 1.5)	< 0.02–< 0.05	< MDC–2.6	0.09				
Northern NJ 1 hr	15	?	?/13		0.3–6.1		April 1993; 1-hr breathing-zone samples (active)	Adsorbed onto carboxen; GC/MS	Workers at service stations and garages for state vehicles	Mohr et al. 1994

(Table continues next page.)

Table 7. Measurements of Nonindustrial Occupational Exposure to MTBE[a] (*Continued*)

Occupation, Activity, Sampling Site, Sampling Period[b]	Oxygenate Content (vol%)	Vapor Recovery System[c]	Samples > MDC/Total Samples	MDC (ppm)	MTBE (ppm)		Sampling Conditions	Collection and Analytic Methods	Miscellaneous Information	Reference
					Range	Median or (Mean)				
MECHANICS (*Continued*)										
Stamford CT 8 hr TWA	13–17	NA	20/28	0.03	< MDC–12.04	0.11	April 1993; full-shift samples (approx. 8 hr)	Adsorbed onto charcoal; GC (FID)	Mechanics with the Department of Public Works and in auto dealers' garages	Buchta 1993
Fairbanks AK 8 hr TWA	15	Not stated	?/10		0.01– 0.81	0.10	Dec. 1992; full-shift samples (approx. 8 hr)	Collected in evacuated canisters in environment where workers spent most of their day; GC		Moolenaar et al. 1994
OTHER VEHICLE-RELATED WORKERS										
Stamford CT 8 hr TWA	13–17	Not stated	0/7	0.03	< MDC	< MDC	April 1993; 8-hr personal breathing-zone samples	Adsorbed onto charcoal; GC (FID)	Workers who spent time in traffic	Buchta 1993
			1/4		< MDC–0.15	< MDC			Workers in various jobs (mostly workers in garages who performed tasks different from the mechanics)	

[a] Asterisks (*) in any column indicate that further explanation is provided in the Miscellaneous Information column.

[b] TWA = time-weighted average.

[c] NA = not applicable.

Table 8. Occupational Exposure to MTBE in the Petroleum Industry[a]

Industry Category	Occupation	Sampling Time	Samples > MDC/Total Samples	MTBE (ppm)	
				Range	Median
MTBE Manufacturing					
Routine operations	Oil refinery and chemical plant manufacturing personnel handling neat MTBE	< 30 min	14/27	0.2–7.8	1.0
		6–9 hr TWA	38/76	0.01–248.7	0.03
		> 9 hr TWA	2/2	0.16–0.17	0.2
Routine maintenance		< 30 min	7/8	0.5–7.2	0.9
		30 min–6 hr	1/1	0.2	0.2
		6–9 hr TWA	4/4	0.0–0.7	0.1
		> 9 hr TWA	2/2	0.16–0.2	0.2
MTBE Blending					
Neat MTBE	Personnel involved in fuel-blending activities	< 30 min	34/35	0.0–97.0	2.9
		30 min–6 hr	12/13	0.2–72.0	1.03
		6–9 hr TWA	7/12	0.0–88.0	2.2
		> 9 hr TWA	0/9	0.2–0.3	0.3
Fuel mixtures		< 30 min	51/98	0.02–100.0	0.3
		30 min–6 hr	5/19	0.03–2.0	0.1
		6–9 hr TWA	34/112	0.02–14.0	0.04
		> 9 hr TWA	9/22	0.0–0.3	0.02
MTBE Transport					
Neat MTBE	Marine barge, pipeline, and rail car personnel; trucking personnel included only for transport of neat MTBE	< 30 min	62/66	0.3–1,050.0	13.8
		30 min–6 hr	23/27	0.04–700.0	2.2
		6–9 hr TWA	9/10	0.03–711.9	0.2
		> 9 hr TWA	1/1	0.3	0.3
Fuel mixtures		< 30 min	60/64	0.001–507.9	2.4
		30 min–6 hr	64/92	0.02–59.4	0.4
		6–9 hr TWA	28/42	0.01–26.2	0.1
		> 9 hr TWA	8/8	0.2–4.5	1.5
MTBE Distribution in Fuel Mixtures					
	Marketing terminal and trucking personnel	< 30 min	93/129	0.0–14.0	0.8
		30 min–6 hr	9/10	0.3–4.1	1.0
		6–9 hr TWA	62/87	0.01–2.2	0.1
		> 9 hr TWA	46/47	0.1–6.2	0.7

[a] Adapted from American Petroleum Institute 1995b. Measurements used different sampling and analytic techniques on both personal breathing zone and area air samples. TWA = time-weighted average.

Table 9. Possible Atmospheric Transformation Products of Oxygenates

Oxygenate	Atmospheric Lifetime (days)[a]	Primary Degradation Products	References
Ethanol	3.5	Formaldehyde	Atkinson 1994
MTBE	4.1	*tert*-Butyl formate, formaldehyde, methyl acetate, acetone	Atkinson 1994; Tuazon et al. 1991
ETBE	1.4	*tert*-Butyl formate, *tert*-butyl acetate, ethyl acetate	Wallington et al. 1988; Smith et al. 1992
DIPE	1.2	Isopropyl acetate	Wallington et al. 1993a
TAME	2.1	*tert*-Amyl formate, methyl acetate, acetaldehyde, formaldehyde	Wallington et al. 1993b; Smith et al. 1995

[a] Assuming a 24-hour average [•OH] to be $1 \times 10^6/cm^3$.

Potential Health Effects of Oxygenates

Metabolism and Disposition

ETHERS

Although the metabolism and disposition of MTBE have not been studied extensively, animal and human studies have provided information about the routes and kinetics of MTBE elimination as well as the pathways involved in its metabolism. Little or no information is available for ETBE, TAME, and DIPE.

Rodent and human studies have shown that MTBE is rapidly absorbed into the circulation following inhalation exposure. Rodent studies also have shown rapid distribution after oral and intraperitoneal exposure; dermal absorption occurs more slowly. Evidence supports metabolic transformation of MTBE to the parent alcohol, TBA, and formaldehyde in rodents and humans. Further oxidative metabolism of TBA seems to be slow, and glucuronidation is a major competing pathway. Formaldehyde metabolism to formate is very rapid; and yet formaldehyde reactivity leads to DNA-protein cross-links (see the Long-Term Effects section). The toxicokinetic parameters of MTBE and TBA depend on the dose and route of administration.

ANIMAL STUDIES WITH MTBE

Routes of Elimination

MTBE labeled with ^{14}C at the central butyl carbon has been used to investigate its disposition following dermal, intravenous, oral, and inhalation exposures in rats (Ferdinandi et al. 1990a,b,c,d). Dermal exposure to MTBE resulted in limited percutaneous absorption of MTBE and low bioavailability (Ferdinandi et al. 1990a). Following both intravenous and oral administration, approximately half of the administered dose was eliminated through the lungs (46% to 54%), and another 25% to 36% was excreted in the urine (Ferdinandi et al. 1990d). These data are supported by the results of another study in which 50% to 55% of a 40 mg/kg body weight dose of MTBE was eliminated in expired air within 3 hours of either intravenous or oral administration (Peterson et al. 1988). Mass balance studies have shown that the radioactivity in expired air following intravenous and oral administration of ^{14}C-MTBE (labeled in the central butyl carbon) was mainly unmetabolized MTBE (Ferdinandi et al. 1990d). The contribution of exhalation relative to that of urinary elimination was somewhat different after inhalation exposure. Following inhalation exposure to 400 ppm MTBE, 65% of the total radioactivity recovered was eliminated in the urine, and only 17% to 22% was detected in expired air (Ferdinandi et al. 1990c). A proportion of the total exhaled radioactivity equal to that associated with MTBE was found to be associated with TBA, indicating some MTBE was metabolized before it was exhaled. A summary of the disposition of MTBE in exhaled air and urine is presented in Table 10.

When the oral and inhalation doses were increased (to 400 mg/kg and 8,000 ppm, respectively), the percentage of the total radioactivity that was eliminated through exhalation relative to the percentage eliminated in urine increased. Also, the percentage of MTBE in exhaled air increased, and that of TBA decreased (see Table 10). In agreement with these results, the ratio of peak blood level to the blood MTBE concentration versus time (area under the curve) was higher for MTBE and lower for TBA than would be expected for the proportional increase in dose (Ferdinandi et al. 1990a). These results suggest that the enzymes responsible for catalyzing the demethylation of MTBE to TBA may be saturated at these higher doses. Pulmonary elimination of MTBE after intraperitoneal administration of 50, 100, or 500 mg/kg in mice supports the view that the amount of MTBE exhaled is dependent on the amount administered (Yoshikawa et al. 1994) with the proportion of MTBE expired in air, compared with other routes of elimination, increasing with dose (Table 10). This is probably a function of the low blood-air partition coefficient of MTBE and the increased blood levels of unmetabolized MTBE at higher doses; the result is a more rapid elimination of radioactivity due to MTBE from the lungs.

Metabolism

Two studies provide information about the metabolic fate of MTBE (Bio/dynamics 1984c; Ferdinandi et al. 1990a). In the Bio/dynamics study, rats were administered ^{14}C-MTBE by intraperitoneal injection. The ^{14}C-MTBE had a specific activity of 10.7 mCi/mmol and was stated to be labeled on the methyl and central carbons of the *tert*-butyl group (Bio/dynamics 1984c). It is important to note that the purity and specific activity of the labeling were questionable. Through analysis at Bio/dynamics, it was determined that

the amount of ^{14}C-MTBE received was 5.5 mCi, rather than the 10.0 mCi reported by the supplier, which indicates a loss of radioactivity upon opening the ampule. It was concluded that essentially all of the ^{14}C from the metabolism chambers was associated with carbon dioxide (CO_2) or MTBE in expired air, and with formate in the urine and feces. Efforts to identify any of the radioactivity with TBA were unsuccessful. Although it was stated that both the methyl carbon and the central carbon of the *tert*-butyl group were labeled with ^{14}C, from the data obtained, it appears that the ^{14}C label was associated with the methyl ether group.

In the other study, rats were administered ^{14}C-MTBE by either the oral or intravenous route (Ferdinandi et al. 1990a). The ^{14}C-MTBE used for this study was provided at a specific activity of 12.1 mCi/mmol with the ^{14}C incorporated into the central butyl carbon atom. In contrast to the Bio/dynamics study, the investigators found that most of the expired radioactivity was ^{14}C-MTBE, not ^{14}C-CO_2, and that approximately 0.5% of the dose was expired as TBA. In addition, the ^{14}C radioactivity in urine was mostly due to 2-methyl-1,2-propanediol and 2-hydroxyisobutyric acid (2-hydroxybutyrate), which are formed from the oxidative metabolism of TBA (Figure 5). Virtually all of the ^{14}C was eliminated within 48 hours from rats treated with ^{14}C-MTBE.

Figure 5 indicates both the specific carbon atoms that were labeled with ^{14}C and the postulated metabolic fate of these carbons. Using an in vitro assay, Cederbaum and Cohen (1980) showed that the first step in the metabolism of MTBE is oxidative demethylation mediated by the liver cytochrome P-450 enzymes. Brady and coworkers (1990) demonstrated that this metabolism of MTBE yields equimolar amounts of TBA and formaldehyde and, using various inducers of cytochrome P-450, found evidence that CYP2E1 and CYP2B1 are involved in the demethylation of MTBE. The apparent Michaelis constant (K_m) for the oxidative demethylation of MTBE in control microsomes was 0.67 mM.

Formaldehyde originates from the ethyl carbon attached to oxygen and is rapidly converted to formic acid. Additional oxidative demethylation of TBA can occur, yielding first the intermediate 2-methyl-1,2-propanediol and then formaldehyde and acetone. 2-Methyl-1,2-propanediol can be oxidized to 2-hydroxyisobutyrate, whose oxidation yields CO_2 and acetone. Further metabolism of acetone yields other oxidation products such as a 2-carbonacetyl group and a formyl group or to 1,2-propanediol and pyruvic acid. The pyruvic acid carbons can contribute to all of the products that arise from pyruvic acid derived through endogenous metabolism.

The data in Table 10 indicate that the route of exposure has an effect on the extent of MTBE metabolism before the radioactivity is expired from the lungs. For example, a higher level of TBA in the exhaled radioactive material during inhalation studies indicates increased metabolism of MTBE before it can be expired from the lungs, compared with lower TBA levels from oral or intravenous exposure. Metabolism of MTBE after inhalation exposure is expected to occur primarily in the lungs and liver because little uptake of MTBE in the nose is predicted from the MTBE blood-air partition coefficient of 11.5 (S. Borghoff, personal communication) or 17.7 (Johanson et al. 1995). This assumption is based on modeling studies of uptake and dosimetry of vapors, which have hypothesized that for compounds with a blood-air partition coefficient of less than 10, most of the compound is taken up in the pulmonary region (Medinsky et al. 1993). Even so, some nasal uptake and metabolism of MTBE cannot be ruled out. Cytochrome P-450dependent monooxygenase, cytochrome P-450 reductase, and formaldehyde dehydrogenases, as well as other

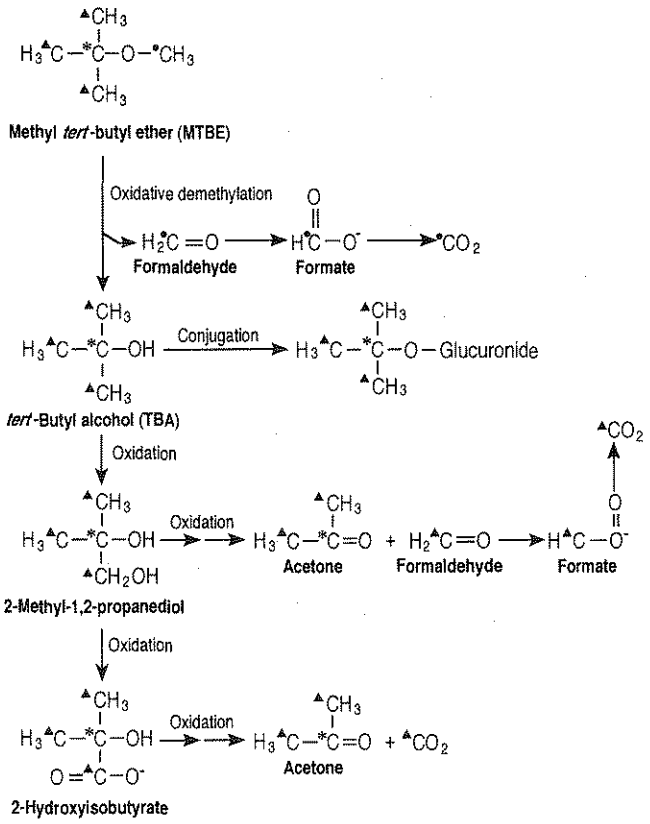


Figure 5. Metabolic pathway of MTBE derived from published studies. The symbols represent the specific carbons that could be labeled and their respective fates: ● = a methyl ether carbon; * = central tertiary carbon of a *tert*-butyl group; ▲ = primary carbons of a *tert*-butyl group.

enzyme activities have been detected in the nasal tissues of mammals, including humans. Nasal microsomes catalyze the production of formaldehyde from a number of substances including cocaine, nicotine, dimethyl ether, and diesel fuel extract (Dahl and Hadley 1983), and both CYP2E1 and CYP2B1 have been reported in the nasal tissues of rats and rabbits (Dahl and Hadley 1991), making it a potential target for chemicals that are metabolized to toxic intermediates (Brittebo 1993). It is not known whether any metabolism of MTBE occurs in the kidney.

Another possible pathway for MTBE metabolism is via cooxidation that can occur during the synthesis of prostaglandins (Smith et al. 1991). Prostaglandin H_2 synthase catalyzes the conversion of arachidonic acid to prostaglandin H_2. Cooxidation is the bioactive mechanism for a variety of compounds, and is important in oxygenating xenobiotics in extrahepatic tissues. Xenobiotics that are substrates for cooxidation include polycyclic aromatic hydrocarbons, aromatic amines, and phenolic compounds (Reed 1987; Smith et al. 1991). However, any discussion of prostaglandin H_2 synthase possibly cooxidizing MTBE or TBA is purely speculative because aliphatic ethers have not been examined as substrates for prostaglandin synthase.

Fate of Formaldehyde The fate of the formaldehyde formed by metabolism of MTBE has not been thoroughly studied; most of the disposition and pharmacokinetics studies to date have used MTBE with the ^{14}C-label on the central butyl carbon and therefore could not detect formaldehyde formation. Moreover, formaldehyde is rapidly metabolized by formaldehyde dehydrogenase to formic acid; McMartin and coworkers (1979) reported a half-life of formaldehyde of 1.5 minutes following intravenous infusion into monkeys. Thus, it is difficult to monitor its disappearance in vivo. Formic acid (formate) combines with tetrahydrofolic acid (THF) to form 10-formyl-THF, which is then oxidized to CO_2 and THF by the enzyme 10-formyl-THF dehydrogenase. Accumulation of a high level of formate in blood and in tissues results in metabolic acidosis and acidification of urine, and causes damage to the retina in humans (Liesivouri and Savolainen 1987; Dorman et al. 1994).

Folate is an essential nutrient required to produce THF; therefore, people with a dietary folate deficiency, such as occurs in some pregnant women and people who are alcoholic, may be more susceptible to the effects of formic acid than normal individuals. The decreased ability of a pregnant woman to metabolize formic acid may also put the fetus at increased risk due to formate accumulating in blood and tissues. In addition, there is a potential that formate metabolism affects the folate status of some individuals already at risk of folate deficiency such as pregnant women. This is of concern because folate deficiency in pregnant women may be linked to an increased rate of neural tube defects (Milunsky et al. 1989).

The contribution of MTBE metabolism to the formate body burden as a function of MTBE dose is not known. The literature on methanol, which is also metabolized to formate, indicates that exposing human subjects to concentrations of methanol up to 200 ppm for 6 hours (the highest concentration tested in chamber studies) does not increase formate levels (Lee et al. 1992). This was shown to be true also for normal and folate-deficient nonhuman primates exposed to methanol for 2 hours at 900 ppm (Dorman et al. 1994). However, the effect of protracted methanol exposure on folate status has not been studied. The data available on methanol suggest that exposing humans to the MTBE levels involved in using oxyfuel would not contribute significantly to the body burden of formate or affect folate levels.

Fate of TBA Unlike many alcohols, including ethanol, TBA is not a substrate for alcohol dehydrogenase (ADH) and is not metabolized to the corresponding aldehyde. Rather, there is evidence that it is metabolized by liver microsomes to yield formaldehyde and acetone (Cederbaum et al. 1983). However, it is not known how much TBA is metabolized; most likely, the extent of TBA metabolism varies among species. Baker and coworkers (1982) administered ^{14}C-TBA (randomly labeled in individual methyl groups; one ^{14}C-methyl group per TBA molecule) to rats by intraperitoneal injection of 0.75 or 2 g/kg body weight and found that 10% of the administered dose was metabolized to acetone, which was excreted in the urine and expired air, and 25% was metabolized to polar products that appeared in the urine. Despite differences in the metabolic pathway between TBA and ethanol, TBA causes intoxication and physical dependence as does ethanol (Faulkner and Hussain 1989; McComb and Goldstein 1979).

The apparent K_m for oxidative demethylation of TBA in control rat microsomes was 30 mM (Cederbaum and Cohen 1980), about 2 orders of magnitude higher than that for MTBE. In vitro experiments in which phenobarbital-induced rat liver microsomes were incubated with 0.1 mM MTBE showed that a negligible amount of the TBA formed (0.048 mM) was oxidized to formaldehyde; in other incubations of microsomes with 0.1 mM TBA, no loss of TBA could be detected during the incubation period (Brady et al. 1990). On the basis of these results, the investigators suggested that the metabolism of TBA to formaldehyde and acetone is negligible if concentrations of TBA are far below the K_m of 30 mM.

Microsomal enzyme activity was induced by approximately 35% in kidney microsomes after exposure of rats to 500 ppm of TBA for 5 days and by about 28% in liver

microsomes after in vivo exposures to 2,000 ppm of TBA for 3 days as a result of proliferation of the smooth endoplasmic reticulum (Aarstad et al. 1985). Similarly, Zahlsen et al. (1985) reported that after exposure to 2,000 or 8,000 ppm TBA, kidney cytochrome P-450 activity increased by about 40% or 43% and liver cytochrome P-450 activity by 10% or 19%, respectively. Induction of enzymes is not expected to substantially lower the K_m value for TBA, and should not increase appreciably the extent of oxidative metabolism, even with exposure to 500 ppm of TBA. Such an exposure may be comparable to an exposure to MTBE between 500 and 1,000 ppm.

Kamil and coworkers (1953) observed that rabbits administered TBA (600 mg/kg body weight) by gavage excreted about 25% of the administered dose as TBA or as TBA conjugated with glucuronic acid. In this detailed study of aliphatic alcohols, the investigators also found that 58% of *tert*-amyl alcohol (the product of TAME oxidation) was excreted as a glucuronide conjugate. The authors concluded that a slow rate of metabolic oxidation of TBA led to extensive glucuronic acid conjugation. No evidence was found for the formation of a sulfate conjugate. The question is whether the glucuronide conjugates would be expected to undergo the type of bioactivation known for some *O*-acyl-glucuronide conjugates. If this did occur, one would expect the glucuronide conjugate of TBA to be converted to isobutene (also known as isobutylene and 2-methylpropene).

In an inhalation study on the metabolic fate of isobutene (at exposure concentrations up to 4,000 ppm), Henderson and coworkers (1993) reported that isobutene was metabolized at exposure concentrations up to 400 ppm. Isobutenediol and 2-hydroxyisobutyric acid were the two urinary metabolites identified. Because isobutene is readily converted to the corresponding epoxide by cytochrome P-450 enzymes, the epoxide is the postulated intermediate for the observed urinary metabolites. Isobutene epoxide is a known mutagen in all bacterial strains tested (Henderson et al. 1993).

In summary, exposure to MTBE at low concentrations should produce TBA concentrations at which one would expect further metabolism to occur primarily by conjugation.

Pharmacokinetics

Peak blood levels of MTBE were reached within 5 to 15 minutes following intravenous and oral administration of a 40 mg/kg dose in rats (Ferdinandi et al. 1990a). Blood levels of TBA peaked approximately 2 hours after MTBE administration.

Inhalation studies in which rats were exposed to 400 ppm ^{14}C-MTBE for 6 hours/day (approximate total dose 285 mg/kg) showed a rapid increase in plasma MTBE levels within 10 minutes of the start of nose-only exposure. Plasma MTBE levels continued to rise until they reached a plateau at approximately 2 hours after the beginning of the exposure. This apparent steady-state concentration was maintained until the end of the 6-hour exposure period (Ferdinandi et al. 1990b). TBA levels, on the other hand, continued to rise after the end of the exposure and peaked 30 minutes after the end of the exposure.

Pharmacokinetic parameters were relatively consistent after all routes of administration; the half-life for eliminating MTBE from the blood ranged between 0.5 and 0.9 hours. In mice, two half-lives (0.75 and 1.3 hour) were determined from two exponential curves of MTBE elimination after 100 or 500 mg/kg MTBE administered intraperitoneally (Yoshikawa et al. 1994). The half-life of TBA was found to be longer than that of MTBE for all routes of MTBE administration in rats; when TBA half-lives were compared across routes of exposure, they were shorter after oral and intravenous exposure than after inhalation exposure. The half-life was found to be approximately 3 hours following inhalation exposure to either 400 or 8,000 ppm MTBE, 0.9 to 1.3 hours after oral or intravenous exposure to 40 mg/kg, and 1.6 to 1.9 hours after oral exposure to 400 mg/kg. After repeated inhalation exposure to 8,000 ppm MTBE, the half-life of TBA was significantly lower (approximately 1.5 to 1.8 hours) than that determined after a single exposure, while the half-life of MTBE was unchanged; this suggests that repeated exposures may induce the metabolism of TBA (Ferdinandi et al. 1990b) consistently with what was observed in vitro.

Summary of Animal Studies

Animal studies provide evidence that MTBE exhibits a pharmacokinetic pattern of rapid peak blood levels, reached at the end of the exposure, and that the metabolite TBA reaches a peak level approximately 0.5 to 2 hours later, depending on the route of administration. The major pathways for elimination of MTBE are exhalation and oxidative demethylation to form TBA and formaldehyde. MTBE metabolism displays saturation kinetics. In vitro experiments showed that both MTBE and TBA are metabolized by the cytochrome P-450 enzymes. TBA can undergo oxidation and produce various metabolites, such 2-hydroxyisobutyric acid, 2-methyl-1,2-propanediol, and acetone, or conjugation to form a glucuronide. The relative contribution of these two pathways will depend on the TBA dose. At low concentrations, the main pathway for eliminating TBA

seems to be conjugation to form a glucuronide, which leads to urinary excretion of TBA.

HUMAN STUDIES WITH MTBE

Preliminary data obtained by Johanson and coworkers (1995) in a study of human volunteers exposed to 5, 25, or 50 ppm MTBE for 2 hours in an environmental chamber suggest that less than half of the MTBE administered (32% to 42%) is absorbed after inhalation exposure and that absorbed MTBE is cleared by both exhalation and metabolism. This is consistent with the results from animal studies. The concentration of MTBE in blood was proportional to the exposure levels, with peak blood levels being reached at the end of the exposure. The decay of MTBE in blood could be separated into three phases with half-lives of about 10 minutes, 1.5 hours, and 19 hours.

Cain and coworkers (1996) exposed four healthy adults to 1.7 ppm MTBE for 1 hour and found that blood levels of MTBE began rising within 2 minutes of the start of exposure and continued to rise, without reaching a plateau, for the duration of the exposure. The peak blood concentration was reached at the end of the 1-hour exposure period and corresponded to a mean of 17.2 µg/L (± 2.0 SD). Blood levels of TBA were not determined during this study. The half-life for the elimination of MTBE was found to be approximately 40 minutes (similar to that in rats). This is in line with studies by Prah and coworkers (1994), in which a clearance half-life of approximately 36 minutes for MTBE was derived using a single-compartment model following a 1-hour exposure of human subjects to 1.4 ppm (5 mg/m^3) MTBE.

Similarly to Johanson and coworkers (1995), both Cain and coworkers (1996) and Prah and coworkers (1994) noted that, after the rapid decay phase (with a half-life ranging from 36 to 90 minutes), MTBE disappeared from human blood at a slower decay rate, which had not been seen in rats. This may indicate that MTBE distributes into tissues or that it binds to blood components. Further research utilizing more subjects and a longer postexposure follow-up is needed to investigate this slower decay component of MTBE disposition. A summary of the relationship between exposure concentration and MTBE and TBA blood levels in the controlled exposure studies is provided in Table 11.

In contrast to MTBE, TBA concentrations gradually increased and reached a plateau concentration that was maintained through the postexposure measurement period of 5 hours in men and 7 hours in women (Prah et al. 1994). In the study by Johanson and coworkers (1995), TBA in blood continued to increase during a 2-hour MTBE exposure of 25 or 50 ppm, then leveled off and began to decline about 6 hours later (Johanson et al. 1995); peak TBA concentrations in blood were proportional to the exposure level. It would appear that the half-life for the clearance of TBA from blood may be longer in humans than in rats, in which the half-life after inhalation exposure is approximately 3 hours (Ferdinandi et al. 1990b). No measurements of formaldehyde or formic acid were made during these studies involving humans. Because the metabolism of TBA is considerably slower than that of MTBE, TBA may be a more appropriate indicator of MTBE exposure.

No information on the absorption, distribution, metabolism, or excretion of TBA in humans was found in a search of the available literature.

In the individuals exposed in the controlled exposure studies just described, MTBE blood levels were similar to the TBA levels and the ratio of MTBE to TBA at the end of the exposure was approximately 1 (see Table 11). The individual MTBE and TBA blood levels for the subjects sampled in the Stamford, CT, community study (White et al. 1995) that will be described in the Short-Term Effects section were provided by Dr. M. White. The mean MTBE and TBA blood levels for the various groups sampled are also reported in Table 11, together with the mean of all the individual MTBE/TBA ratios. Two observations can be derived from this set of data. First, the individual blood levels of MTBE and TBA are highly variable (reflecting variations in the individual exposures and possibly individual metabolic differences). Second, the MTBE blood levels relative to TBA levels are lower than what might be predicted from the controlled exposure studies; with one exception, they are only 3% to 40% of the TBA levels. This discrepancy between the controlled exposure studies and the community studies may be due to delays in obtaining blood samples at the end of the exposure period in the field.

ANIMAL STUDIES WITH ETBE

Limited information on the metabolism and disposition of ETBE in rats and mice after inhalation exposure has recently become available. In one study, rats were exposed to 500, 1,000, 1,750, 2,500, and 5,000 ppm ^{14}C-ETBE for 6 hours (Sun and Beskitt 1995a). The amount of radioactivity in exhaled air, blood, and urine was determined at different times after the end of the exposure. However, the composition of the radioactive material was not characterized. As with MTBE, a fraction of the radioactivity inhaled was exhaled. This fraction increased from 38% to 60% with increasing concentrations of ETBE from 500 ppm to 1,750 ppm, then remained constant while the fraction of radioactivity in urine decreased from 60% to 38%. The fraction of radioactivity exhaled as CO_2 was approximately 2% of the total radioactivity eliminated at all exposure concentra-

tions. The formation of ^{14}C-CO_2 requires complete metabolism of the tertiary group via the formation of TBA followed by oxidative metabolism to CO_2 from the central carbon of TBA. The remaining radioactivity was found associated with the feces. All the radioactivity was eliminated by 48 hours.

The level of radioactivity in blood was found to peak at the end of the exposure period. The peak blood level of ETBE increased with the exposure concentration up to 1,750 ppm and then remained constant. Taken together, these data suggest that saturation of ETBE metabolism at doses higher than 1,750 ppm may be a consequence of saturation of uptake. The level of radioactivity in the kidney did not increase at exposures greater than 2,500 ppm. The uptake and disposition of ETBE after repeated exposures was not studied; therefore, it is not known whether saturation of uptake occurs also after repeated exposures.

A similar pattern of ETBE metabolism and disposition was noted in mice (Sun and Beskitt 1995b). The proportion of radioactivity in exhaled air increased from 11% to 44% as the ETBE concentration increased from 500 to 1,750 ppm and then remained unchanged. The proportion of radioactivity in urine decreased from 74% to 46%. Uptake of ETBE, determined as the level of ETBE in blood at the end of the exposure, increased up to an exposure level of 1,750 ppm. Similarly, the level of ETBE in liver increased only up to 1,750 ppm. Blood levels were 37 μg/mL in rats and 154 μg/mL in mice after exposure to 500 ppm, and 124 μg/mL in rats and 481 μg/mL in mice after exposure to 1,750 ppm. Thus, saturation of uptake occurs at higher blood levels in mice than in rats.

ETHANOL

Ethanol metabolism and disposition have been studied extensively in both animals and humans, but have never been characterized after inhalation of concentrations in the range of those that might be encountered as a result of ethanol's use in oxyfuel.

Although ethanol blood levels have traditionally been expressed as mg% (that is, mg/100 mL), they will be reported in this section as mg/L (or g/L) for consistency with data reported for MTBE blood levels; mg/L can be converted to mg% by dividing by 10.

METABOLISM

Because of its solubility in both lipids and water, ethanol is readily absorbed across most epithelial surfaces, including those of the nose, lungs, and gastrointestinal system. When ethanol is inhaled, the respiratory tract absorbs approximately 60% of it on average; this fraction seems to be independent of the inspired concentration and rate of ventilation (Lester et al. 1951; Kruhoffer 1983). After absorption, ethanol enters the blood stream and is rapidly distributed by cardiac output into total body water, with an apparent volume of distribution of 0.7 L/kg body weight (Jones and Neri 1985). Factors that increase cardiac output (such as physical exertion or pregnancy) increase the rate at which equilibrium is achieved (Lester et al. 1951). Ingested ethanol is rapidly absorbed by the gastrointestinal tract and enters the blood stream (Jones and Neri 1985).

Ethanol is removed from the blood primarily by metabolism in the liver, with about 1% eliminated unchanged via the urine or the lungs (Lewis 1986). In the liver, three pathways metabolize ethanol to acetaldehyde. The major pathway is mediated by ADH (a cytoplasmic enzyme). A second pathway is mediated by the microsomal ethanol-oxidizing system in the endoplasmic reticulum. Some evidence suggests that the capacity of this oxidative system, which is dependent on cytochrome P-450, increases with the exposure concentration and after repeated exposures. However, it is unclear whether the liver cytochrome P-450–dependent oxidative system ever becomes the principal pathway (Pohorecky and Brick 1987). A third pathway, which utilizes catalase within the liver peroxisomes, is thought to contribute very little to ethanol metabolism in the liver (Lieber and Pirola 1982). Acetaldehyde is metabolized via mitochondrial acetaldehyde dehydrogenase to acetate, which is released from the liver and metabolized peripherally (Pohorecky and Brick 1987). Acetaldehyde dehydrogenase has a very low K_m and a high reaction rate (International Agency for Research on Cancer 1988).

Upon inhalation, substantial ethanol uptake is expected to occur in the nose due to the high blood-air partition coefficient of ethanol, 2,000, which is 2 orders of magnitude greater than that of MTBE (Medinsky et al 1993; Morris et al. 1993). Because only a thin barrier exists between the brain and the olfactory bulb, which is traversed by the olfactory neurons, it has been suggested that these neurons may participate in transporting chemicals into the brain from the nasal cavity, thereby providing a route of administration of chemicals through the brain that circumvents the blood-brain barrier (Dahl and Lewis 1993). Thus, some inhaled ethanol may be transported directly to the brain from the nose, bypassing liver metabolism; some also may be metabolized in the nose. Alcohol dehydrogenase and acetaldehyde dehydrogenase have been found in the nasal mucosa of hamsters and rats, respectively (Dahl and Hadley 1991). Metabolism in the upper respiratory tract leads not only to high levels of metabolites in this region, but also to

a decreased amount of parent compound available for absorption into the blood stream.

PHARMACOKINETICS

Widmark (1932) first proposed that ethanol in blood is eliminated at a constant rate. A detailed examination by Lewis (1986) led to a refinement of Widmark's hypothesis, and more recently it has been confirmed that the metabolic rate of ethanol is not constant, but varies in a predictable way with blood ethanol concentrations and can be represented by Michaelis-Menten elimination (Campbell and Wilson 1986; Holford 1987). Based on the Michaelis-Menten kinetic model, the elimination of ethanol is dependent on ethanol blood levels when they are lower than the K_m for ADH (first-order kinetics), and becomes independent of ethanol blood levels when they are much greater than the K_m for ADH and approach the maximal velocity (V_{max}) (zero-order kinetics). Different values of the K_m for ADH have been reported in the literature. They include 30 mg/L blood (Campbell and Wilson 1986), 95 mg/L (Forrest 1986), and 128 mg/L (Holford 1987). A weakness of the model is that it does not reflect cytochrome P-450 oxidation and direct excretion at higher ethanol concentrations. Using the Michaelis-Menten equation, Forrest estimated a maximal rate of ethanol elimination of 230 mg/L/hour, similar to those determined by Holtzman and coworkers (1985) of 170 mg/L/hour in men and of 210 mg/L/hour in women. However, Holtzman also identified individuals with slower ethanol metabolism. Pohorecky and Brick (1987) reported an average rate of 150 mg/L/hour and a range from 100 to 340 mg/L/hour. In one study in which human subjects ingested 5 g of ethanol, it was eliminated from blood with first-order kinetics with an estimated half-life of 16 minutes (Jones 1985).

Controversy exists concerning the degree to which ethanol inhalation can contribute to the ethanol body burden because very few studies have examined the kinetics of ethanol uptake and elimination following inhalation exposure. Blood alcohol concentrations of human volunteers after inhaling ethanol vapor at a concentration of 1,000 ppm remained below the detection limit of 20 mg/L (or 2 mg%) (Table 12), even after 3 hours of exposure (Campbell and Wilson 1986). However, the limited sensitivity of the analytic method may have contributed to the inability to detect an increase of ethanol levels in this study. Kruhoffer (1983) agreed with Lester and coworkers (1951) that inspired ethanol may cause an elevation of blood alcohol concentrations that would depend, in addition to the ethanol concentration and the duration of exposure, on the rate of ventilation and the individual weight.

Kruhoffer (1983) observed an increase of 50 mg/L in ethanol blood levels in subjects exposed to 5,000 ppm for 60 minutes with exercise, and Lester and coworkers measured an increase in blood levels in subjects exposed to 8,000 ppm for 3 hours with exercise (of 250 to 450 mg/L) and without exercise (of 20 to 65 mg/L) (see Table 12). Lester and coworkers concluded that if the amount of ethanol taken up by an individual does not exceed the rate of ethanol oxidation, no increase in ethanol blood level should be expected.

In subjects administered 0.68 g/kg ethanol orally, absorption was complete after 50 minutes, at which time ethanol blood levels peaked (the increase in blood level was around 900 to 1,000 mg/L). Blood levels then began to drop and returned to normal after 7 hours (Jones and Neri 1985). Lewis (1986) suggested that the blood alcohol concentration depends to some extent on the length of the absorption period and the prevailing blood alcohol concentration during exposure. At low blood concentrations, metabolism in the liver will result in lower than expected ethanol blood concentrations, although at concentrations of ethanol near the V_{max}, some ethanol will escape metabolism in the liver and reach the general circulation. Frezza and coworkers (1990) found that, after ingestion of 0.38 g/kg ethanol, the ethanol blood concentration versus time (area under the curve) was lower in men than in women. This was interpreted as being due to presystemic ethanol metabolism in men, probably occurring in the gastrointestinal tract. These investigators based their conclusion that the bioavailability of ingested ethanol is much greater in women than in men on the finding that in women the first-pass effect of gastric metabolism is lower because of lower gastric ADH activity. However, gastric metabolism would not contribute to overall ethanol elimination following inhalation exposure.

Daily consumption of moderate doses of ethanol (45 g/day) for 3 weeks did not appear to increase the blood alcohol concentration at the end of the exposure period above the levels observed after a single exposure, or affect the rate of disappearance of ethanol from blood in either men or women (Holtzman et al. 1985).

Ethanol is the product of many endogenous metabolic pathways. As a result, endogenous blood levels of ethanol can vary among individuals over a wide range of values. Endogenous blood levels were reported to be between 9 and 27 mg/L (Lester et al. 1951); by extrapolation from breath samples, Jones (1985) reported concentrations of 0.2 to 0.85 mg/L in fasting subjects and Lester (1962) reported concentrations below 1.5 mg/L. Thus, endogenous blood levels range between 0.2 and 27 mg/L (see Table 12).

Any additional ethanol burden should be viewed in light of the endogenous blood levels at the time of the exposure.

The blood level that would be expected from a possible exposure scenario can be estimated from the ethanol concentration, the duration of exposure, and the ventilation rate. For a typical refueling exposure scenario of 1 ppm (1.9 mg/m^3) for 3 minutes, and assuming that 60% of inhaled ethanol is taken up by the body, the resulting dose is equal to 1.9 $mg/m^3 \times 0.05$ hours $\times 0.6 \times 0.83$ m^3/hour (or 14 L/minute, ventilation rate with minimal exercise) = 0.05 mg.

Assuming an average body weight of 70 kg, the dose per kilogram is 0.05 mg/70 kg = 0.0007 mg/kg (0.7 μg/kg). This dose would distribute in a volume of 0.7 L/kg (Jones and Neri 1985). Thus, the corresponding incremental blood level would be 0.7 μg/kg:0.7 L/kg = 1 μg/L.

For an extreme exposure scenario of 10 ppm ethanol for 15 minutes, the estimated incremental blood level is 40 μg/L. (These calculations do not take into account the amount of alcohol metabolized during the 15-minute period.) The resulting incremental blood levels are below the range of endogenous blood levels (0.3 to 27 mg/L), so ethanol would not significantly increase in blood under either of these exposure scenarios.

Studies with humans have also pointed out that some individuals metabolize ethanol more slowly than others; however, differences in the rate of ethanol elimination are not expected to be noticeable when exposure levels are similar to those in the scenarios discussed above.

DISPOSITION OF ETHANOL DURING PREGNANCY

Several studies have investigated the transfer of ethanol from the maternal circulation to the fetus in laboratory animals such as mice, sheep, and nonhuman primates. These studies, which are summarized below, used exposure routes other than inhalation; however, the information they provide is useful in illustrating the rapid transfer of ethanol to the fetus and the potential for the fetus to be exposed to ethanol for possibly lengthy periods of time.

Animal Studies

Studies of pregnant sheep 2 weeks before term (Cumming et al. 1984) indicated that ethanol was rapidly transferred to the fetus, achieving peak blood levels in both maternal and fetal blood (1.8 and 1.9 g/L, respectively) within 2 hours after the end of a 2-hour infusion (of a dose of 1.2 g/kg body weight).

In pregnant mice near term administered ethanol by gavage, peak ethanol concentrations of 1.03 and 0.94 g/L, respectively, were reached in the fetus after 5 minutes and in amniotic fluid after 25 minutes and were lower than the maternal blood levels at the same times (Kaufman and Woolam 1981). After 60 minutes, the levels of ethanol in each compartment had fallen; concentrations were equal in maternal blood and the fetus (0.46 and 0.41 g/L), but slightly higher in amniotic fluid (0.54 g/L), suggesting that amniotic fluid can act as a reservoir for ethanol, and movement from the fluid back into the fetus could result in fetal exposure to ethanol for an extended period of time.

In nonhuman primates administered ethanol at 0.8 to 1.5 g/kg body weight intravenously over a 30-minute period during gestational days 106 to 160 (the third trimester), ethanol appeared in the fetal circulation almost immediately, but the time required to attain peak concentrations varied among fetuses (Hill et al. 1983). By 60 minutes, the maternal and fetal blood ethanol levels were equal. For example, after administering ethanol at 1 g/kg body weight to a monkey in its 134th day of gestation, the maternal and fetal blood ethanol levels were 1.2 g/L. The elimination rates of ethanol from maternal and fetal blood were similar among monkeys that received 0.8, 1.0, or 1.5 g/kg body weight. Using the same monkey at 134 gestational days as an example, both the maternal and fetal elimination rates were 0.22 g/L/hour. Because ethanol is not metabolized substantially more rapidly in the fetal compartment, compared with the maternal compartment, fetal blood ethanol elimination is probably determined by the rate of ethanol elimination from maternal blood.

In these nonhuman primates, however, a marked difference in elimination rates of ethanol was noted between mothers and their neonates delivered 2 hours after infusion of ethanol in the mothers. Neonatal monkeys eliminated ethanol from their blood at approximately 25% the rate seen in maternal blood. This pattern was consistent in each of three mother-neonate pairs studied. The values for a representative pair were 0.14 g/L/hour for maternal elimination and 0.04 g/L/hour for neonatal elimination. The finding that the elimination rate of ethanol from neonatal monkey blood was significantly lower than from maternal blood is remarkably similar to that in neonatal humans reported below by Idanpaan-Heikkila and coworkers (1972).

Three conclusions can be drawn from these animal studies: (1) ethanol is rapidly transported across the placenta to the fetus and reaches an equilibrium with maternal levels within 1 to 2 hours after the end of the exposure; (2) amniotic fluid may act as a reservoir for ethanol after its concentration in maternal blood decreases; and (3) ethanol is cleared from fetal blood at a rate similar to the rate of elimination from maternal blood, but is cleared from the blood of neonates more slowly than from maternal blood.

Human Studies

Many studies have suggested that consumption of large amounts of ethanol affects fetal development. Because of its fetotoxic effects, few investigations have been conducted into the disposition of ethanol administered to women early in pregnancy. However, because of its use in stopping premature labor during the 1960s through the 1980s, information has been obtained on maternal, fetal, and neonatal blood alcohol concentrations during the second and third trimesters of pregnancy. In one study, the placental transfer and time course of ethanol distribution in maternal, fetal, and neonatal blood were studied in pregnant women at term who received an intravenous infusion of a saline solution containing 8% ethanol (by volume) for 1 hour (Idanpaan-Heikkila et al. 1972). An equilibrium in the distribution of ethanol between the mother and the fetus was achieved at the end of the infusion period (approximately 800 mg/L blood). From birth to 4 hours of age, the mean elimination rate of ethanol was 0.08 g/L/hour in the newborn infant and 0.14 g/L/hour in the mother. Eight hours after birth, the mean ethanol concentration in neonatal blood (0.11 g/L) remained significantly higher than in the mother (0.06 g/L). The authors proposed that this may have been due to lower levels of ADH in the neonate, or the higher water content in the tissues of the newborn.

In another study, the appearance of ethanol in amniotic fluid was studied in pregnant women early in the second trimester (admitted to a hospital for elective abortion) after ingestion of ethanol at 0.3 g/kg body weight (equivalent to 2 oz of 40% distilled spirits or 18 g) (Brien et al. 1983). The mean maternal blood ethanol concentration decreased to 0.01 g/L by 3.5 hours after oral ingestion, and blood levels were below the detection limit in three of the six women. Ethanol appeared in the amniotic fluid between 15 and 45 minutes after ingestion; the time to maximum ethanol concentration in amniotic fluid (0.09 to 0.31 g/L) ranged from 1.5 to 2.5 hours. Ethanol was cleared from amniotic fluid at half the rate seen in maternal blood so that it was still present in amniotic fluid at concentrations ranging from 0.06 to 0.24 g/L after 3.5 hours, when the levels of ethanol in maternal blood had fallen significantly. Measurements in one subject indicated that ethanol was no longer present in amniotic fluid 5 hours after she ingested a single oral dose.

Thus, there was a differential disposition of ethanol in maternal blood and amniotic fluid following maternal ingestion of a single dose of ethanol during the second trimester of human pregnancy. The relatively high concentration of ethanol in amniotic fluid after it was virtually eliminated from maternal blood suggests that the fetus, placenta, or amniotic fluid can act as a reservoir for ethanol in utero. Thus, a fetus can be exposed to ethanol and subjected to its effects for a longer time period than would be predicted from measurements of maternal venous blood ethanol concentrations (Brien et al. 1983).

CONCLUSIONS

ETHERS

Inhaled or ingested MTBE is quickly taken up into the blood stream and distributed to body water. At the range of concentrations relevant to human exposures, the level of MTBE in blood appears to be directly proportional to the MTBE concentrations in the air. This is assumed to be true also for ingested MTBE. Once taken up, MTBE is either exhaled as such or metabolized. The extent to which MTBE is metabolized before it is expired depends on the route of exposure and the dose administered. At comparable MTBE blood levels, a higher proportion of MTBE is exhaled after oral and intravenous exposure than after inhalation exposure. Oxidative demethylation by cytochrome P-450–dependent enzymes is the first step in metabolizing MTBE, which yields formaldehyde and TBA. TBA is detected in both blood and urine after exposure to MTBE. MTBE has a short half-life in blood (ranging from 0.5 to 1.5 hours depending on the study); TBA appears to be metabolized more slowly, with glucuronidation being an important pathway for eliminating TBA. Formaldehyde is metabolized very rapidly, and its level in blood has not been followed. Levels of TBA in blood are similar or higher than the corresponding levels of MTBE. Although both MTBE and TBA appear to be reliable indicators of MTBE dose, further studies to establish the time course of TBA metabolism as a function of exposure concentrations need to be conducted before TBA can be used as a marker of exposure in human studies.

Inhaled ETBE is also eliminated by exhalation or through the urine. As with MTBE, the fraction of radioactivity exhaled increases with increasing exposure concentrations. Saturation of uptake may occur at exposure concentrations greater than 1,750 ppm.

ETHANOL

Ingested ethanol and a portion of inhaled ethanol (about 60%) are rapidly absorbed into the blood stream and distributed to body water. Although the uptake and disposition of ingested ethanol (at amounts comparable to those contained in alcoholic beverages) have been fairly well studied, no such studies at concentrations of ethanol that could be encountered in ambient air when ethanol is used as an oxygenate additive have been conducted. Thus, the

added body burden and resulting blood levels from such exposures can only be estimated. Estimates of incremental blood levels were made for two exposure scenarios for the public blood: a typical refueling scenario, 1 ppm for 3 minutes, and an extreme exposure scenario, 10 ppm for 15 minutes. Even the extreme scenario is expected to result in an incremental blood level that is insignificant compared with the endogenous blood levels resulting from normal metabolism.

Studies on the disposition of high levels of ethanol in pregnant animals and humans indicate that (1) ethanol crosses the placenta rapidly, and an equilibrium between maternal and fetal circulation is quickly established; and (2) the concentration of ethanol in the amniotic fluid of humans remains high after the maternal blood alcohol levels have fallen. These observations have been made after ethanol exposure by ingestion of more than 10 g, after which marked increases in blood levels were observed. Because exposure to ethanol from its use in gasoline is not expected to cause an increase in maternal blood ethanol levels above the endogenous level, no increase in exposure to the fetus is expected.

Table 10. Disposition of Methyl *tert*-Butyl Ether in Rats[a]

Exposure Level	Gender	MTBE			TBA	
		Peak Blood Level (mg/L)	Percentage of Radioactivity		Percentage of Radioactivity	
			Exhaled from 0 to 48 Hours	Released in Urine from 0 to 48 Hours	Exhaled from 0 to 3 Hours	Exhaled from 0 to 48 Hours
Inhalation						
400 ppm for 6 hours	M	15[b]	17[c]	65[c]	28[c]	65[c]
	F	14	22	65	31	65
8,000 ppm for 6 hours	M	556[b]	54[c]	22[c]	10[c]	42[c]
	F	513	59	17	7	35
Intravenous						
40 mg/kg	M	12[d]	42[c]	26[e]	4[e]	26[e]
	F	8	46	24	4	26
Oral						
40 mg/kg	M	17[d]	46[e]	35[e]	4[e]	35[e]
	F	11	54	27	4	27
400 mg/kg	M	123[d]	65[e]	16[e]	1[e]	16[e]
	F	115	69	10	1	11

[a] Adapted from Costantini 1993.

[b] Ferdinandi et al. 1990b.

[c] Ferdinandi et al. 1990c.

[d] Ferdinandi et al. 1990a.

[e] Ferdinandi et al. 1990d.

Table 11. Relationship Between Exposure Concentration and Blood Levels of MTBE and TBA in Humans

Study	MTBE Concentration in Air (ppm)	Duration of Exposure (hours)	MTBE (ppm × hour)	Mean MTBE Concentration in Blood at End of Exposure[a] (μg/L)	Mean TBA Concentration in Blood[a] (μg/L)	Mean MTBE/ Mean TBA[a]
Cain et al. 1996	1.7	1	1.7	17.2 ± 2 (4)		
Prah et al. 1994	1.4	1	1.4	6.1[b] (1 Male) 10.9[b] (1 Female)	7.8 7.8	0.78 1.4
Johanson et al. 1995	5	2	10	114[c] (10 Males)		
	25	2	50	572[c] (10 Males)	518[c]	1.1
	50	2	100	1,144[c] (10 Males)	925[c]	1.2
Stamford CT Study[d]						Mean MTBE/TBA
Service station attendants (n = 3)	ND[e]			17.2 ± 10.8 (7.6–28.9)	59.2 ± 34 (20–82)	0.31 ± 0.1 (0.18–0.38)
Mechanics, non-smokers (n = 13)	0.03–12	8	0.24–96	5 ± 9.7 (0.2–36)	17.6 ± 9 (2.7–34)	0.19 ± 0.27 (0.03–1.1)
Mechanics, smokers (n = 8)				2.40 ± 4.4 (0.2–13)	14.6 ± 11.5 (2.4–32)	0.16 ± 0.15 (0.04–0.4)
Commuters, non-smokers (n = 11)	ND			0.39 ± 0.8 (0.03–2.61)	2.7 ± 2.3 (0.8–7.8)	0.09 ± 0.08 (0.04–0.33)
Commuters, smokers (n = 3)	ND			0.22 ± 0.17 (0.09–0.4)	2.45 ± 0.54 (1.8–2.8)	0.08 ± 0.05 (0.05–0.14)

[a] Number of subjects or range of concentrations is given in parentheses.

[b] Data were reported as ppb (or μL/L blood). They were converted to μg/L by multiplying by the density (0.74 for MTBE and 0.78 for TBA).

[c] Data were reported as μmol/L. They were converted to μg/L by multiplying by the molecular weight (88 for MTBE and 74 for TBA).

[d] M. White (personal communication, 1996). Values from this study are presented as means ± SD.

[e] ND = not determined.

Table 12. Ethanol Blood Levels in Humans After Various Exposure Concentrations and Durations

Ethanol Exposure[a]	Route of Exposure	Dose Inhaled[b] (g)	Increase in Blood Level (mg/L)	Number of Subjects	Reference
1,000 ppm for 3 hr (7 L/min)	Inhalation	2.9	< 20 (MDC)	1	Campbell and Wilson 1986
3,000 ppm for 80 min[c] (30 L/min with exercise)	Inhalation	6.5	50[d]	1	Kruhoffer 1983
8,000 ppm for 3 hr (7 L/min without exercise) (24 L/min with exercise)	Inhalation	11 39	20 to 65[d] 250 to 450	3 2	Lester et al. 1951
45 g	Ingestion	45	900[d]	1	Holtzman et al. 1985
0.68 g/kg body weight	Ingestion	~ 48	880 to 1,000[d]	48	Jones and Neri 1985
10 ppm for 15 min (14 L/min)	Inhalation	0.0024	0.04[e]	NA[f]	Estimated
1 ppm for 3 min (14L/min)	Inhalation	0.00005	0.0001[e]	NA	Estimated
Endogenous levels			9–27 < 1.5 0.2–0.85[d]	3 19 1	Lester et al. 1951 Lester 1962 (estimated from breath levels) Jones 1985 (estimated from breath levels)

[a] When known, ventilation rate is given in parentheses.

[b] Calculated from the following equation using the ventilation rates reported in column 1:
g = (g/m^3 [ppm × 1.9] ×) duration of exposure [minutes] × ventilation rate [L/min] × 0.6 [% absorbed])/1,000.
For comparison, one alcoholic beverage contains 11 to 14 g ethanol.

[c] This exposure occurred 4 hr after an oral exposure to 55 g of ethanol.

[d] Estimated from a graph in the original publication.

[e] Calculated from the inhaled dose divided by the body weight (70 kg) and the volume of distribution (0.7 L/kg).

[f] NA = not applicable.

Potential Health Effects of Oxygenates

Short-Term Effects

Gasoline containing MTBE or ethanol as an octane booster (0.2% to 9% by volume) has been used since the late 1970s. Ethanol has been studied extensively because of its widespread consumption in alcoholic beverages; the information available on MTBE is less plentiful. Most of the studies conducted on ethanol have assessed the consequences of oral exposure, which is the predominant route; information on the effects of inhaling ethanol is lacking. Animal studies on MTBE were first undertaken during the 1970s to evaluate acute toxicity. More extensive testing was carried out in the 1980s particularly after designation for testing by the Interagency Testing Committee; however, studies to evaluate possible risks from exposure to MTBE in humans were not initiated until after the widespread introduction of oxyfuel in 1992. Information on other ethers is limited. This section reviews the data available on MTBE, other ethers, and ethanol regarding the potential short-term effects that result from using these compounds as oxygenates in gasoline.

ETHERS

ANIMAL STUDIES

General Toxicity Studies

The dose or concentration necessary to produce death in half of the animals in a study (LD_{50} or LC_{50}) is a gross measure of a chemical's toxicity. For MTBE, the rat LD_{50} is approximately 3.9 g/kg body weight, and the LC_{50} has been calculated to be between 40,000 ppm for 4 hours (ARCO 1980) and 180,000 ppm for 6 minutes (Shamprogetti 1986). At such high doses, death is preceded by ocular and mucous membrane irritation, ataxia, and central nervous system (CNS) depression. For comparison, the ethanol LD_{50} for rats is 10 g/kg body weight (Merck Index 1989).

To evaluate the systemic, neurotoxic, and irritant effects of ethers, short-term studies have been conducted in laboratory animals exposed to nonlethal doses of MTBE. An MTBE inhalation study conducted by Bio/dynamics (1984d) demonstrated inflammation in the nasal mucosa of rats after exposure to 3,000 ppm for 6 hours/day for 9 days. In another study, Sprague-Dawley rats were exposed by gavage to 0.4, 0.7, 1.0, or 1.4 g/kg for 14 days (Robinson et al. 1990). Both males and females exposed to the high dose exhibited anesthesia at the end of each dosing and decreased daily food intake; diarrhea was noted in all exposed animals. The major effects noted after 14 days were decreased mean body weight in females and lower mean body weight gain in both males and females at 1.4 g/kg. Absolute lung and spleen weights were reduced in both genders at 1.4 g/kg, and liver weights relative to body weight were increased at this same dose in females. In males, mean relative kidney weights and renal tubular disease (nephropathy), characterized by increased hyaline droplets, were increased at the highest concentration.

Studies of 28-day exposures of rats have been conducted with MTBE, ETBE, and TAME. One MTBE study and the only ETBE study involved inhalation exposure; another MTBE study and a TAME study involved oral exposure (by gavage and drinking water, respectively). Inhalation exposure levels were 400, 3,000, and 8,000 ppm for MTBE (Chun and Kintigh 1993); 500, 2,000, and 4,000 ppm for ETBE (IIT Research Institute 1991). Oral doses were 0.09, 0.44, and 1.75 g/kg for MTBE and 0.125, 0.5, and 1 g/kg for TAME (Daughtrey and Bird 1995) Table 13 summarizes the exposure concentrations and study results. It should be noted that MTBE was tested in F344 rats for inhalation exposure and in Sprague-Dawley rats for gavage exposure. ETBE and TAME were tested in Sprague-Dawley rats.

Both MTBE and ETBE, but not TAME, caused hypoactivity and ataxia at some of the concentrations tested. Inhaled MTBE caused a decrease in relative and absolute spleen weight in both genders at 8,000 ppm, consistent with the finding of the 14-day study. All ethers caused an increase in the weight of kidneys, adrenal glands, and livers in at least one gender. Specifically, effects on all three organs were observed after exposures to 3,000 and 8,000 ppm MTBE and after exposure to 1.75 g/kg MTBE in both genders; liver weights increased after exposure to 4,000 ppm ETBE in both genders; kidney and adrenal weights increased in male rats after exposure to 4,000 ppm ETBE or after exposure to 0.5 and 1 g/kg TAME. In the F344 rats, kidney lesions (nephropathy) were observed in all exposure groups, including the control group.

MTBE caused an increase in protein accumulation and cell proliferation in the kidneys of male F344 rats after inhalation exposure to 3,000 or 8,000 ppm. However, this

increase was not accompanied by an increase in the level of $\alpha_{2\mu}$-globulin (for a discussion of the possible role of this protein in kidney tumors, see Long-Term Effects section). MTBE administered by gavage also caused an increase in hyaline droplet formation in the kidneys of male Sprague-Dawley rats at doses of 0.44 and 1.75 g/kg (similar effects were observed in males after 14-day exposure to 1.4 g/kg MTBE). Based on the limited pharmacokinetic data on MTBE, a gavage dose of 1.75 g/kg corresponds to an inhalation dose of approximately 8,000 ppm for 6 hours (see Table 10 in the Metabolism and Disposition section). The male kidney appears to be the primary target of MTBE. No histopathologic changes were noted in the kidneys after exposure to ETBE or TAME, but no detailed analyses of protein changes were conducted.

Determining the relative toxicity of MTBE, ETBE, and TAME would require making several assumptions about their uptake, metabolism, and disposition and extrapolating from various routes of exposure. The Oxygenates Evaluation Committee thought that too many uncertainties remain for such an assessment to have much validity, but noted that similar exposure levels resulted in similar effects for the the ethers.

The subchronic toxicity of TBA was evaluated in a 90-day study with $B6C3F_1$ mice and F344 rats of both genders in which TBA was administered in drinking water (Lindamood et al. 1992). Dose levels of TBA were 0%, 0.25%, 0.5%, 1%, 2%, and 4% (w/v); some lethality was observed in both genders and species at the highest concentration tested. A decrease in weight gain, ataxia, and hypoactivity were noted at the two highest doses in both species. Kidney mineralization and nephropathy were observed in rats of both genders. These and other findings led to the conclusion that, in rodents, the kidney is a target organ, and that males are more sensitive to TBA toxicity than females.

Because effects on the kidney also were observed with MTBE, the systemic toxicity of MTBE may in part be due to TBA. Given its chemical structure, ETBE is also predicted to be metabolized to TBA. If so, one would expect ETBE to induce nephropathy, contrary to what has been observed. More information about the dosimetry, metabolism, and pharmacokinetics of ETBE is needed to interpret these findings.

In another 28-day inhalation study, Sprague-Dawley rats were exposed to gasoline vapors containing approximately 20% MTBE (Amoco 1992). The concentrations of MTBE were 100, 270, and 620 ppm. At the middle and high doses, the investigators noted hyaline droplet formation in the kidneys of male rats, an increase in relative liver weight in male and female rats, and increases in relative weights of kidneys, adrenal glands, brains, and lungs in male rats. No CNS depression was recorded in clinical observations, and no effects on functional observational batteries were observed. Although the effects of exposure to gasoline alone were not characterized in this study, it is more likely that the effects observed were caused by gasoline than by MTBE because the gasoline concentrations were 4- to 5-fold higher than the MTBE concentrations.

In summary, the increases in liver, kidney, and adrenal gland weight and CNS depression were observed in rats exposed for 28 days to exposure concentrations of MTBE, ETBE, and TAME that are much higher than those likely to be encountered by the general public. Thus, these effects are not expected to occur after brief exposures by inhalation, even if they are repeated over time. However, protracted ingestion of ethers at sufficient levels through contaminated drinking water may present a health risk.

Studies of Neurotoxic and Other Effects

The only systematic data available on MTBE neurotoxicity come from two unpublished studies conducted at the Bushy Run Research Center: a single-exposure study (Gill 1989) and a 13-week exposure study (Dodd and Kintigh 1989). The results of these studies are summarized in Table 14.

In the acute-exposure study, rats were exposed to concentrations of 0, 800, 4,000, and 8,000 ppm MTBE for 6 hours. One subgroup was then evaluated for motor activity during a 5-hour period following the termination of exposure. Another subgroup underwent examination with a functional observational battery of tests at 1, 6, and 24 hours after termination of exposure.

Motor activity, an almost universal element of neurotoxicity screening batteries, is prescribed by the EPA for that purpose. In the two Bushy Run experiments, it was measured by a device that counts interruptions of photocell beams directed in two dimensions, and at two heights, in an enclosed space. In such an apparatus, untreated animals typically are most active, exploring the enclosure directly after their insertion. Activity then gradually wanes. Rats of both genders exposed to 8,000 ppm MTBE showed a decrease in activity at first, then an increase, and then another decrease; males exposed to the two lower levels showed an initial increase in activity. For male rats exposed to 800 ppm MTBE, total activity counts were significantly higher than those of control animals during the 5-hour test period. The pattern seen for the two lower concentrations is commonly found with neurotoxic solvents such as toluene (Wood and Colotla 1990).

Functional observational batteries, which comprise a standardized array of observations, are also commonly included in preliminary neurotoxicity screening (Moser

1994). The Bushy Run functional observational battery included phenomena such as convulsions, tremor, stereotyped behaviors, piloerection, gait, vocalizations, grip strength, muscle tone, surface righting reflex, and others. Only the first hour following the end of exposure yielded biologically significant findings. Ataxic gait and a phenomenon called duckwalk appeared after exposure to 4,000 and 8,000 ppm MTBE. At 8,000 ppm, other effects appeared including lacrimation, labored respiration, decreased muscle tone, increased hind limb splay, and decreased performance on a treadmill.

In the longer exposure study, rats were exposed to MTBE at the same concentrations as the single-exposure study (0, 800, 4,000, and 8,000 ppm) for 5 days/week for 13 weeks. Functional observational batteries were conducted in weeks 1, 2, 4, 8, and 15. Motor activity was measured in weeks 4, 8, and 13. Both sets of measurements took place on weekends, at a time when MTBE blood levels had presumably fallen to insignificant levels. Functional observational battery measurements revealed few differences between control and MTBE-exposed animals throughout the 13-week period. Only motor function indices suggested adverse effects, but these seemed inconsistent. Motor activity patterns as a function of dose varied through the experiment, but no sharp differences among exposure groups emerged. No neuropathology was evident.

In summary, motor activity was affected by exposure to 800 ppm MTBE (the lowest concentration tested), and other effects such as sedation and ataxia were observed at higher concentrations. Work with toluene has shown that the measure of motor activity is not generally the most sensitive indicator of behavioral effects. Exposure to toluene in rats caused a biphasic change in activity, increasing it at lower concentrations (from 560 to 1,780 ppm) and decreasing it at high concentrations (3,000 ppm), similar to the pattern observed with MTBE (Wood and Colotla 1990). In contrast, effects of toluene on conditioned performance (complex behavior) were observable in rats at concentrations below 300 ppm and close to the threshold limit value (TLV) (Wood and Cox 1995). In humans, toluene has been shown to impair performance at concentrations as low as 100 ppm (Dick et al. 1984).

Thus, the motor effects observed with MTBE are of concern because we do not have a no-observable-adverse-effect level (NOAEL) for them and they suggest the possibility of effects on more complex functions of the CNS at lower levels. Overall, the studies of MTBE support its classification as a neurotoxicant and suggest that its primary effect is likely to be in the form of acute impairment. In the absence of animal data at concentrations lower than 800 ppm, the relevance of the effects observed with MTBE for assessing effects in humans is unclear even though humans are typically exposed to concentrations of MTBE several orders of magnitude lower than those used in these studies.

ETBE was also evaluated for neurotoxic effects in rats exposed to 400, 2,000, and 4,000 ppm, 6 hours/day for 28 days (IIT Research Institute 1991). Only minor effects were observed at the highest concentration tested, 4,000 ppm; however, motor activity, the behavior most affected by MTBE exposure in other studies, was not measured in this study.The potential for MTBE to irritate the respiratory tract also has been investigated in animals. Tepper and coworkers (1994) assessed the effects of MTBE exposure on breathing frequency in mice. Exposures ranged from 80 to 8,000 ppm for 1 hour. The breathing frequency decreased with increasing concentrations up to a 52% decrease at 8,000 ppm. For all concentrations except 8,000 ppm, the breathing frequency returned to normal after 10 minutes of exposure. The respiratory waveform indicated that pulmonary irritation did not occur at concentrations below 8,000 ppm, and levels of markers of lung injury (total protein and lactate dehydrogenase) in bronchoalveolar lavage fluid (from the animals exposed to 8,000 ppm MTBE) were not different from those in control animals. From this information, the investigators calculated a TLV for sensory irritation in humans of 140 ppm (according to the model for sensory irritants of Alarie 1981).

HUMAN STUDIES

Oxyfuel was introduced in Fairbanks, AK on October 15, 1992. In November and December 1992, residents of the community voiced complaints about health symptoms, increased odor, mechanical problems with vehicles, and reduced gas mileage. At first, complaints were voiced on Talk Radio (Gordian et al. 1995), and then on a telephone hotline set up by a private citizen (M. White, personal communication). The health symptoms most often reported were eye irritation, headaches, and nausea. People also complained about the $0.14 increase in the price of gasoline. Within 2 months from the introduction of oxyfuel, the governor of Alaska, on the basis of the results of a community study conducted jointly by the Alaska Division of Public Health and the National Center for Environmental Health of the Centers for Disease Control and Prevention (CDC) (Beller et al. 1992), suspended the oxyfuel program in Fairbanks. In Anchorage the program continued through February 1993, but was not implemented in the winter of 1993/1994. In the winter of 1994/1995, Anchorage used ethanol exclusively for its oxyfuel program. During the brief period in which gasoline containing MTBE was used in Fairbanks in 1992/1993, the State of Alaska Department of Health and Social Services and the CDC conducted studies to evaluate

the complaints. During the same winter, health complaints also were reported by citizens in other states, most notably Montana and New Jersey.

On January 1, 1995, RFG was introduced in the nine areas that were most out of compliance with federal regulations for ozone (for details, see Exposure Assessment section). As with the introduction of oxyfuel in 1992, this action was accompanied by outbreaks of complaints in some areas (for example, Wisconsin and Maine). In addition to health symptoms, people in Wisconsin complained of damage to their engines and poor gas mileage. Just after RFG was introduced, the results of a third carcinogenicity study with animals (Belpoggi et al. 1995) had been released; this study reported an increase in leukemia and lymphomas in rats exposed to MTBE by stomach tube for a lifetime. Heightening the public's concern, an ABC News segment on the show "Day One" early in January 1995 suggested that serious illnesses such as cancer resulted from exposure to MTBE. Reports of citizens' complaints in other states, including North Carolina and New Jersey, also appeared in the media during that period.

In response to widespread public concern in certain locales, community investigations and controlled human exposure studies were conducted with the goal of identifying whether a relationship exists between exposure to oxyfuel and health symptoms. Informal surveys also were undertaken in some areas as a screening tool to investigate complaints or to assess whether adverse health effects had occurred.

Informal Surveys

When MTBE (8% by volume) was first introduced in the Denver area in 1987, a few complaints about odor and health effects were received by the Colorado Department of Public Health and Environment. The total number of complaints declined over the duration of the program from 28 during the first winter (1987/1988) to 2 in 1993/1994 (Livo 1995). However, a hotline was not established specifically for reporting health complaints. In 1988, the Department of Public Health and Environment surveyed hospitals in Colorado Springs and Denver and did not find "sufficient evidence to confirm linkage between MTBE and health complaints" (Livo 1995).

A retrospective study of outpatient insurance claims conducted in Alaska compared claims received during the months in which oxyfuel was being used in the winter of 1992/1993 (2 months for Fairbanks and 4 months for Anchorage) with claims in the same months of the two previous winters. The percentage of claims for illnesses, including hospital admissions for respiratory ailments and asthma, was stable over the 3-year period studied. However, the investigators noted that the percentage of claims in the 1992/1993 winter peaked in December and declined in January and February in both cities (Gordian et al. 1995).

In Missoula, MT, another area where citizens reported complaints, a survey of physicians was conducted to determine whether illnesses had increased that winter (Missoula City-County Health Department 1993). The information provided by the physicians did not indicate that an outbreak of illnesses occurred during the period, but confirmed that people were aware of oxyfuel health issues and were concerned.

An informal survey of workers in two U.S. oil refineries (Mehlman 1995) indicated that some workers exposed to gasoline containing MTBE experienced several symptoms during an unspecified period of time. Because of the lack of a formal sampling design, the prevalence of symptoms among the workers in those refineries was not documented. A survey of health-related complaints received by oil companies was conducted by the API (McCoy et al. 1995) during the period from January 1, 1992 through April 30, 1994, and involved 18 member companies. In 1992, the highest numbers of complaints had been received during the months of October (14/42) and November (21/42). In 1993 there were fewer complaints; the highest numbers were reported in January (3/15) and February (3/15). A total of 71 employee complaints were received during the study period. Although the symptoms seem to have occurred while the wintertime oxyfuel was in use, according to the API the manufacturing and transporting of MTBE began months before the starting date of the program. The primary symptoms reported were headache, dizziness, and nausea. Distribution workers reported symptoms most frequently (31/71), followed by service station attendants (13/71) and MTBE production workers (11/71). The total number of workers potentially exposed and the relative number of workers in each job category are not indicated in the report.

Community Studies

Formal epidemiologic investigations designed to test specific hypotheses concerning MTBE added to gasoline and specific human health or human comfort endpoints have not been undertaken. The majority of the community studies were conducted in response to the public concerns that followed the widespread introduction of oxyfuel during the winter of 1992/1993, and the introduction of RFG during the winter of early 1995. The studies were not designed to elucidate underlying mechanisms or to specifically test the plausible alternatives of direct versus odor-triggered toxicity. Table 15 describes this set of primarily cross-sectional studies. These studies were conducted with little advance planning because of time constraints and

were limited in scope by funding. Generally considered to be pilot studies, they were to be followed by more definitive studies. The studies compared the prevalence of self-reported symptoms across groups that were assumed to be exposed to different levels of gasoline exhaust, vapors, or both. The study populations consisted of convenience samples of individuals selected by their occupation and ease of recruitment, or randomly selected by telephone. In all studies, the subjects were asked to complete a questionnaire on health symptoms inquiring about the frequency and severity of the symptoms and the situations in which the symptoms occurred.

The list of symptoms used in most of these studies was first developed by medical epidemiologists from the State of Alaska Department of Health and Social Services, Division of Public Health, and the National Center for Environmental Health of the CDC. It was based on interviews with persons who reported being ill, on reports of symptoms to the Fairbanks mayor's office or the citizen telephone hotline, and on the symptoms that might be expected to occur as a result of exposure to gasoline emissions (J. Middaugh, personal communication, 1995). The symptoms were eye irritation, burning sensation in the nose or throat, headache, nausea or vomiting, cough, a sensation of "spaciness" or disorientation, and dizziness (Moolenaar et al. 1994). A number of studies have labeled these symptoms as "key health complaints." Symptoms assumed not likely to be attributable to MTBE, such as fatigue, fever, sweats or chills, diarrhea, fainting or blackout spells, skin irritation or redness, muscle aches, and difficulty breathing, were included in some of the questionnaires to control for symptoms of viral illnesses and also as a negative control (Moolenaar et al. 1994).

Table 16 indicates the composition of the populations studied and summarizes the prevalence of these symptoms in all the community studies. In the first two Alaska studies (Beller et al. 1992; Chandler and Middaugh 1993), a case definition that excluded individuals with symptoms likely to be due to viral illness was used. In the other community studies, the prevalences of one or more individual key symptoms were compared across exposure groups. In the Wisconsin study, the prevalences of all symptoms were combined. Because of differences in the way questions were asked and in the times of the year of these studies, quantitative comparisons of the symptom prevalences across the studies are not meaningful.

Exposure assessment in most studies was limited. In a few studies, measures of MTBE in the microenvironment or in the personal breathing zone were obtained for a subgroup of the study population. In other studies, the investigators classified exposure on the basis of job description, the time spent in a situation where gasoline was used or in a vehicle, or the use of a certain type of gasoline. Table 17 summarizes the MTBE exposure data and the range and median levels of MTBE. MTBE was measurable in occupational settings, at service stations, and inside cars. Mechanics and service station attendants had, on average, the highest exposures, but the personal breathing zone levels were highly variable (ranging from 0.2 to 50 ppm for mechanics and from 0.2 to 70 ppm, for service station attendants in one study) (White et al. 1995). MTBE was detected in the blood of commuters, mechanics, and service station attendants. The MTBE concentrations in the blood samples correlated with the MTBE concentrations measured in the breathing zone or in the microenvironment.

Alaska Studies The first community studies were conducted in Alaska at the beginning of the first oxyfuel season shortly after the outbreak of complaints (December 1992). Middaugh and coworkers conducted two cross-sectional investigations, one in Fairbanks (n = 203) (Beller et al. 1992) and one in Anchorage (n = 162) (Chandler and Middaugh 1993), comparing the symptom prevalences among individuals from different occupations who spent different amounts of time in motor vehicles (taxi drivers, workers in neighborhood health clinics, workers in hospitals, or students). Subjects were considered to have symptoms potentially related to oxyfuel exposure if they had an increase in headaches, or if they had two other complaints such as nausea, burning of the nose or mouth, cough, dizziness, or spaciness. Individuals who also reported diarrhea, fever, sweats or chills, or muscle ache were not included. The symptom prevalences in the different groups from Fairbanks and Anchorage are summarized in Table 16. The highest prevalence of cases was found among taxi drivers, considered to have the highest MTBE exposures, and the lowest among students (assumed to be the least-exposed individuals). More taxi drivers in Anchorage reported symptoms than in Fairbanks (46% versus 33%, respectively). For all groups except students, more people reported symptoms while traveling than while refueling (see Table 18).

In the winter of 1992/1993, a longitudinal study was also conducted in Fairbanks by the State of Alaska Department of Health and Social Services and the CDC (Centers for Disease Control and Prevention 1993b; Moolenaar et al. 1994). This study involved two different populations: randomly selected residents of Fairbanks, who were surveyed by telephone, and occupationally exposed workers (10 mechanics and 8 individuals who spent the day in motor vehicles), who answered a symptom questionnaire at the end of a workday (see Table 15 for details about administration of the questionnaire). The study was conducted in two

phases: during the period when oxyfuel was being used (December 1992, Phase I), and during the 2-month period after the program had been discontinued (February 1993, Phase II). A limited number of blood samples was obtained during both phases at the beginning and end of a work shift or a 1-hour commute. Workers interviewed in Phase I had a higher prevalence of symptoms than workers interviewed in Phase II, as did individuals sampled in the telephone surveys. With the exception of a few workers, different people were surveyed during the two phases. Blood levels of MTBE in December were significantly increased at the end of the shift or after a 1-hour commute and were higher than those measured in February, indicating greater exposure to MTBE during Phase I. Workers had a higher prevalence of symptoms than any of the groups previously studied in Alaska (see Table 16). For example, the prevalence of headache was 67% in the workers recruited by Moolenaar and coworkers (1994), and 25% and 44% in taxi drivers studied by Middaugh and coworkers in Fairbanks and Anchorage, respectively. Blood MTBE levels (postshift minus preshift) were proportional to workplace levels of MTBE. Workers with MTBE blood levels in the upper quartile (> 9.6 µg/L) were more likely to report one or more symptoms of MTBE exposure than workers with lower blood levels, but this difference was statistically unstable (odds ratio = 2.2, calculated using an adjustment for small sample size, 95% CI = 0.1, 49) (see Table 17).

Studies in Connecticut, New York, and New Jersey The results of Phase I of the Fairbanks study (Moolenaar et al. 1994) can be compared with the results of a similar study conducted in Stamford, CT, in April 1993 (CDC 1993a; White et al. 1995), at a time when oxyfuel containing MTBE was still being used (see Tables 16 and 17). The two studies were conducted in different seasons and also differed because of the absence of media attention in Stamford as compared with the high degree of publicity in Fairbanks. The Stamford study population included workers exposed occupationally to MTBE (mechanics, service station attendants, people who spent time in traffic) and commuters. Personal breathing zone air samples and blood samples were collected to measure MTBE in a subset of workers and commuters. The highest MTBE exposures and blood levels were measured in three service station attendants (who had a median MTBE blood level of 15.2 µg/L). The median MTBE blood level in mechanics (1.7 µg/L) was similar to that in workers in the same job in Fairbanks (1.8 µg/L), but the Stamford workers had a lower symptom prevalence. The levels of MTBE in the breathing zone were associated with levels of MTBE and TBA in the blood.

The Stamford study offered two findings: (1) exposure classification based on occupation served as a poor measure of MTBE exposure; and (2) actual blood levels of MTBE were related to symptom prevalence. Individuals from all groups with blood levels in the upper quartile were more likely to report symptoms than individuals with lower blood levels. Subjects in the upper quartile had blood levels higher than 2.4 µg/L when the commuters were included in the analysis and higher than 3.8 µg/L when they were excluded. These levels are substantially lower than the upper quartile in Fairbanks (higher than 9.6 µg/L).

A comparison population for the Stamford population with similar socioeconomic characteristics was selected in Albany, NY, where oxyfuel was not being used (Centers for Disease Control and Prevention 1993c). As shown in Table 17, MTBE concentrations in ambient air and blood were very low. The prevalence of symptoms was similar for the groups studied: garage workers and service station attendants, workers who spent time in traffic or garages, and office workers. Although some symptoms were more prevalent in Stamford than in Albany, the pattern of elevation was not consistent. In addition, some symptoms not thought to be related to MTBE, such as diarrhea and muscle aches, were also more prevalent in Stamford than in Albany. Subjects who reported a cold, flu, or allergies in the past month were more likely to report the presence of a key symptom.

In New Jersey, workers from state-operated garages in areas using oxyfuel containing MTBE (northern New Jersey) were compared with those in areas where oxyfuel was no longer used (southern New Jersey) (Mohr et al. 1994). The participation rate was 79% in northern New Jersey and 81% in southern New Jersey. The exposure assessment was based on passive sampler measurements over a period of 3 days on a subsample of workers (11% of the study population in the north and 3% of the study population in the south). Workers in the south were generally exposed to MTBE concentrations lower than 0.8 ppm, and workers in the north were frequently exposed to concentrations between 0.8 and 6 ppm. Symptom reporting by the two groups of workers was similar. In a separate analysis, 11 workers from the north who pumped gasoline more than 5 hours/day were matched with 11 similar workers from the south who had low or nondetectable exposures to MTBE. There were no differences between the groups in symptom prevalence.

As with many environmental pollutants, there is likely to be a range of sensitivity to MTBE. One study has investigated the response of a small group of potentially sensitive individuals, with multiple chemical sensitivities or chronic fatigue syndrome (Fiedler et al. 1994). These individuals reported significantly more symptoms (both MTBE-related and non-MTBE-related) than the control group, and these

symptoms were not confined to situations in which MTBE was present. Further efforts to identify and characterize sensitive individuals are needed.

Wisconsin Studies In 1995, the Wisconsin Department of Health and Social Services conducted two studies to investigate public concerns regarding the safety of RFG (Anderson et al. 1995a,b). The first study consisted of a random-digit-dial telephone survey of individuals in two areas using RFG (Milwaukee, WI, and Chicago, IL) and in an area not using RFG (the remainder of Wisconsin). The investigators reported that about half of the RFG sold in Milwaukee and Chicago contained MTBE; the other half contained ETBE or ethanol. However, this conclusion was based on very limited measurements of MTBE in the gasoline of some service stations. Awareness of RFG (defined as having heard of MTBE) in the three areas varied substantially, with individuals in Milwaukee being the most aware (54%), followed by individuals in the rest of Wisconsin (40%), and then by individuals in Chicago (23%). The participation rate in the survey also was different among the areas: 74% in Milwaukee, 74% in the remainder of Wisconsin, and 42% in Chicago (Table 19). Exposure to RFG was estimated by using the location of the subject's residence and car ownership. Thus, individuals who owned cars were assumed to be exposed to RFG if living in Chicago or Milwaukee and not to be exposed if living in the rest of Wisconsin. The investigators also attempted to estimate individual exposure to RFG or to a specific oxygenate based on the self-reported information about whether the subject bought specific brands of gasoline for which the researchers had measured MTBE levels, or whether the gasoline purchased had been labeled as containing MTBE or ethanol or as being RFG. However, the majority of the Chicago residents could not assess their exposure on the basis of these criteria, and risk ratios of individual symptoms by type of exposure could not be calculated.

As in the earlier community studies, this study analyzed the frequency of self-reported symptoms. However, two major methodologic differences must be noted relative to previous studies. First, individuals were initially asked whether they had experienced unusual symptoms in the previous months, and only in the event of a positive answer were they asked about the frequency of specific symptoms. This approach may explain the lower symptom prevalence, independent of the type of gasoline, relative to prevalences reported in most previous studies (with the exception of Phase II of the study in Fairbanks). In addition, interpretation of the questions concerning "unusual symptoms" may have varied in the different regions. Second, in the data analysis, the investigators considered all unusual symptoms, including many that had been excluded in other studies because they were thought not to be associated with MTBE (such as diarrhea, muscle ache, rashes, and sinus congestion).

The symptom prevalence data, which are summarized in Tables 16 and 19, show that the prevalence of all symptoms categorized as unusual was significantly higher in Milwaukee (23%) than in the other two areas (6% in both Chicago and Wisconsin). In both Milwaukee and Chicago, people who reported buying RFG in the previous months also reported more unusual symptoms (relative risk 2.8 and 2.3). However, residents of Milwaukee were more likely to report eye irritation, headache, or sinus problems while pumping gas or driving as compared with respondents in Chicago and the remainder of Wisconsin. These differences are likely to be attributable to different levels of awareness about exposure to MTBE. A comparison less likely to be contaminated by the effects of media publicity is between respondents from Chicago and those from Wisconsin (outside Milwaukee). Although the absolute numbers were small, respondents in Chicago (which used RFG) reported a slightly higher prevalence of eye irritation, headache, and sinus problems while pumping gas or driving than the respondents from Wisconsin (outside Milwaukee). The investigators also assessed the risk factors associated with symptom reports; for Milwaukee residents, the most consistent predictor of risk was report of a cold or flu, suggesting that such illnesses may have confounded these results. Because overall symptom prevalence in Chicago (where RFG was in use) did not differ from that in Wisconsin (where RFG was not in use), the investigators concluded that factors other than RFG contributed to the higher symptom prevalence in Milwaukee.

Overall, this cross-sectional study requires cautious interpretation. The participation rate was lower in Chicago (42%) than in Milwaukee (74%) and the rest of Wisconsin (74%). Using Chicago as a comparison population for Milwaukee may not be appropriate because the two areas also differed in their awareness of RFG. Similarly, comparisons of the rest of Wisconsin with Milwaukee or Chicago could have been confounded by rural-urban differences in factors that affect questionnaire responses in general. The media publicity alleging major adverse health effects from oxygenate fuel additives may have influenced the reporting of symptoms. Residents of Milwaukee may also have been sensitized to possible new health hazards from the environment because 2 years earlier they had experienced a widespread outbreak of *Cryptosporidium* infection transmitted through the public water supply, which had affected about 25% of the population (MacKenzie et al. 1994).

The Oxygenates Evaluation Committee concurs with the investigators' conclusions and the statement of the scien-

tific panel that reviewed this study that "the study does not support a conclusion that exposure to RFG is associated with widespread or serious acute health effects," and that the study "does not rule out subtle effects or the possibility that some individuals may have greater sensitivity to RFG mixtures." However, problems with the structure of the questionnaire, the choice of the control population, and lack of actual measures of personal exposures preclude drawing any firm conclusions regarding symptom prevalence and RFG.The other study conducted in Wisconsin consisted of interviews with individuals who had initially reported health complaints to government agencies in Milwaukee (Anderson et al. 1995b). The goal of this study was to describe the range of complaints and to identify factors that may be predictive of symptom occurrence. The total number of Milwaukee residents interviewed was 1,280. This group had a higher median age and a much higher prevalence of symptoms (71%) than the Milwaukee residents interviewed in the random-digit-dial telephone survey (23%). For this study population, the strongest predictors were age, having allergies, and having a cold or flu. Exposure to RFG, determined by self-reported information on the type of gasoline purchased, was not a predictor of symptoms.

Summary of Community Studies Evidence on the health effects of exposure to MTBE in the community setting is limited and largely derived from cross-sectional studies conducted with few resources. Some of these studies, for example, those in Alaska, New Jersey, and Wisconsin, were performed in response to outbreaks of complaints concerning symptoms after MTBE in gasoline had been introduced; in contrast, the studies in Connecticut and in New Jersey were carried out at times when outbreaks of complaints were not occurring.

General limitations of the community studies largely reflect the exigencies of the circumstances under which they were performed. The sampling strategies were often dictated by feasibility considerations; for example, the occupational groups were selected on a convenience basis rather than by random sampling. Only a few studies, the telephone surveys conducted by the Wisconsin Department of Health in Wisconsin and by the State of Alaska Department of Health and Social Services and the CDC in Fairbanks and the community study conducted by Mohr and coworkers in New Jersey, incorporated a formal design for sampling participants. Sample sizes were small in many studies, and in all studies statistical power was not adequate for outcomes of interest at effect levels of public health concern. In all studies data were collected by questionnaires based on self-reported symptoms and illnesses. The pattern of symptoms first reported in Alaska guided the development of questionnaires for subsequent studies; adopting the Alaska symptoms as a starting point for most subsequent community studies (with the exception of the Wisconsin study) may have limited the characterization of responses to MTBE. Finally, the Wisconsin study emphasized symptom patterns considered to be "unusual" by the respondents, an approach that may have led participants not to report increases in common symptoms that often occur chronically such as headache or eye irritation. Sensitization of communities to the presence of a new form of gasoline also may have contributed to the symptom outbreaks, perhaps by heightening public concern and awareness of a new gasoline with an unfamiliar unpleasant odor. Moreover, the studies did not use appropriate statistical methods for analyzing outcomes that may not have been independent.

The studies also were limited in assessing exposures of the participants: some studies incorporated presumed gradients of exposure based on exposure surrogates (such as job description, time spent in a certain situation, or types of gasoline used), but concurrent measurements of MTBE levels in the personal breathing zone or blood were limited.

In spite of these limitations, some of the community studies document symptom outbreaks in locations where MTBE was used in oxyfuel or RFG. Some studies suggest, although others do not, that exposure to MTBE in gasoline is associated with a higher prevalence of symptoms, as documented by limited measurements of blood levels or by presumed levels of exposure. The symptoms were generally mild and of short duration; no evidence of associated clinical morbidity has appeared, although such outcomes were not specifically addressed. In a sample of 18 exposed workers in Fairbanks, symptoms were more common in people with MTBE blood levels in the top quartile than in subjects with lower blood levels (the findings were statistically unstable due to the small sample size, CI = 0.1, 49). The same pattern of response was evident in the Stamford, CT, study (odds ratio 8.9 relative to people with lower blood levels; 95% CI = 1.2, 76), in spite of much lower upper-quartile MTBE blood concentrations compared with those in the Fairbanks study. In the Stamford study, frequent headaches and dizziness also occurred more commonly in two occupational groups with presumably higher exposure levels than commuters. However, most other key symptoms based on the Fairbanks criteria were not associated with exposure. In New Jersey, workers from an area using oxyfuel were not more likely to report symptoms than workers from an area without oxyfuel. In the Fairbanks 1992/1993 study, the prevalence of symptoms reported by workers exposed occupationally was substantially higher than in the other studies during the period of oxyfuel use, and substantially

lower after oxyfuel was removed; in the Wisconsin study the prevalence of symptoms was lower than in all the other studies in all three population groups (see Table 16).

By themselves, the studies described here do not provide definitive evidence for an association between exposure to MTBE and symptoms, nor do they provide insight into the mechanisms that could produce the reported symptoms. They do provide an imperative for further research and offer a starting point for designing more informative studies. Well-designed prospective studies, capable of detecting changes in symptom prevalence associated with MTBE, should be conducted in populations exposed to oxygenates. Among the mechanisms to investigate are those by which odors may trigger symptoms, whether by psychophysical mechanisms or by direct irritation. Also to be considered is the possibility that MTBE exacerbates the effects of other health factors. Individuals with preexisting respiratory health conditions or allergies and older people are among the groups who may be more sensitive. Exposure to MTBE is coincidental with exposure to other components of gasoline, and comparing the consequences of exposure to two complex mixtures, MTBE-enriched gasoline and gasoline without MTBE, challenges the capabilities of epidemiologic methods.

In summary, although more thorough research is needed, these studies provide an indication that some individuals exposed to emissions from automotive gasoline containing MTBE may experience acute symptoms such as headache or eye and nose irritation.

Controlled Exposure Studies

Three controlled human exposure studies were carried out (Prah et al. 1994; Johanson et al. 1995; Cain et al. 1996). The goal of these studies was to determine whether exposure to MTBE alone caused an increase in self-reported symptoms and in more objective measures of effects on the eyes, the nose, and the CNS and to characterize the disposition of MTBE and TBA in humans (see Table 19 for details on the measurements made in each study and their conclusions). Two studies were conducted with very similar protocols and involved exposing healthy human volunteers to a single concentration of MTBE vapors, 1.7 ppm in the study by Cain and coworkers (1996) and 1.4 ppm in the study by Prah and coworkers (1994), for 1 hour. In the third study, subjects were exposed to 5, 25, or 50 ppm MTBE for 2 hours (Johanson et al. 1995). In the Cain study, the subjects were exposed separately to a mixture of volatile organic compounds (VOCs) (at a concentration of 7.1 ppm), which was intended to approximate exposure to gasoline vapor.

Subjects exposed to MTBE did not report symptoms more frequently than under control conditions in any of the three studies, although in all three studies some subjects were able to detect an odor when exposed to MTBE. In the studies by Cain and coworkers and by Prah and coworkers, women were more likely than men to note greater odor intensity and to rate the air quality as worse during exposure to MTBE (and to VOCs) than to clean air. No effects on the measures of eye or nose irritation or on CNS function were observed. Exposure to VOCs led to positive effects on the number of polymorphonuclear neutrophilic leukocytes (PMNs) in nasal lavage fluids sampled on a delayed basis. The study by Johanson and coworkers (1995) did not include neurobehavioral assessments, although they used higher levels of MTBE than other investigators and an exposure period of 2 hours.

In all three studies, the blood levels of MTBE measured at the end of the exposure were much higher than the upper-quartile levels measured in workers and commuters in Stamford (Table 20).

In summary, the controlled exposure studies have failed to demonstrate either subjective symptoms (other than an air quality rating of "poor") or objective measures of eye or nose irritation and CNS dysfunction in healthy young adults exposed to MTBE vapor alone, at levels higher than those reported in the community studies. However, these studies used only healthy volunteers and did not examine exposure to MTBE as a component of a gasoline mixture, which limits the interpretation of their findings. In each of these studies, subjects were exposed to MTBE alone. Under actual conditions, however, MTBE exposure usually occurs in conjunction with exposure to gasoline vapor and combustion emissions, which are a complex mixture, and exposure is repetitive. A hydrocarbon mixture simulating gasoline did produce a delayed minor inflammatory reaction in the upper airways after exposure. Thus, on the basis of these three studies and barring a distinct supraadditive effect, one would expect vapor from gasoline containing MTBE to produce, at most, modest symptoms of irritation or inflammatory changes that do not differ significantly from those produced by gasoline vapor alone. This hypothesis, however, has not yet been studied directly.

Therapeutic Use of MTBE

Occasionally in clinical settings, MTBE has been used for the therapeutic treatment of gallstones. Patients were usually administered MTBE at doses ranging from 0.01 to 0.2 g/kg body weight through a catheter inserted either in the bile duct or the gallbladder. The duration of the infusion varied from 1 to 30 minutes. The dose actually absorbed by the patients was not determined. The patients were re-

ported to display transient side effects of perspiration, hypotension, bradycardia, sedation, and an elevation of liver enzymes (Costantini 1993). These effects may be of concern if MTBE were ingested through contaminated drinking water at sufficient levels and for prolonged periods of time.

Effects of Ethers on Odor Thresholds

Several studies were conducted to evaluate the effects of ethers on the odor threshold of gasoline (American Petroleum Institute 1993a,b; TRC Environmental Corporation 1993; Smith et al. 1994). All studies involved presenting to a panel of subjects a series of dilutions of vapor samples of gasoline, gasoline with an ether, or ether. Each dilution was presented to the panelists in a glass funnel in conjunction with two other funnels from which filtered air flowed. The detection threshold was the concentration at which a panelist was capable of determining the difference between the sample and filtered air; the recognition threshold, as defined by the authors, was the concentration at which a panelist was capable of rating the intensity of the odor relative to butanol. The study by Smith and coworkers primarily evaluated temperature effects on gasoline with and without MTBE; one study by the API (1993a) compared the effects of various ethers. The other two studies focused on ETBE and TAME. Except for the studies at low temperatures, which were conducted at the University of Alaska in Fairbanks, all the other studies were conducted by TRC Environmental Corporation.

Effects of MTBE on the Hedonic Rating of Gasoline The hedonic rating measures the pleasantness of an odor: 1 = very pleasant and 9 = extremely unpleasant. Adding MTBE did not significantly affect the hedonic rating of two different gasoline blends (Mapco and API) at any of three low temperatures tested: +25°F, 0°F, and −25°F (Smith et al. 1994). Without MTBE, the API blend had a slightly higher rating (more unpleasant odor) than the Mapco gasoline (ratings ranged from 5.9 to 6.3 for the Mapco blend and from 6.5 to 6.6 for the API blend). (Mapco gasoline is one of the two gasoline blends sold in Alaska; it contains a greater proportion of aromatics and a lower proportion of low molecular weight alkanes and alkenes than the API blend.)

Effects of Temperature on MTBE Concentration in the Headspace No significant differences were found in the headspace MTBE concentration in two blends of gasoline (Mapco and API) containing 15% MTBE by volume as a function of temperature (Table 21); however, the percentage of MTBE in the headspace of the Mapco blend was overall lower than that observed in the API blend (Smith et al. 1994).

Effects of Temperature on the Odor Threshold With and Without MTBE The odor thresholds for two blends of gasoline (API and Mapco) alone and with 15% MTBE were compared at temperatures of −24°F, 0°F, and +25°F (Smith et al. 1994). No consistent temperature-related effects were observed within this temperature range (see Table 22). A blend of the Amoco, Exxon, and Atlantic gasolines had lower odor thresholds at 75°F than the average odor threshold of the API gasoline at the three low temperatures (0.43 and 1.01 ppm, respectively, for the detection threshold, and 0.97 and 1.7 ppm, respectively, for the recognition threshold). Adding MTBE had a greater effect in lowering both the detection and recognition odor thresholds at 75°F than at the lower temperatures (−67% change in both thresholds when MTBE was added to the blend of Amoco, Exxon, and Atlantic gasolines versus a −10% to −40% change when MTBE was added to the API blend at −24°F to +25°F). Because the two blends differed in composition, it is not clear that this difference is due to an effect of temperature (no difference was seen in the percentage of change in thresholds between Mapco gasoline at low temperatures and at 75°F).

Effects of MTBE on the Odor Thresholds of Different Gasoline Blends Among the various blends of gasoline tested, the two Alaskan blends, Mapco and Tesoro, had the highest odor threshold, indicating that they were the least odorous (see Tables 22 and 23). However, adding MTBE caused a greater reduction in both detection and recognition thresholds for the Alaska blends than it did for the other blends. After adding MTBE, the odor thresholds for the Alaska gasolines were lower than the corresponding thresholds for the other gasoline blends, indicating they had become more odorous when MTBE was added. MTBE of higher purity caused a greater reduction than MTBE of lower purity; and the amount of reduction in the odor threshold of gasoline was dependent on the amount of MTBE added.

Effects of MTBE, ETBE, and TAME on the Odor Threshold Gasoline blends defined as winter or summer were tested to determine the effect of these three ethers on the odor thresholds (American Petroleum Institute 1993a,b; TRC Environmental 1993; Smith et al. 1994). The winter blends had a higher vapor pressure than the summer blends because short-chain alkenes had been added to them. Adding MTBE, ETBE, or TAME to gasoline consistently decreased both the detection and recognition odor thresholds of the gasoline blends tested (see Table 23). Using equal concentrations of ether, the greatest reduction in both detection and recognition thresholds was observed when ETBE was added to the two Alaska gasolines.

Summary of Effects of Ethers on Odor Thresholds The odor studies described above were conducted using small groups of well-trained and experienced individuals. Although the individuals were chosen to reflect a distribution of olfactory sensitivity representative of the general population, their reactions as experienced panelists may be different from those of the general population. Nevertheless, these results indicate the relative odor thresholds of different compounds and mixtures, and provide information that can aid in interpreting the community studies.

The ethers used in the studies described above have high vapor pressures, and their odor is detectable at very low concentrations. These ethers were shown to have odor thresholds approximately 2 orders of magnitude lower than those of various gasoline blends. All the gasoline blends tested in these studies had similar odor thresholds except for the two Alaska blends, which had higher odor thresholds by themselves.

Adding MTBE, ETBE, or TAME caused a substantial reduction (ranging between 50% and 90%) in both odor detection and recognition thresholds of all gasoline blends. Thus, any gasoline containing 10% to 15% of an ether is more odorous than the same gasoline without the ether. When MTBE was added to the Alaska gasoline, it caused a greater reduction in the odor threshold (it became more odorous) than for any other gasoline. This phenomenon may help explain the higher symptom prevalence in the Fairbanks workers, if such symptoms are triggered by a reaction to odor. The effect of MTBE in reducing the odor threshold of gasoline did not appear to be dependent on temperature, and temperature did not affect the headspace concentration of MTBE.

ETHANOL

As with MTBE, environmental levels of ethanol vapor stemming from its use as a component of gasoline are relatively low. At such levels, the primary question for adult exposure is the degree to which it might impair performance, which has been shown to be affected after ingestion of alcoholic beverages. Thus, this section will focus on the neurotoxic effects of ethanol.

ANIMAL STUDIES

As in humans, an abundant literature indicates that the endpoints most sensitive to acute ethanol exposure in animals lie in the realm of CNS function. The levels of exposure or dose and their effects on laboratory animals are summarized below by type of behavior tested and are also presented in Table 24.

Naturalistic Behaviors

Motor Activity Simple measures of motor activity in rats and mice are commonplace in neurotoxicity screening and were used to assay ethanol as well as MTBE. The most common finding after a single exposure has been an elevation of spontaneous locomotor activity at low doses of ethanol (0.1 to 0.5 g/kg in rats and 1 to 2 g/kg in mice) and a diminution at high doses (1 g/kg in rats and 15.5 to 3 g/kg in mice) (Middaugh et al. 1992; Moore et al. 1993; Criswell et al. 1994). MTBE exhibited a dose-response pattern similar to that of ethanol (Gill 1989); that is, the lowest concentration tested (800 ppm) tended to increase spontaneous motor activity, and a higher concentration (8,000 ppm) tended to suppress it.

Coordination Ataxia and other indications of motor impairment are a familiar accompaniment of alcohol intoxication in humans. Similar reactions are evoked in animals. Le and Israel (1994) assessed coordination by requiring rats to maintain their balance on an oscillating wooden bar. Gavage doses of 0.5 to 1.5 g/kg impaired performance. In dogs ataxia was observed after administration of 1 or 2 g/kg by gavage (Weiss and Laties 1964).

Aggression Under certain circumstances, animals will attack others of the same species. Weerts and coworkers (1993) examined such responses in squirrel monkeys (*Saimiri sciurea*) and rats. Dominant male squirrel monkeys were tested by pairing them with males from a different social group. Low doses of alcohol in the range of 0.1 to 0.3 g/kg increased aggressive behaviors. Higher doses (1 to 3 g/kg) decreased such behaviors and also produced coordination deficits. The monkeys were tested by introducing a male "intruder" into the home cage. Similar dose-related effects appeared in the rats.

Operant Behaviors

Operant behavior, or schedule-controlled operant behavior, is a primary approach to the evaluation of complex performance owing to its versatility and its ability to provide a "predictive explanation of behavior" (Iversen and Iversen 1981).

A schedule of reinforcement describes a relation between responses by the subject, such as lever-presses by a rat, and their consequences, such as the delivery of food pellets. Moser and Balster (1985, 1986) exposed trained mice to various concentrations of ethanol vapor, measured their ability to press a lever that would yield a water or milk reward, and measured their response on two different schedules. In the first study (Moser and Balster 1985), 100 lever-presses had to be emitted to obtain a small amount of water (fixed-ratio-100 schedule); the rate of responding (number of lever-presses) fell significantly at 20,000 ppm.

For comparison, Middaugh and coworkers (1992) observed reduced rates in mice on a fixed-ratio-20 schedule after exposure to 1 to 3 g/kg. In the other study Moser and Balster (1986) tested rats on a fixed-interval-60-second schedule (meaning that lever would yield milk delivery 1 minute after the previous delivery); the typical pattern of responding consisted of suppression of lever-pressing at the beginning of the session succeeded by elevated rates of lever-pressing later in the session. Such a pattern was noted beginning with the lowest concentration tested, 12,000 ppm, but a statistically significant effect was noted only at the the highest concentrations, 36,000 and 48,000 (inhalation) and 2 g/kg (gavage). At a dose of 4 g/kg, the response rate was suppressed during the whole duration of the session.

Results such as these are typically interpreted as indicating that both responsiveness to environmental contingencies and discriminative processes have been altered. The effects can take other forms. Laties and Weiss (1962) administered alcohol to rats trained to space lever-press responses by a minimum interval of 20 seconds. Doses from 0.25 to 1.0 g/kg progressively lowered response rates, primarily by the intrusion of long intervals of more than 40 seconds. An experiment with dogs (Weiss and Laties 1964) required the subjects to accumulate 60 seconds of responses by pressing their snouts against a panel to obtain a food reward. From 0.5 to 2.0 g/kg, a dose-dependent decrease in the response duration within the 60-second interval was observed; this also meant that the number of responses for each food reward rose correspondingly. Avoidance of aversive electric shock by lever-pressing is another performance assay. In an experiment in rats by Galizio and coworkers (1984), a dose of 1.5 g/kg reduced the avoidance response rate and led to more shocks.

Impairment of memory function is another indicator of neurotoxicity. Melia and coworkers (1990) tested the ability of rats to recall the site of a previous lever-press (left or right) after an imposed delay. Such a scheme is known as a delayed-matching-to-position paradigm. A dose of 0.25 g/kg reduced the rate of forgetting during the delay period, and doses above 0.5 g/kg accelerated the rate. In a variant of this paradigm, subjects were required to alternate response sites on each trial to obtain rewards; for example, right lever, left lever, right lever, etc. (Elsner et al. 1988). Ethanol doses of 0.25 to 1.0 g/kg administered intraperitoneally to rats produced a dose-dependent decrease in accuracy and an increase in response latency (or decrease in reaction time).

One variant of the delayed response is known as delayed match-to-sample, and requires the subject to respond to the same stimulus (for example, a geometric shape) presented before the delay. Geller and coworkers (1985) recorded slower response times, but no changes in accuracy of the response, in 2 of 4 baboons exposed to ethanol vapor concentrations of 20,000 ppm. A reward schedule termed fixed consecutive number also contains a memory component. As used by Doty and coworkers (1992), 13 responses had to be emitted on one of two levers to activate the second lever. A response on the second lever then delivered a food pellet. Doses of ethanol between 0.3 and 1.7 g/kg elicited decreases in response rates, but did not impair accuracy.

Fine motor control is seldom studied in animals. In fact, it is even a rarity in human studies. Newland and Weiss (1991) undertook an experiment to measure the effects of ethanol on tremor in squirrel monkeys. The study derived from evidence that ethanol diminished essential tremor, as determined clinically. The monkeys were trained to grip and to hold in position a bar attached to a lever that, in turn, was coupled to a rotary variable-displacement transducer that allowed small variations in position to be recorded. The positioning behavior was maintained by juice rewards. The monkeys received oral doses of ethanol at concentrations of 0.125, 0.25, 0.5, and 1.0 g/kg. The primary performance measure consisted of power spectra computed to yield distributions of power (variance) over tremor frequency. The lowest dose to modify the power spectrum was 0.25 g/kg. It reduced the size of the tremor modal frequency that lay between 8 and 10 Hz.

Summary of Animal Studies

Even with the brief review above, ethanol is seen to exert diverse types of CNS effects as revealed in behavior. The literature suggests a NOAEL for a dose of about 0.25 g/kg given orally. The data, however, are diminished in usefulness for risk assessment because they lack two features. First, blood alcohol concentrations are rarely reported, making interspecies extrapolation or even endpoint comparisons somewhat speculative tasks. Second, results tend to be reported in terms of statistically significant differences, but for dose-response relationships, it would prove far more useful to compute a measure, such as a 10% change in performance, which is the precursor to a benchmark dose calculation. If further experiments on MTBE are undertaken with the aim of expanding the information about neurobehavioral toxicity currently available, these defects in the ethanol literature should be addressed.

HUMAN STUDIES

Most of the data on the effects of ethanol in humans have been obtained after oral ingestion of relatively high doses of alcohol. Recently, the results of a community study of health symptoms were published (Egeland and Ingle 1995). The literature on alcohol effects in humans is summarized below.

Community Studies

A longitudinal study was conducted to evaluate the symptom prevalence in a group of Anchorage residents in the winter of 1994/1995 during the period in which oxyfuel containing ethanol was being used (Egeland and Ingle 1995). The study consisted of telephone surveys, conducted over a period from December 1994 to the end of March 1995, in which people were asked to report their symptoms in the past week. Symptoms had been selected based on those reported by Alaskan residents in 1992, when oxyfuel containing MTBE had been introduced, although it is not clear that ethanol exposure would result in the same set of symptoms as exposure to MTBE. The data obtained (prevalence of symptoms while driving or within hours of pumping gas) were grouped into three categories that corresponded to time periods when ethanol was not present, was being phased in or out of gasoline, or was present in all the gasoline sold. The results of the survey are reported in Table 25 together with those from a similar survey conducted in Fairbanks in 1992/1993 when oxyfuel contained MTBE (Centers for Disease Control and Prevention 1993b). For the Anchorage study, the prevalences of symptoms reported in the two situations were combined, although some individuals may have reported symptoms in both.

Because of differences in the survey questionnaire and the data analysis, comparisons between the two studies have limited value. The questionnaire administered to Anchorage residents in 1994/1995 inquired about symptoms that occurred during the previous week; the one administered to Fairbanks residents inquired about symptoms in the previous 6 to 8 weeks. More importantly, in the Anchorage study, individuals who reported illnesses as well as symptoms in the previous week (26% of the respondents) were excluded from the analysis. In the Fairbanks study, individuals were asked to report only symptoms that were not explained by a cold or flu, but individuals who reported symptoms possibly associated with colds and flu were not excluded from the analysis (see Table 17).

In general, it appears that the symptom prevalence in Anchorage at all ethanol phases was lower than that in Fairbanks after MTBE was removed from gasoline, suggesting that adding ethanol did not trigger health complaints. However, during the ethanol phase a higher percentage of people noted an unusual odor while pumping gasoline. It should be mentioned that the frequency of symptoms in the Anchorage study was higher while driving than within an hour of pumping gas. Similarly, in previous studies with oxyfuel containing MTBE, more people reported symptoms while driving than while refueling, suggesting that, independent of the type of gasoline, driving is a situation that triggers more complaints.

Experimental Studies

In humans, alcohol has been shown to influence numerous biological processes. Although modest doses (in the range of 1 to 2 g/kg) may affect upper airway reflexes (Erskine et al. 1994) and hemodynamics (Kelbaek et al. 1988), the most sensitive functional outcome of acute exposure is impaired performance. Because the combination of performance competence and alcohol is such an inflammatory social and legal topic, the research issues, despite an already vast literature, continue to attract experimenters. In addition, workers in neurotoxicology find ethanol an effective positive control, especially for agents such as organic solvents because they can calibrate the magnitude of solvent effects against the dose-response function for ethanol. For example, Echevarria and coworkers (1991) used the alcohol calibration strategy to evaluate the behavioral toxicity of toluene. They chose toluene concentrations of 0, 75, and 150 ppm, with an exposure period of 7 hours, to bracket the then prevailing TLV of 100 ppm, and ethanol doses of 0, 0.33, and 0.66 g/kg to bracket the blood alcohol concentration of 50 mg% blood (or 500 mg/L) often alluded to as the lowest concentration associated with an increased rate of automobile accidents. Peak blood levels with the two alcohol doses were estimated at 30 and 63 mg%, respectively. At the higher dose, performance fell on the following neurobehavioral tests: digit span, symbol-digit substitution, finger tapping, target tracking, hand steadiness, and continuous performance. Most of the tests were administered while blood alcohol levels were rising; because of acute tolerance effects, this is generally the period during which test performance is degraded the most.

Simulation of automobile driving is a frequent choice for assessing performance deficits induced by alcohol, an understandable target given that alcohol is responsible for a huge proportion of accidents. West and coworkers (1993) studied subjects who drove with an observer. They administered doses of alcohol designed to achieve blood levels of 0, 25 (low), and 50 (moderate) mg%. The moderate dose increased the mean time taken to respond to traffic hazards from 2.5 to 3.2 seconds, but did not change driving speed. They speculated that part of the increased risk of accidents stemming from alcohol ingestion is attributable to a lengthened response time to relatively infrequent hazards. For comparison, the legal ethanol limit in blood when driving in most states is 100 mg%.

After reviewing the literature on alcohol and driving ability, Ferrara and coworkers (1994) concluded that investigators need sufficiently complex performance tests to

reveal the effects of low blood alcohol concentrations. Because most evaluations of performance impairment rely on a battery of relatively quick neuropsychological tests, they may tend to sacrifice depth of assay to breadth, which may not be the optimal strategy for estimating the consequences of exposure to low levels of ethanol.

The alcohol literature points to vigilance and monitoring as sensitive endpoints. In a review of alcohol studies based on automobile driving, Perrine (1973) concluded that the tasks most vulnerable to performance impairment were those requiring sustained attention. Moskowitz (1984) came to a similar conclusion. Although some authors distinguish between vigilance and attention, no clear distinction is possible given the diverse definitions of these terms. Vigilance often is applied to situations in which the subject is asked to detect the occurrence of infrequent signals; a sonar operator on a submarine is an example. Attention is often applied to situations in which the subject has to make choices in response to stimuli. Driving an automobile and piloting an airplane obviously contain elements of both. Koelega (1995) published an extensive review of the relationship between alcohol and vigilance. Two convincing conclusions emerge from this review. First, the potential for alcohol-related impairment cannot be viewed in isolation as simply a function of blood alcohol level. Alcohol and time of day strongly interact. Performance measures during the early morning hours seem to be more gravely depressed by alcohol than performance during the daylight hours. Second, Koelega (1995) argues that even blood levels as low 12 mg% may induce performance degradation under less than optimal conditions (such as low illumination).

CONCLUSIONS

ETHERS

Systemic effects were observed at concentrations of 3,000 ppm for MTBE, 4,000 ppm for ETBE, and 0.5 g/kg for TAME when administered to rats for a period of 4 weeks. These effects include an increase in the weight of livers, kidneys, and adrenal glands, and signs of ataxia and hypoactivity. Neurotoxic effects, primarily in the form of activity modification, were observed at 800 ppm MTBE. The effects of MTBE on activity are by themselves indicators of possible effects on important brain functions (Burbacher 1993) and raise the possibility that effects on more sensitive behavioral endpoints may be observed at lower doses; they support the classification of MTBE as a neurotoxicant. Little investigation of the potential neurotoxicity of other ethers has been carried out. For ETBE, minor effects were seen only in rats exposed to 4,000 ppm for 28 days (the highest concentration tested); however, activity, a more sensitive endpoint for MTBE, was not measured.

In rats, MTBE did not seem to be a strong sensory irritant, in terms of inhibiting respiratory function. Such inhibition was noted after exposure to 8,000 ppm, but not at lower concentrations. On the basis of this information and the Alarie model, a TLV for sensory irritation in humans was estimated to be 140 ppm MTBE (Tepper et al. 1994). Consistent with the study in rats showing that MTBE alone is a weak respiratory irritant, the controlled human exposure studies failed to document significant sensory irritation—either subjective or objective—from MTBE alone. Likewise, controlled human exposure studies failed to reproduce the symptoms of headaches and nausea reported in some community studies and did not show neurotoxic effects, but the simplified exposure scenario did not fully replicate the likely odor impact from gasoline containing MTBE or of climatic conditions such as temperature and humidity. In addition, it is not clear whether the controlled exposure studies had sufficient power to detect neurotoxic effects at the low concentrations tested even though the blood levels of MTBE in the subjects exposed in the chamber studies were substantially higher than those determined in subsamples of individuals in the community studies.

The community studies provided conflicting results on symptom prevalence among individuals when evaluations were based on presumed exposures to oxyfuel containing MTBE or to RFG containing MTBE, ethanol, or ETBE. On the one hand, two studies that measured MTBE blood levels (Moolenaar et al. 1994, for Fairbanks; White et al. 1995, for Stamford) confirmed that (1) people exposed to MTBE had increased MTBE blood levels, although the levels were highly variable; and (2) job title is poorly related to actual exposure as measured by MTBE blood levels. Furthermore, their results indicated that people with higher blood levels of MTBE were more likely to report symptoms. On the other hand, two groups of New Jersey state garage workers with substantially different MTBE exposures showed no difference in symptom prevalence or intensity. Furthermore, some studies, for example, those in Albany and Wisconsin, have shown that subjects who reported a cold, flu, or allergies were more likely to report symptoms independently of their exposure classification. Overall, the community studies, despite their limitations, raise the possibility of an association between exposure to MTBE and symptoms.

Ethers are odorous compounds that can be detected in air at very low concentrations. The odor detection thresholds are 53 ppb for MTBE, 13 ppb for ETBE, and 27 ppb for TAME (see Table 2 in the General Properties of Oxygenates

section). Adding MTBE at a concentration of 15% by volume dramatically lowered subjects' odor detection thresholds for gasoline (by 54% to 80%, depending on the type of gasoline); for ETBE, the effect was even more dramatic (89% reduction in odor threshold). The effect of MTBE in lowering the odor threshold was more pronounced in the Alaska gasolines than in other gasoline blends.

Ambient air contains many compounds. Formaldehyde, which often increases in automotive emissions as a result of adding MTBE to gasoline, is a known sensory irritant. It is also a metabolite of MTBE. A product of the atmospheric transformation of MTBE, *tert*-butyl formate, is also an irritant. Symptoms typical of acute formaldehyde exposure are among those found in the community studies in Fairbanks and other cities where the oxyfuel program had been implemented. Furthermore, some studies (not reviewed here) have shown that people may manifest symptoms, particularly headache and nausea, in response to odors as such (Shusterman et al. 1991; Shusterman 1992).

Overall, the available data suggest that most people do not experience unusual symptoms or significant acute medical consequences when inhaling MTBE in fuel, but some may experience acute symptoms under some circumstances. For other ethers, no conclusions can be drawn at this time.

The short-term effects of ingesting MTBE are difficult to predict, especially in the absence of data on possible levels of MTBE in drinking water.

ETHANOL

The research on ethanol provides a vast pool of data on which to base estimates of its potential health effects as a constituent of gasoline. The consequences of acute ingestion are overwhelmingly the result of its action on the CNS. They range from the recognized signs of intoxication such as slurred speech and ataxia, to subtle impairment of performance detectable only by neurobehavioral testing. As is typical of toxicants, sensitivity varies enormously among individuals even when body weight is accounted for. On the basis of the available literature, it can be projected that blood levels as low as 10 mg% (100 mg/L) may induce performance deficits in some people under some conditions. Functions such as vigilance and attention seem to be those most affected at low levels, but complex decision processes and similar high-level cognitive abilities have not been examined fully.

Although the animal literature is replete with behavioral data, rarely are they accompanied by blood concentrations, so that comparisons with human data remain elusive. A survey of this literature, however, indicates that oral ethanol doses of 0.25 g/kg are often sufficient to produce behavioral effects; by extrapolation, such doses are likely to yield blood levels in the vicinity of 30 mg% (300 mg/L). Consequently, the sensitivity to alcohol of laboratory animals studied under appropriate experimental conditions seems to be close to that of humans. The two behavioral studies of mice based on inhalation exposures showed some effects at concentrations beginning at about 12,000 ppm (although statistically significant differences were noted only at 36,000 and 48,000 ppm). In rats, Nelson and coworkers (1988) measured blood levels of 50 mg% (500 mg/L) after inhalation exposure to 16,000 ppm. If the parallels with humans are maintained under this route of exposure, humans would not be expected to incur adverse effects from ethanol vapors in oxyfuel because the increments in blood levels would be lower than endogenous levels.

In summary, there is a large difference between the lowest blood levels of ethanol at which neurotoxic effects have been reported in humans (10 mg%) and the predicted blood levels arising from inhalation of gasoline containing ethanol. In exposure scenarios encountered by the general public, it is unlikely that an increase in ethanol blood levels will be measurable. On the basis of one community survey of symptoms conducted in Alaska, it does not appear that ethanol-containing fuel causes an increase in prevalence of symptoms.

Table 13. Summary of the Effects of 28-Day Toxicity Studies with MTBE, ETBE, and TAME and Rats

	MTBE						ETBE			TAME		
Study investigators	Chun and Colleagues 1992			IIT Research Institute 1992			IIT Research Institute 1991			Daughtrey and Bird 1995		
Animal strain and exposure protocol	F344 rats, inhalation, 6 hours/day, 5 days/week			Sprague-Dawley rats, gavage, 5 days/week			Sprague-Dawley rats, inhalation, 6 hours/day, 5 days/week			Sprague-Dawley rats, gavage, 7 days/week		
Exposure level	400 ppm	3,000 ppm	8,000 ppm	0.09 g/kg	0.44 g/kg	1.75 g/kg	500 ppm	2,000 ppm	4,000 ppm	0.125 g/kg	0.5 g/kg	1 g/kg
Gross effects		Hypoactivity, ataxia, lack of startle reflex in M & F			Hypoactivity & ataxia in M; salivation in M & F				Sedation & ataxia in M & F			
Body weight gain			↓ In M & F	No effects			No effects					↓ in M
Organ weight[a] Kidney		↑ Absolute & relative in F	↑ Absolute in F & relative in M & F	↑ Relative in F	↑ Relative in M	↑ Relative in M & F			↑ Absolute in M		↑ Absolute & relative in M	
Adrenal gland		↑ Absolute & relative in F	↑ Absolute & relative in M & F			↑ Relative in M			↑ Absolute in M		↑ Absolute & relative in M	
Liver		↑ Absolute & relative in M & F	↑ Absolute & relative in M & F			↑ Relative in M & F		↑ Relative in F	↑ Absolute & relative in M & F			
Spleen			Å Absolute & relative in M & F									
Necropsy observations	No changes observed			No changes observed			No changes observed					
Histopathologic changes		↑ Protein accumulation and cell proliferation in kidney in M			Kidney lesions with hyaline droplets formation in M		No changes observed; cell proliferation and protein accumulation were not studied			No changes observed; cell proliferation and protein accumulation were not studied		
Hematologic changes			↑ Sodium in M			↑ Cholesterol level		↑ White blood cell count				↓ Serum glucose
Urine changes			↑ Volume ↑ pH	Not measured			Not measured			Not measured		

[a] The change in organ weight is presented as the absolute value, or the value relative to body weight.

Table 14. Comparison of the Neurotoxic Effects of MTBE and ETBE

	MTBE		ETBE
Study investigators	Gill 1989	Dodd and Kintigh 1989[a]	IIT Research Institute 1991
Concentrations	800, 4,000, or 8,000 ppm	800, 4,000, or 8,000 ppm	800, 2,000, or 4,000 ppm
Exposure duration	Single exposure, 6 hours	6 hours/day, 5 days/week, for 13 weeks	6 hours/day, 5 days/week, for 4 weeks
Activity (cumulative counts)	↑ at 800 ppm in M ↓ at 8,000 ppm in M	↑ at 800 & 4,000 ppm in F (8th week) ↑ at 4,000 ppm in F (13th week)	Not measured
Gait (ataxia and duckwalk)	At 4,000 ppm in F At 8,000 ppm in M & F	No effect	At 4,000 ppm
Hind leg splay	↑ at 8,000 ppm in M	No effect	Change in trend toward an ↑ at 4,000 ppm in M & F
Hind limb grip strength	↓ at 4,000 and 8,000 ppm in F	↓ at 4,000 ppm in M (4th week) and in M & F (13th week)	No effect
Treadmill	↓ at 8,000 ppm in M	No effect	Not measured
Body temperature	↓ at 4,000 ppm in F ↓ at 8,000 ppm in M & F	↑ at 8,000 ppm in M (1st week) ↑ at 4,000 and 8,000 ppm in F (13th week)	↓ at 4,000 ppm in M (5th day)

[a] Assessments were conducted on weekends when animals were not exposed to MTBE.

Table 15. Characteristics of the Designs of Field Studies

	When and How Symptom Questionnaire Was Administered	Period of Symptom Occurrence	Participation Rate	Type of Exposure Assessment	Concentration of MTBE in Gasoline
Fairbanks 1992[a]	December 7-8, 1992 by personal interviews	Between October 15 and the interview (generally 2 months)	Not reported	Based on type of job and number of hours spent in the car	15%
Anchorage[b]	December 16-18, 1992 by personal interviews	Between November 1 and the interview (generally 1.5 months)	Not reported	Based on type of job and number of hours spent in the car	15%
Fairbanks 1992/1993[c] Workers	Early December 1992 and February 1993 by personal interviews	Between October 1, 1992 and the interview (generally 2 months) and during the day of the interview for subjects who had blood drawn	25% of those surveyed responded	Samplers in the room where worker spent the most time or in motor vehicle; MTBE and TBA blood levels at the end of the shift	15%
General population	Early December 1992 and February 1993 by telephone survey	Between October 1, 1992 and the interview (generally 2 months)	Of those telephoned in December, 45% responded; of those telephoned in February, 63% responded, and 85% of those agreed to participate	Based on whether oxygenated gasoline was used	15%
Stamford[d]	April 1993 by personal interviews	For workers, in the month before the interview and during the day of the interview (at the end of the work shift); for commuters, after a 1-hour commute	Not reported	Breathing zone samples for 32 garage workers; MTBA & TBA blood levels in 27 garage workers, 3 service station attendants, and 14 commuters	15% for 90% of the gasoline
New Jersey[e]	April 1993 in northern NJ, May 1993 in southern NJ by questionnaires filled out by workers	In the month before and during the day of the interview	In the north 79%; in the south 81%	Ambient air sampling and personal samplers	15%
Albany[f]	May 1993 by personal interviews	In the month before and during the day of the interview	Not reported	Personal air samplers for 24 workers, ambient samplers at 10 outdoor locations and at 11 work sites for 7 to 10 hours	0% to 9.2% (median 1.5%) in regular gas, 0.5% to 6.4% (median 1.4%) in plus gas, 0.3% to 6.2% (median 1.9%) in super gas
Wisconsin-Chicago[g]	March 1995 by telephone survey	Between December 1, 1994 and the interview (generally 4 months)	Of the completed phone calls, 41% agreed to participate in Chicago, 76% in Milwaukee, and 74% in the remainder of Wisconsin	Based on living in an area with or without RFG	9% to 12%; authors state that approximately 50% of the gasoline contained MTBE and the remainder ethanol or ETBE

[a] Beller et al. 1992.

[b] Chandler and Middaugh 1993.

[c] Centers for Disease Control and Prevention 1993b; Moolenar et al. 1994.

[d] White et al. 1995.

[e] Mohr et al. 1994

[f] Centers for Disease Control and Prevention 1993c.

[g] Anderson et al. 1995a.

Table 16. Prevalence of Symptoms in Community Studies[a]

Study Population	*n*	Eye Irritation	Nose & Throat Burning	Headache	Nausea	Cough	Spaciness	Dizziness	Sleepiness	Diarrhea	Fainting	Fever	Skin Irritation	Sweats & Chills	Difficulty Breathing	Muscle Aches	Fatigue
Fairbanks 1992[b] Taxi drivers	12	8	0	25	25	8	8										
Health care workers	90	10	2	23	10	9	1										
University students	101	3	3	10	6	3	2										
Anchorage[c] Taxi drivers	25	32	20	44	20	16	16										
Health care workers	29	7	7	21	3	7	0										
Hospital employees	108	10	10	23	7	10	3										
Fairbanks 1992/1993[d] Service station attendants, mechanics, and drivers During oxyfuel use in 1992	18	67	50	72	33	28	33	44									
After oxyfuel use ended in 1993	28	7	0	4	4	0	0	0									
General population During oxyfuel use in 1992	41	37	29	34	15	22	12	15		15	5	5	17	10	2	5	10
After oxyfuel use ended in 1993	100	18	12	10	2	8	6	4		9	2	2	6	4	6	1	13
New Jersey[e] Service station attendants & mechanics North, during oxyfuel use	115	22		36	27	12			13	14		4		9		34	
South, after oxyfuel use had ended	122	27		39	35	17			24	20		4		8		35	

(Table continues next page)

Table 16. Prevalence of Symptoms in Community Studies[a]

Study Population	*n*	Eye Irritation	Nose & Throat Burning	Headache	Nausea	Cough	Spaciness	Dizziness	Sleepiness	Diarrhea	Fainting	Fever	Skin Irritation	Sweats & Chills	Difficulty Breathing	Muscle Aches	Fatigue
Stamford[f] Service station attendants & mechanics	48	21	15	27	2	15	10	6		8	0	0	13	2	10	21	15
Other workers (e.g., meter readers)	12	17	33	42	17	42	8	17		25	0	0	0	0	0	33	33
Commuters	59	19	15	25	0	15	3	2		9	0	2	3	3	9	17	24
Albany[g] Service station attendants & mechanics	34		6	21	6	15	0	10		6	0	0	9	6	0	15	18
Policemen, garage & toll booth workers	48		4	47	6	25	4	12		8	0	4	10	12	14	18	16
Students, office workers	182		13	24	8	20	7	3		12	2	2	9	7	14	20	24
Wisconsin-Chicago[h] Milwaukee	527	7	9	13	7		7	8		5		3	4		5	4	
Chicago	485	2	2	3	1		1	1		1		1	1		1	1	
Wisconsin	501	4	1	2	1		0	1		1		1	1		1	1	

[a] Symptom data are presented as percentages of subjects studied. The dark vertical rule after Sleepiness separates a group of key symptoms, thought to be associated with MTBE exposure, from other symptoms not likely to be attributable to MTBE. In the Wisconsin-Chicago study, all unexplainable symptoms were included in the analysis.

[b] Beller et al. 1992.

[c] Chandler and Middaugh 1993.

[d] Centers for Disease Control and Prevention 1993b; Moolenaar et al. 1994.

[e] Mohr et al. 1994.

[f] White et al. 1995.

[g] Centers for Disease Control and Prevention 1993c.

[h] Anderson et al. 1995a. Only those subjects who reported symptoms unexplained by illness or other factors were asked for specific symptoms.

Table 17. MTBE Exposure Concentrations, Blood Levels, and the Conclusions from Community Studies That Included MTBE in Air, or Blood, or Both

	n[a]	Range and (Median) MTBE Blood Levels (µg/L)	Exposure Concentration (ppm)	Headache (% of Subjects)	Major Conclusions of the Investigators
Fairbanks 1992/1993[b] Service station attendants, mechanics, and drivers					• Air levels of MTBE and symptom prevalence were both higher during oxyfuel use than after it ended • MTBE concentrations in the workplace correlated with (postshift minus preshift) MTBE blood concentrations • Workers in the upper quartile of postshift blood concentrations (> 9.6 µg/L) were more likely to have reported one or more symptoms on the day of testing (4/4) than the other workers (9/14); this finding was not statistically significant
During oxyfuel use in 1992	18	0.2–37.0 (1.8)	0.005–0.8 (median 0.1), workplace samples	72	
After oxyfuel use had ended in 1993	28	0.05–1.44 (0.24)	0–0.14 (median 0.04), workplace samples	4	
New Jersey[c] Service station attendants & mechanics					• The two groups reported symptoms at the same rate during the month before the survey • No difference was noted in symptom prevalence across the work-shift among workers who pumped gas > 5 hours/day
North, during oxyfuel use	115	ND[d]	< 0.8–6, personal samples	36	
South, after oxyfuel use had ended	122	ND	< 0.8–6, personal samples	39	
Stamford[e]					• Subjects (from all groups) (11/44) or workers (8/30) in the upper quartile of postshift blood levels (> 2.4 µg/L and > 3.8 µg/L, respectively) had higher prevalence of one or more key symptoms • Personal breathing-zone MTBE concentrations were strongly correlated with MTBE blood levels
During oxyfuel use Service station attendants & mechanics	21/48	Mechanics: 0.2–37 (1.7) Attendants: 7.5–29 (15.2)	Mechanics: < MDC (0.03)–12.00, breathing zone samples	52	
Other workers (e.g., meter readers)	6/12	< 0.05–0.5 (0.15)	< MDC (0.03), breathing zone samples	67	
Commuters	14/59	< 0.05–26 (0.12)	< MDC (0.03), breathing zone samples	42	
Albany[f]					• No difference was noted in symptom prevalence among groups with different exposures
Using conventional gasoline Service station attendants & mechanics	11/34	0.09–1.5 Smokers: (0.46) Nonsmokers: (0.38)	< MDC (0.03)–0.14, breathing zone samples	21	
Policemen, garage and toll booth workers	9/48	< MDC (0.05)–0.11 (0.08)	< MDC (0.03), breathing zone samples	47	
Students, office workers	18/182	< MDC (0.05)	ND	24	

[a] When two values are given, the first one is the number of subjects who provided blood samples and the second is the total number of subjects studied.

[b] Moolenaar et al. 1994.

[c] Mohr et al. 1994.

[d] ND = not determined.

[e] White et al. 1995.

[f] Centers for Disease Control and Prevention 1993c.

Table 18. Prevalence of Symptom Reporting in Fairbanks and Anchorage in December 1992

Study Population	Number of Cases Given as the Percentage of Interviews	People Reporting Symptoms While Traveling Given as the Percentage of Total Cases	People Reporting Symptoms While Fueling Given as the Percentage of Total Cases
Fairbanks 1992[a]			
Taxi drivers	33% (4/12)	75% (3/4)	25% (1/4)
Health care workers	29% (26/90)	42% (11/26)	35% (9/26)
University students	15% (15/101)	20% (3/15)	20% (3/15)
Anchorage 1992[b]			
Taxi drivers	46% (12/25)	100% (12/12)	75% (9/12)
Anchorage Health Center employees	25% (7/29)	71% (5/7)	43% (3/7)
Hospital employees	27% (29/108)	76% (22/29)	55% (16/29)

[a] Beller et al. 1992.

[b] Chandler and Middaugh 1993.

Table 19. Wisconsin Study[a]

	Metropolitan Milwaukee	Metropolitan Chicago	Remainder of Wisconsin
Type of gasoline used	RFG[b]	RFG[b]	Conventional
Number of respondents	527	485	501
Response rate	71% City 76% Suburbs	42% City 41% Suburbs	74%
Familiarity with MTBE as a fuel additive	54%	23%	40%
Number of individuals reporting any unusual symptoms since November 1, 1994	119 (23%)	30 (6%)	32 (6%)
Number of individuals perceiving unusual smells while refueling	275 (52%)	96 (20%)	77 (15%)
Number of individuals who associated:			
Eye irritation with			
Pumping gas	22 (4%)	4 (1%)	0 (0%)
Driving	24 (5%)	8 (2%)	1 (0%)
Headache with			
Pumping gas	43 (8%)	6 (1%)	0 (0%)
Driving	51 (10%)	8 (2%)	3 (0%)
Sinus problems with			
Pumping gas	22 (4%)	2 (0%)	0
Driving	28 (5%)	3 (1%)	0

[a] Adapted from Anderson et al. 1995a.

[b] Authors note that approximately 50% of the RFG in use contained MTBE and the remainder contained ethanol or ETBE based on a very limited number of gasoline samples analyzed by the EPA. In Milwaukee, up to 65% of the samples contained MTBE at some time in 1995.

Table 20. Controlled Exposure Studies

	Cain and Coworkers 1996	Prah and Coworkers 1994	Johanson and Coworkers 1995	Stamford Community Study (White et al. 1995)[a]
n	22 M, 21 F, 43 T	20 M, 20 F, 40 T	10 M	
Exposure concentration	1.7 ppm for 1 hour	1.4 ppm for 1 hour	5, 25, or 50 ppm for 2 hours	
Peak MTBE blood levels[b]	17.1 µg/L (n = 4)	8.8 µg/L (n = 2)	144, 572, or 1,144 µg/L (n = 10)	> 2.4 µg/L (upper quartile n = 11)
Endpoints	• Subjective symptoms and indices of mood and air quality • Indices of eye inflammation (ocular hyperemia-redness, tear film breakup time, epithelial cell turnover, number of PMNs in tear fluid) • Indices of nose inflammation (presence of inflammatory cells in nasal lavage fluid) • Measures of CNS function (symbol-digit substitution, switching attention, mood state)	• Subjective symptoms and indices of air quality • Indices of eye inflammation (ocular hyperemia, tear film breakup time, presence of mRNA for inflammatory mediators, number of PMNs in the conjunctiva) • Indices of nose inflammation (presence of neutrophils and inflammatory mediators in nasal lavage fluid) • Measures of CNS function (symbol-digit substitution, switching attention, mood state)	• Subjective symptoms • Indices of eye inflammation (blinking frequency, redness, tear film breakup time, epithelial damage) • Indices of nasal inflammation (nasal and oral peak expiratory flow, nasal swelling, changes in cell count and certain proteins in nasal lavage fluid)	• Subjective symptoms
Findings	No effects on any of the endpoints studied, although subjects could detect MTBE by smell	No effect on any of the endpoints studied, although women reported worse air quality during MTBE exposure than during clean air exposure	No effect on any of the endpoints studied	Subjects in the upper quartile of postshift blood concentrations (> 2.4 µg/L) had higher prevalence of one or more key symptoms

[a] Study reported for comparison; data not from controlled exposures.

[b] The n values here reflect subjects providing blood samples.

Table 21. Concentration of MTBE in Headspace for Two Gasoline Blends[a]

Temperature (°F)	Mapco (vol%)	API Blend (vol%)
−25	20	30
0	30	30
+25	26	45

[a] From Smith et al. 1994.

Table 22. Effect of Temperature on Odor Thresholds of Gasolines With and Without MTBE[a]

Temperature	Concentration of MTBE in Gasoline (Gasoline Blend)	Odor Detection Threshold			Odor Recognition Threshold		
		Gasoline Alone	Gasoline + MTBE	Percentage of Change	Gasoline Alone	Gasoline + MTBE	Percentage of Change
−24°F	15% (API) 15% (Mapco)	1.28 1.3	1.16 0.71		1.85 2.54	1.28 0.72	
0°F	15% (API) 15% (Mapco)	0.90 1.51	0.6 0.36		1.71 2.0	0.80 0.42	
+25°F	15% (API) 15% (Mapco)	0.85 2.2	1.00 0.34		1.57 4.94	1.00 0.53	
Average of the above three temperatures	15% (API) 15% (Mapco)	1.01 1.67	0.91 0.39	−10 −71	1.7 3.16	1.02 0.56	−40 −82
75°F	15% (Blend of Amoco, Exxon, Atlantic)	0.43	0.14	−67	0.97	0.32	−67
	15% (Average of Mapco and Tesoro)	1.1	0.17	−85	3.1	0.53	−83

[a] From Smith et al. 1994.

Table 23. Effect of Ethers on Odor Thresholds of Various Gasoline Blends at 75°F

Type of Gasoline and Oxygenate Content (vol%)	Odor Detection Threshold (ppm)			Odor Recognition Threshold (ppm)		
	Gasoline Alone	Gasoline + Ether	Percentage of Change	Gasoline Alone	Gasoline + Ether	Percentage of Change
Average of Mapco Regular and Tesoro Regular + 15% MTBE[a]	1.325	0.250	–81	3.79	0.671	–83
Average of Amoco, Exxon, and Atlantic (regular and premium) + 15% MTBE[a]	0.433	0.139	–68	0.97	0.325	–66
Summer blend + MTBE (97% purity)[b] (3% MTBE) (11% MTBE) (15% MTBE)	0.576	 0.5 0.275 0.264	 –13 –52 –54	0.802	 0.696 0.710 0.686	 –13 –11 –14
Summer blend + 15% MTBE (99% purity)[b]	0.576	0.113	–80	0.802	0.358	–55
Summer blend + 15% ETBE (99% purity)[b]	0.576	0.064	–89	0.802	0.139	–83
Summer blend + 15% TAME (94% purity)[b]	0.576	0.114	–80	0.802	0.207	–74
Winter blend + 15% MTBE (97% purity)[b]	0.479	0.219	–54	1.121	0.398	–64

[a] From Smith et al. 1994.

[b] From American Petroleum Institute 1994.

Table 24. Summary of the Neurotoxic Effects of Ethanol in Laboratory Animals

Behavior	Exposure or Dose	Species	Effect and Concentration at Which Effect Was Seen	Reference
Spontaneous locomotor activity	0.5, 1, 1.5, 2, 2.5, or 3 g/kg (i.p.)	Mouse	↑ At 1, 1.5, and 2 g/kg ↓ At 2.5 and 3 g/kg	Middaugh et al. 1992
	0.125, 0.25, 0.5, or 1.0 g/kg (i.p.)	Rat	↑ At 0.25 g/kg in one strain No effect at 0.125 and 0.5 g/kg ↓ At 1 g/kg in all strains	Criswell et al. 1994
	0.1 or 0.5 g/kg (i.p.)	Rat	↑ During 0-10 minute interval at 0.1 g/kg ↓ During 30-40 minute interval at both dose levels	Moore et al. 1993
Coordination (ability to maintain balance)	0.5, 1, or 1.5 g/kg (i.p. or gavage)	Rat	Dose-dependent ↓	Le and Israel 1994
Ataxia	0.5, 1, or 1.5 g/kg (gavage)	Dog	Observed at 1 and 2 g/kg	Weiss and Laties 1964
Aggression	0.1, 0.3, 0.6, 1.0, or 1.5 g/kg (gavage)	Squirrel monkey	↑ At 0.1 and 0.3 g/kg ↓ At 1 and 1.5 g/kg	Weerts et al. 1993
Operant behavior	20,000 ppm	Mouse	↓ Response rate (lever presses) at 20,000 ppm	Moser and Balster 1985
	12,000, 24,000, 36,000, or 48,000 ppm × 40 minutes (inhalation)	Mouse	↓ Response rate (lever presses to obtain milk) in the first 3 minutes of a session at 36,000 and 48,000 ppm	Moser and Balster 1986
	1, 2, or 4 g/kg (gavage)		A 2g/kg, ↓ response rate in first 3 minutes of session, then ↑ during remainder of session ↓ Response rate during whole session at 4 g/kg	
	0.25, 0.5, 1, 1.5, 2, or 3 g/kg (i.p.)	Mouse	Dose-dependent ↓ in response rate (lever presses)	Middaugh et al. 1992
	0.25, 0.5, or 1 g/kg (i.p.)	Rat	↓ Response rate (lever presses to obtain water) and accuracy of timing behavior at 1 g/kg	Laties and Weiss 1962
	0.5, 1, or 1.5 g/kg (i.p.)	Rat	↓ Response rate (lever presses to avoid shock) at 1.5 g/kg	Galizio et al. 1984
	0.5, 1, or 2 g/kg (gavage)	Dog	Dose-dependent ↓ in response duration (lever presses)	Weiss and Laties 1964

(Table continues next page.)

Table 24. Summary of the Neurotoxic Effects of Ethanol in Laboratory Animals *(Continued)*

Behavior	Exposure or Dose	Species	Effect and Concentration at Which Effect Was Seen	Reference
Memory or accuracy of performance	0.25, 0.5, or 0.75 g/kg (i.p.)	Rat	↓ Rate of forgetting at 0.25 g/kg and ↑ at 0.75 g/kg	Melia et al. 1990
	0.25, 0.5, 0.75, or 1 g/kg (i.p.)	Rat	Dose-dependent ↓ in response accuracy and ↑ in response latency (slower reaction time)	Elsner et al. 1988
	0.3, 0.56, 0.75, or 1.7 g/kg (i.p.)	Rat	Dose-dependent ↓ in response rate; no effect on response accuracy	Doty et al. 1992
Memory or accuracy of performance	5,000, 10,000, 15,000, or 20,000 ppm × 3 hours (inhalation)	Baboon	↓ Response rate (lever presses to obtain food using a matching task) at 20,000 ppm ↑ number of errors at 15,000 and 20,000 ppm	Geller et al. 1985
Fine motor control	0.125, 0.25, 0.5, 1.0, or 1.5 g/kg (gavage)	Squirrel monkey	Dose-dependent ↓ in tremor	Newland and Weiss 1991

Table 25. Symptom Prevalence from Telephone Surveys in Two Areas Using MTBE or Ethanol in Oxyfuel[a]

Locale	*n*	Eye Irritation	Nose & Throat Burning	Cough	Spaciness	Headache	Dizziness	Nausea
Fairbanks 1992/1993[b]								
During oxyfuel use in 1992	41	37	29	22	12	34	15	15
After oxyfuel use had ended in 1993	100	18	12	8	6	10	4	2
Anchorage 1994/1995[c]								
No ethanol	316	6	5	4		9	0	
Partial ethanol	218	7	4	4		13	2	
Total ethanol	654	7	3	4		11	0	

[a] Values are expressed as the percentage of the population surveyed, given in the *n* column.

[b] Centers for Disease Control and Prevention 1993b.

[c] Egeland and Ingle 1995.

Potential Health Effects of Oxygenates

Reproductive and Developmental Effects

The introduction of any new chemical into the environment carries with it the potential that the embryo, fetus, or neonate may be significantly more sensitive to its toxic effects than more fully developed humans. Because of its widespread consumption, ethanol has been studied extensively not only in animals, but also in humans. MTBE is the only ether so far that has been studied for effects on reproduction and embryonic development; these studies have been conducted only with laboratory animals as part of the toxicity testing of MTBE mandated by the Toxic Substances Control Act. Most of the studies of ethanol on reproduction and development investigated the effects of ingested ethanol, whereas the studies of MTBE investigated the effects of inhaled MTBE. Studies on TAME are currently planned.

MTBE

RAT STUDIES

Three studies of reproductive and developmental effects of MTBE have been conducted with rats. They are summarized below and in Table 26. Biles and coworkers (1987) exposed male and female rats to 0, 300, 1,300, or 3,400 ppm MTBE prior to mating for 6 hours/day, 5 days/week, for 12 weeks (males) or 3 weeks (females). Exposures continued through the mating period, after which males continued being exposed to MTBE until a second mating period. Pregnant females continued being exposed for 7 days/week during gestational days 0 through 21 and for 5 days/week during lactational days 5 through 20. After weaning, the investigators produced a second litter from the same adults, using the same mating and exposure protocols. In total, adult males were exposed to MTBE for approximately 28 weeks and females for 16 weeks. Overall, exposure to MTBE concentrations as high as 3,400 ppm for an extended period had no effect on male reproductive indices (reproductive function or histological changes in the organs of the reproductive tract). Some effects, such as dilated renal pelves and a slight, but not statistically significant, decrease in the pregnancy rate were observed after both matings in the mothers exposed to the highest concentration. No effects on reproductive organ pathology and gestational length were noted. A small, but statistically significant reduction in offspring viability (from 99% viability to 95.5%) was seen in the second litter from dams exposed to 1,300 or 3,400 ppm MTBE.

In another single-generation study (Bio/dynamics Institute 1984b; Conaway er al. 1985), pregnant rats were exposed to 0, 250, 1,000, or 2,500 ppm MTBE for 6 hours/day, but only during gestation days 6 through 15, the period of embryonic organogenesis. The results, summarized in Table 26, show that exposing rats to a concentration of MTBE as high as 2,500 ppm was not toxic to the mothers, did not affect reproductive indices such as pregnancy rate and number of resorptions, and was not teratogenic (American Petroleum Institute 1984b; Conaway et al. 1985). The exposure period was much shorter in this study than in the one just described and did not include the mating period and first 5 days of pregnancy.

Neeper-Bradley (1991) exposed male and female rats to 0, 400, 3,000, or 8,000 ppm MTBE over two generations, one litter per generation. Female rats were exposed for 6 hours/day, 5 days/week, for 3 weeks prior to mating, and during mating, gestation (for 7 days/week), lactation, and weaning. Male rats were exposed for 10 weeks prior to mating and during mating. After weaning, the first-generation pups continued being exposed to MTBE for at least 8 weeks prior to mating and producing the second-generation pups. All exposures, including those for the mating, gestational, and postnatal periods, were similar to those for the adults that produced the first-generation litter. The results of these exposures are shown in Table 26. The investigators reported a consistent pattern of adult toxicity, such as hypoactivity, ataxia, and loss of startle reflex, after exposure to 8,000 ppm MTBE and less striking effects after exposure to 3,000 ppm. First-generation pups from parents exposed to 8,000 ppm MTBE exhibited statistically significant reductions in body weight on postnatal days 14 through 28; significantly reduced body weight in litters from parents exposed to 3,000 ppm MTBE were seen only on postnatal day 14. Significant reductions in body weight were observed on gestational days 7 through 28 in second-generation pups from parents exposed to 8,000 ppm MTBE, and on postnatal days 14 through 28 in the second-generation pups from parents exposed to 3,000 ppm. Increased perinatal deaths were seen only in second-generation litters from parents exposed to 8,000 ppm MTBE on postnatal day

4. In utero exposure to 3,000 or 8,000 ppm MTBE produced hypoactivity and reduced startle reflex in the second generation-offspring after weaning; ataxia was seen in this group only after parental exposure to 8,000 ppm MTBE.

MOUSE STUDIES

Two studies of developmental toxicity have been conducted with mice. In one, Tyl and Neeper-Bradley (1989) exposed pregnant mice to 0, 1,000, 4,000, or 8,000 ppm MTBE for 6 hours/day during gestational days 6 through 15 (Table 27). Both maternal and fetal toxicity were seen after pregnant mice had been exposed to 4,000 or 8,000 ppm MTBE. Hypoactivity, ataxia, lacrimation, and reduction in body weight were reported in the mothers exposed to either 4,000 or 8,000 ppm; pregnant animals exposed to 8,000 ppm had fewer viable implantations owing to the increased number of resorptions and dead fetuses. Fetal effects included decreased body weight and increased incidence of skeletal malformation after exposure to both 4,000 and 8,000 ppm MTBE, and cleft palate after 8,000 ppm. The significant increases in the incidence of several skeletal malformations seen at these two concentrations indicate a reduced level of ossification in the fetus. However, the authors point out that the increased level of cleft palate may have resulted from the maternal stress induced by the exposure. Maternal stress originating from many sources has been shown to cause terata, specifically cleft palate, in mice. This effect is believed to be related to stress-induced elevated blood levels of corticosteroids, which are known to produce cleft palate in susceptible strains of mice (Tyl and Neeper-Bradley 1989).

In another study, pregnant mice were exposed to 0, 250, 1,000, or 2,500 ppm MTBE for 6 hours/day during gestational days 6 through 15 (Bio/dynamics Institute 1984a; Conaway et al. 1985). No effects on maternal reproductive indices or on fetal malformations were observed, which suggests that concentrations of 2,500 ppm MTBE or lower during gestation are not toxic to the mother or the fetus in mice.

RABBIT STUDIES

Tyl (1989) exposed pregnant rabbits to 0, 1,000, 4,000, or 8,000 ppm MTBE for 6 hours/day during gestational days 6 through 18. The results of these exposures are presented in Table 27. Some evidence of maternal toxicity was noted at 8,000 ppm MTBE such as hypoactivity, ataxia, and reduced liver weight gain (relative to body weight). Maternal body weights were decreased at both 4,000 and 8,000 ppm. However, there were no effects on the fetuses.

SUMMARY OF MTBE STUDIES

Maternal effects were observed in rats exposed to 3,000 or 8,000 ppm (but not to 400 ppm), and in mice and rabbits exposed to 4,000 ppm or 8,000 ppm MTBE (but not to 1,000 ppm). Pregnant rats exposed to 4,000 or 8,000 ppm MTBE showed a reduced number of viable fetal implantations. Small, but statistically significant, decreases were observed in the viability of offspring from pregnant rats exposed to 1,300 or 3,400 ppm MTBE when compared with control animals.

Rat pups exposed in utero to 8,000 ppm MTBE (and, to a lesser extent, from those exposed to 3,000 ppm) showed statistically significant decreases in body weight during lactation, compared with control pups and pups exposed in utero to 400 ppm. Effects on the central nervous system (hypoactivity, ataxia, and loss of startle reflex) were seen in adult rats exposed in utero to 3,000 or 8,000 ppm MTBE. No malformations were reported in the fetuses examined in the three studies of rats described above. However, increased frequencies of skeletal malformations were found in fetuses from mice exposed to 4,000 or 8,000 ppm MTBE. When administered to mice at lower concentrations (1,000 ppm), MTBE was not teratogenic, nor was it toxic to the mother or the fetus.

ETHANOL

A large body of literature on ethanol's effects in animals and humans has identified it as a developmental toxicant. Although ethanol's contribution to birth defects had been recognized marginally sometime earlier, it was not until the compelling studies of Lemoine and coworkers (1968) in France and Jones and Smith (1973) in the United States that ethanol's teratogenic potency attained wide acknowledgment. These two studies described the case criteria for what is now known as fetal alcohol syndrome (FAS), and led to explosive growth in the ethanol literature, and to subsequent observations of more subtle effects from lower levels of ethanol consumption during pregnancy, known as *fetal alcohol effects*. Although experiments in rodents have managed to clarify some of the mechanisms involved in FAS and have expanded the range of functional outcomes associated with early developmental ethanol exposure, both pharmacokinetic and pharmacodynamic differences between rodents and humans make extrapolation between the species a challenging issue. The following text summarizes some of the results from animal and human studies.

ANIMAL STUDIES

Malformations induced by ethanol in animals require high exposure levels. Because extreme dose levels are not

the issue with environmental exposures, the focus of this section is on functional (or neurobehavioral) outcomes, which are more sensitive measures of developmental effects than gross malformations. However, it should be noted that the behavioral endpoints evaluated in animals are not as complex as those that can be studied in humans.

Gentry and Middaugh (1988) and Middaugh and Gentry (1992) studied behavioral effects in mice. They used 90-day-old mice whose mothers had received ethanol as part of their diet during gestational days 5 through 17 (Table 28). These studies used a behavioral test, called schedule-controlled operant behavior, that has shown sensitivity in experiments with other agents. However, to detect a behavioral effect, relatively high blood ethanol levels had to be achieved. Also shown in Table 28 are the adverse behavioral outcomes in the offspring of pregnant rats fed ethanol as part of their diet (Chen et al. 1982; Meyer and Riley 1986). The dose-response relation observed in rats was comparable to that seen in mice. In summary, prenatal ethanol exposure, at high levels (20% to 35% of maternal caloric intake), which produced blood levels of ethanol of 260 to 300 mg%, caused adverse behavioral outcomes in rats and mice; however, low levels, comparable to what is termed light or moderate intake in humans, have rarely been studied.

Nelson and coworkers (1985) exposed pregnant rats to 0, 10,000, 16,000, and 20,000 ppm ethanol by inhalation for 7 hours/day throughout gestation. No statistically significant increase in fetal malformations was noted despite blood ethanol levels that reached 200 mg% in dams exposed to 20,000 ppm ethanol and that produced toxic effects, such as narcosis and a reduced food intake in the mothers. Nelson and coworkers (1988) extended this study to include paternal exposure to ethanol and an assessment of functional and neurochemical changes in the offspring of pregnant rats. Male rats were exposed to 10,000 or 16,000 ppm ethanol for 7 hours/day, 7 days/week, for 6 weeks and allowed to breathe clean air for 2 days before mating. Female rats were exposed to ethanol only after mating, on gestational days 1 through 19, for 7 hours/day. Behavioral testing between days 10 and 90 after birth showed that offspring from paternally or maternally exposed rats performed as well as control animals in tests of neuromotor coordination, activity levels, and learning ability. However, only a limited set of behavioral tests were used, some of which may not have been sensitive enough to detect subtle behavioral changes.

To account for the behavioral effects of prenatal ethanol exposure, experimenters have investigated changes in neurotransmitters or damage to particular brain structures. In the study described above by Nelson and coworkers (1988), although behavioral changes were not observed, the investigators found statistically significant changes in the levels of some neurotransmitters (dopamine, acetylcholine, ß-endorphin, norepinephrine, metenkephalin, and substance P) in various brain regions of 21-day-old rat pups; however, the effects seen in a given brain region were seldom related to the level of ethanol exposure, and changes in neurotransmitters were often not consistent across brain regions.

West and his collaborators have focused on the cerebellum (see Table 29). For example, they examined the Purkinje cells of rat pup cerebellum after administering ethanol at 5 g/kg body weight daily to pregnant dams, or 2.5 g/kg daily to neonates (Marcussen et al. 1994) . The results of these exposures are presented in Table 29. Briefly, the investigators noted that Purkinje cells in the cerebellar area of the rat pups were unaffected when exposed in utero to ethanol late in gestation, but were reduced when they were treated orally with ethanol after birth. Hamre and West (1993) found that postnatal days 4 to 5 were the most sensitive period for Purkinje cell loss, which also corresponded to the period of greatest granule cell loss. Transient astrogliosis, detected by glial fibrillary acidic protein assays, also was produced by ethanol treatment during the sensitive postnatal period (Goodlett et al. 1993). Dose levels in the range described above also lead to enduring deficits in motor function as measured by rotarod performance. In these studies, the ethanol blood levels measured in the pups ranged from 200 to 550 mg%.

Studies in which pups were exposed by inhalation after birth have also shown effects in the pups brains. Bauer-Moffett and Altman (1977) observed brain damage in preweanling rats exposed for 90 minutes, twice a day, to 34,000 to 40,000 ppm ethanol vapor (on 2 days/week only one exposure occurred). Phillips and Cragg (1982) also reported changes in the brain tissue of rat pups exposed to ethanol by inhalation (Table 29).

The rationale for postnatal exposure lies in the asynchrony between brain development in rodents and that in humans. Rats and mice are born at a time roughly equivalent in brain development to the second human trimester. Thus, much of their brain growth spurt occurs postnatally and postnatal exposure captures the impact of ethanol on late brain development.

Studies of late brain development in nonhuman primates capitalize on certain similarities between the human and nonhuman primate brain: both develop in utero along the same timeline, and the brain structures correspond with each other. Clarren and coworkers focused on a primate model of binge drinking because of the evidence that FAS is a product of such an intake pattern (Clarren and Astley

1992; Clarren et al. 1992). Pregnant *Macaca nemestrina* monkeys received weekly doses of ethanol ranging from 2.5 g/kg to 4.1 g/kg during weeks 5 through 24, or doses of 0.3 g/kg to 1.8 g/kg throughout gestation (weeks 1 through 24). (If administered early in pregnancy, the higher doses tended to produce nonviable pregnancies.) Peak plasma levels after doses of 2.5 g/kg reached 250 mg%. Clarren and coworkers (1988) measured a variety of endpoints in the infants. In those animals exposed throughout pregnancy, hints of impaired cognitive function and motor development began to appear at blood levels of about 25 mg%, close to the levels that would be attained following human consumption of 0.5 oz (15 mL) of absolute ethanol, or one drink (35 to 45 mg%) (Jacobson and Jacobson 1995). At the higher doses, corresponding to blood levels of 140 to 250 mg%, cognitive impairment was unmistakable. None of the animals displayed the full gamut of FAS outcomes, although some of its features could be found in 16 of the 28 infants, most consistently in those exposed to peak blood levels of 140 mg% and above. Such levels, the authors point out, correspond to maternal consumption of about 2.5 to 3.0 oz of ethanol weekly. These studies indicated that the first 6 to 8 weeks of pregnancy represent a sensitive developmental period in nonhuman primates.

In summary, postnatal exposure to high levels of ethanol, orally or by inhalation, produces cellular changes in the brain and behavioral deficits in rodent pups. Exposure to high levels of ethanol by inhalation did not produce malformations in rats, as determined by conventional structural teratology. The offspring of pregnant monkeys that had received ethanol at a level that produced a blood ethanol concentration of about 25 mg% showed hints of impaired cognitive and motor development. This blood ethanol level is close to the postulated threshold of 0.5 oz/day needed to develop behavioral deficits in humans.

The results of binge-type drinking by nonhuman primates during the first 2 months of pregnancy suggest that this activity presents as great a hazard to the developing fetus as drinking during the entire gestational period. However, studies of rodents indicate that drinking during the late stages of pup brain development is more dangerous to the fetus than if drinking is confined to the early stages of pregnancy. Because brain development progresses from neurogenesis to subsequent stages during which cells migrate and form the extensive connections underlying complex function, later drinking is likely to exact the more subtle consequences of excessive ethanol exposure.

HUMAN STUDIES

The developmental effects of ethanol abuse during pregnancy result in the appearance of both characteristic malformations and functional deficits that are manifestations of brain damage. The physical markers include short palpebral fissures, a flat midface, a thin upper lip, a long and often smooth filtrum, and other facial anomalies. Microcephaly is also observed. Intrauterine growth retardation is a common and distinguishing sign. Children with FAS exhibit underdeveloped motor and cognitive functioning and a variety of learning disabilities, attention problems, and conduct disorders. Their IQ scores tend to fall into the subnormal range.

The FAS, however, is not germane to the question of environmental exposure to ethanol. It is a product of maternal ethanol abuse, particularly in the form of binge-type drinking, which is defined as consuming several drinks within a relatively short time on a single occasion. More germane to environmental exposure are fetal alcohol effects. The term originated to describe the more subtle outcomes of maternal ethanol consumption during gestation. These outcomes are not the dramatic malformations of FAS, but appear as deficits in performance on neuropsychological tests, as behavioral imbalances such as hyperactivity, and as lowered intellectual capacity in general. These symptoms are produced by levels of ethanol intake lower than those that can induce FAS.

Subtle functional disturbances have now become the focus of ethanol research investigating the hazards of maternal consumption during gestation. The shift in emphasis from overt FAS came about partly because both health professionals and the public posed a rather simple query: How much ethanol is it safe to drink during pregnancy? In response, investigators began to explore low-dose levels and to refine their outcome measures. Their findings make it clear that even modest ethanol consumption during pregnancy can lead to adverse consequences in the offspring, and that a simple answer to the question is unattainable because an unambiguous threshold cannot be identified.

The most extensive work on ethanol and developmental toxicity has been conducted at the University of Washington by Streissguth and her collaborators. The investigation centered on the functional consequences of ethanol ingestion during pregnancy. It sought to establish correlations between prenatal ethanol consumption and a variety of behavioral outcomes. To fulfill this goal, the researchers recruited more than 1,500 pregnant women to participate in a longitudinal study of the consequences of maternal ethanol consumption (Streissguth et al. 1981).

From the beginning, the investigators were aware of the numerous confounding variables that might intrude on their findings. They selected a sample consisting primarily of middle-class women who had enrolled in prenatal care programs, a choice designed to overcome the problems

associated with populations at high risk of adverse pregnancy outcomes. They were sensitive to the question of dose-response relationships, and devised several techniques for ascertaining estimates of ethanol intake. Because of the necessity to include an adequate representation of all dose levels, they strove to overrepresent in their final cohort women who could be classified as heavier drinkers Finally, they recorded or determined a large number of covariates, such as socioeconomic status, that might be correlated with either ethanol ingestion or with behavioral outcomes in the offspring. The final cohort numbered approximately 500 singleton births.

The members of this cohort have been followed intensively since the original enrollment period in 1974 to 1975, a period before ethanol's threat to fetal development had gained widespread public recognition. The offspring of these mothers are now approximately 20 years of age. Throughout this study, as the children passed through the successive stages of development, they manifested various neurobehavioral changes resulting from prenatal ethanol exposure (Streissguth et al. 1994). The key findings, according to age, are described in Table 30. To analyze such an enormous, multidimensional data set, in which multiple outcomes are the product of multiple influences, novel statistical procedures were developed (Bookstein et al. 1996). Dose, the central independent variable, was not treated as a unitary measure, but as a multivariate index that combined factors, such as timing, pattern, magnitude, and duration of consumption, into a single net summary index. In a similar fashion, the multitude of behavioral outcomes, some of which are described above, also were combined into a net outcome score. The relation among these indices, determined by a technique termed partial least squares, is the basis for the conclusions reached by the investigators (Bookstein et al. 1996).

One of the important factors to emerge from this research is the overwhelming influence of drinking pattern on outcomes. Binge drinking exerts greater adverse effects, resulting in FAS, than a comparable net intake of ethanol in a pattern of steady drinking. In addition, as confirmed in the primate studies of Clarren and coworkers (1992), doses early in pregnancy exert greater adverse effects than equivalent doses in mid-pregnancy.

Although the investigation undertaken by Streissguth and her collaborators is the most extensive, and has yielded the most longitudinal data, its primary findings have been supported and enlarged by a voluminous and expansive literature. The overall findings are, first, that prenatal ethanol exposure exerts effects that primarily reflect deficits in brain development; indices such as birth weight are of little use as guides. Second, these effects do not fade with time. Rather, they take on a different character as development proceeds. Third, adverse outcomes are discernible at low exposure levels, and a statistically cogent threshold is not identifiable.

Because environmental human exposures to ethanol invoke questions about adverse effects at low doses, the slope of the dose-response function in this range is the core issue.

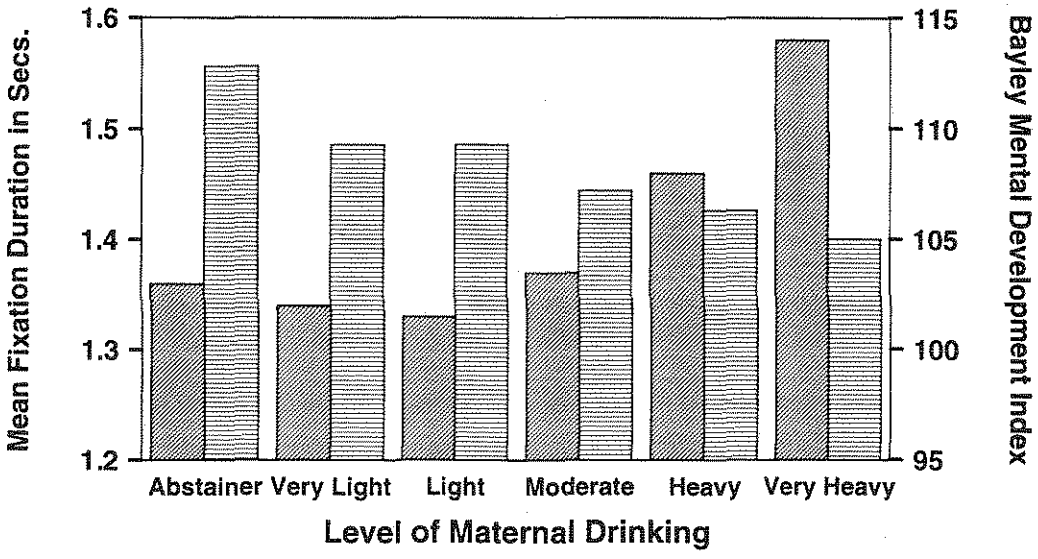


Figure 6. Relation between level of maternal drinking and infant performance. Performance was measured on the Fagan test of fixation duration, in seconds (bar on the left in each pair), and on the Mental Development Index of the Bayley Scales of Infant Development (bar on the right in each pair). Adapted from Jacobson and coworkers (1993) and Jacobson and Jacobson (1995).

Jacobson and Jacobson (1995) proposed that dose-response relationships yield different kinds of patterns depending on the endpoint studied; for example, one test instrument might suggest a threshold pattern and another might show a monotonic rise of response with an estimated increase in dose.

This approach, which examines dose-response relationships for individual tests, differs from that of Bookstein and coworkers (1996), which attempted to deal with the problem that both dose and response are multidimensional in character by analyzing jointly dose, effects, and duration of consumption. Methods that acknowledge these multivariate contributions are essential for both proper study design and subsequent data analysis. For environmental ethanol exposures, the multidimensional features of ingested dose are augmented by adding inhalation exposure; neither exposure source can be viewed in isolation. Risk assessments of environmental ethanol based on developmental indices need to deal with what, in many instances, will be the contribution of incremental doses.

Despite the questions raised by extracting individual results from a complex matrix, such examinations can convey useful information. Figure 6 depicts two different relationships between level of drinking and outcome. One relates maternal drinking level to scores on the Mental Development Index of the Bayley Scales of Infant Development. It reflects a pattern that Jacobson and Jacobson (1995) describe as "linear with no apparent threshold." The other relates an infant's duration of fixation on a novel stimulus (the Fagan test) to the maternal drinking level and, in contrast, shows a distinct threshold.

On the basis of their own studies as well as their surveys of the literature, Jacobson and Jacobson (1995) contend that, for most neurobehavioral endpoints, a practical threshold seems to fall at approximately 0.5 oz (or 11 g) of absolute ethanol (one drink) daily. It is important to emphasize that this level of alcohol intake does not allow the number of drinks per week to be averaged over 7 days. For a 60-kg woman, it means a dose of 0.18 g/kg and a corresponding peak blood level of about 35 mg% (350 mg/L). The discussion of ethanol in the Metabolism and Disposition section describes the study of Brien and coworkers (1983), which demonstrated that ingesting the equivalent of 2 oz of 40% (80 proof) distilled spirits, approximately 1 oz of absolute ethanol, produced peak blood ethanol levels ranging from 23 to 73 mg% in pregnant women in their second trimester, bringing them across the estimated threshold for neurobehavioral effects in the offspring. In addition, after 3.5 hours, mean blood ethanol levels fell to 1 mg%; however, ethanol was still present in amniotic fluid at levels ranging from 6 to 24 mg%. Thus, there was a differential disposition of ethanol in maternal blood and amniotic fluid following maternal ingestion of a single dose of ethanol during the second trimester of pregnancy. The relatively high concentration of ethanol in amniotic fluid, after it was virtually eliminated from maternal blood, suggests that amniotic fluid can act as a reservoir for ethanol in utero. As a consequence, Brien and coworkers (1983) suggest that a fetus can be exposed to ethanol for a longer total time than would be predicted from measurements of maternal blood ethanol concentrations.

In summary, fetal alcohol effects are more subtle outcomes of maternal ethanol consumption during gestation than the dramatic malformations seen in FAS. These effects appear as deficits in performance on neuropsychological tests, as behavioral aberrations, and as lowered intellectual capacity. They are the product of levels of ethanol intake lower than those that induce FAS. For most neurobehavioral endpoints, Jacobson and Jacobson (1995) contend that a practical threshold seems to fall at about 0.5 oz of absolute ethanol (one drink) daily. However, as Figure 6 demonstrates, not all developmental indices are consistent with even a threshold model. As noted above, Bookstein and coworkers (1996) assert that consumption patterns need to be described in multidimensional terms.

CONCLUSIONS

MTBE

Maternal toxicity and effects on reproductive indices have been observed in female rats exposed for extended periods to 3,000 or 8,000 ppm MTBE, and in mice and rabbits exposed to 4,000 or 8,000 ppm. In the offspring, developmental effects in the form of skeletal malformations were noted at 4,000 ppm in mice, CNS effects at 3,000 ppm in rats, decreased body weight at 3,000 ppm in rats, and decreased viability at 1,300 ppm in rats. No effects were observed at lower concentrations (300 or 400 ppm). These findings must be interpreted in the context of the measured MTBE levels in scenarios that might provide the greatest opportunity for human exposure; these are at least 3 orders of magnitude lower than the concentrations at which no effects on fetal development were seen. The measured concentrations of MTBE at service stations or in urban air rarely exceed 1 ppm except for brief periods during refueling. Thus, it is unlikely that effects on reproduction and fetal development would occur in humans from such exposures.

In considering the MTBE studies, it should be noted that a large number of studies indicate that the most sensitive endpoints in the offspring of humans and animals exposed

to ethanol lie in the realm of CNS function. In contrast, the small number of studies on MTBE's effects on CNS function in laboratory animals during their early development do not provide comparable information on neurobehavioral endpoints that can be applied to environmental (including occupational) exposures to this oxygenate.

ETHANOL

Data from studies of both animals and humans demonstrate conclusively that ethanol is a potent developmental toxicant that damages the CNS. Fetal alcohol effects are subtle outcomes that arise from lower levels of human ethanol consumption during gestation than levels that cause FAS. They are evidenced by functional deficits that are manifestations of brain damage. Although the data do not justify a statistically cogent threshold, some investigators contend that, for most neurobehavioral endpoints, a rough threshold seems to fall at about 0.5 oz of ethanol (one drink) daily, although no statistically verifiable threshold can be calculated. For a woman weighing 60 kg, this would mean a peak blood level of about 35 mg%. Pregnant rats that inhaled 20,000 ppm ethanol for an extended time showed a blood ethanol level of 200 mg% (Nelson et al. 1988). An extrapolation from these data suggests that achieving a blood alcohol level of 40 mg% would require inhaling 5,000 ppm ethanol for 4 hours, a level far above that which might be expected from human exposure resulting from adding ethanol to gasoline. Measurements of ethanol in the breathing zone during refueling were usually under the minimal detectable concentration of 1 ppm. A high concentration of 46 ppm was recorded under unusual circumstances (see Exposure Assessment section). Because exposure to ethanol within this range is not predicted to increase the ethanol blood level above the endogenous level, effects on the fetus from maternal exposure by inhalation of ethanol are not expected.

Table 26. Studies of MTBE and Its Reproductive and Developmental Effects on Rats[a]

Exposure Concentrations and Regimens	Effect and Concentration at Which Effect Was Observed
Single-Generation Study[a] 300, 1,300, or 3,400 ppm, 6 hr/day, 5 days/week (except during gestation) Females: before mating, and during gestation (7 days/week), lactation, and second gestation (7 days/week) Males: exposed in parallel for the duration of female exposures	Adult males: no effects Adult females: dilated renal pelvis at 300 and 3,400 ppm Pups: first litter, no effects; second litter, ↓ viability at 1,300 and 3,400 ppm
Single-Generation Study[b] 250, 1,000, or 3,300 ppm 6 hr/day, 7 days/week, during gestational days 6–15	Adult females: no effects Pups: no effects
Two-Generation Study[c] 400, 3,000, or 8,000 ppm, 6 hr/day, 5 days/week Females: 3 weeks before mating, and during mating, gestation (7 days/week), and lactation (F_1) Males: 10 weeks before and during mating First-generation pups (F_1): from age 28 days for 8 weeks before mating, and during mating, gestation (7 days/week), and lactation (F_2)	Adults: ↓ body weight at 8,000 ppm in males; hypoactivity and loss of startle reflex at 3,000 and 8,000 ppm in males and females; ataxia at 8,000 ppm in males and females F_1 pups: ↓ body weight gain during lactation at 3,000 and 8,000 ppm F_1 adults: ↓ body weight in premating period in males and females at 8,000 ppm F_2 pups: ↑ perinatal death at postnatal day 4 at 8,000 ppm; ↓ body weight after weaning at 3,000 and 8,000 ppm F_2 adults: ↑ liver weight at 8,000 ppm; hypoactivity and loss of startle reflex at 3,000 and 8,000 ppm; ataxia at 8,000 ppm

[a] Data from Biles et al. 1987.

[b] Data from Bio/dynamics 1984b.

[c] Data from Neeper-Bradley 1991.

Table 27. Studies of MTBE and Its Reproductive and Developmental Effects on Mice and Rabbits

Exposure Concentrations and Regimens	Effect and Concentration at Which Effect Was Observed
Mouse[a] 1,000, 4,000, or 8,000 ppm 6 hr/day during gestational days 6–16	Adult females: hypoactivity and ataxia at 4,000 and 8,000 ppm; ↓ body weight at 8,000 ppm; ↓ number of viable implantations at 8,000 ppm Fetuses: ↑ number of reabsorbed and dead fetuses at 8,000 ppm; ↓ body weight at 4,000 and 8,000 ppm; ↑ skeletal malformations at 4,000 and 8,000 ppm; ↑ incidence of cleft palate at 8,000 ppm
Mouse[b] 250, 1,000, or 2,500 ppm 6 hr/day during gestational days 6–15	Adult females: no effects Fetuses: no effects
Rabbit[c] 1,000, 4,000, or 8,000 ppm 6 hr/day during gestational days 6–18	Adult females: hypoactivity and ataxia at 8,000 ppm; ↓ weight gain at 4,000 and 8,000 ppm; ↑ liver weight relative to body weight at 8,000 ppm Fetuses: no effects

[a] Data from Tyl and Neeper-Bradley 1989.

[b] Data from Bio/dynamics 1984a.

[c] Data from Tyl 1989.

Table 28. Studies of Maternal Ethanol Ingestion and the Neurobehavioral Outcomes Observed in Rodent Pups

Species	Ethanol Intake	Blood Ethanol Concentration	Effects
Mouse[a]	25% of maternal caloric intake on gestational days 5–10, 12–17, or 5–17	260 mg% on gestational day 8; 100 mg% on gestational day 17	No effects from exposure on gestational days 5–10 Decreased fixed ratio performance (in M more than F) from exposure on gestational days 12–17 or 5–17
Rat[b]	35% of maternal caloric intake on gestational days 6–19	300 mg%	Lower latency to nipple attachment and hyperactive during early development Elevated or depressed motor activity and impaired avoidance performance during later development

[a] From Gentry and Middaugh 1988 and Middaugh and Gentry 1992.

[b] From Chen et al. 1982 and Meyer and Riley 1986.

Table 29. Studies of Ethanol and the Changes It Induces in the Rat Pup Brain

Route, Time of Exposure, and Dose	Blood Ethanol Level (mg%)	Effects
Inhalation on gestational days 1–19, 16,000 ppm for 7 hours/day[a]	Maternal: 150–200	No changes in these brain neurotransmitters: norepinephrine, dopamine, acetylcholine, met-enkephalin, β-endorphin, substance P
Oral on gestational days 13–18, 5 g/kg each day[b]	Maternal: 266	No change in cerebellar Purkinje cell density
Inhalation on postnatal day 3 or days 3 and 4 (dose not specified)[c]	Pups: > 550	Damaged Purkinje cells
Inhalation on postnatal days 3–20, 34,000–40,000 ppm for 3 days[d]	Pups: 239	Brain growth stunted, particularly at cerebellar area
Oral on postnatal days 4–9, 2.5 g/kg each day[b]	Pups: 205	Reduced cerebellar Purkinje cell density

[a] Data adapted from Nelson et al. 1988.

[b] Data adapted from Marçussen et al. 1994.

[c] Data adapted from Phillips and Cragg 1982.

[d] Data adapted from Bauer-Moffett and Altman 1977.

Table 30. Studies of Maternal Ethanol Ingestion During Pregnancy and the Neurobehavioral Outcomes Observed in Children at Different Developmental Stages

Developmental Stage	Effects[a]
Early postnatal infancy	• Poorer habituation and low arousal on the Brazelton Neonatal Assessment Scale • Lower sucking pressure as measured on a specially-designed nipple
Later infancy	• Dose-dependent slowing of reaction time (Jacobson et al. 1994)
Age 4 years	• Attention deficits, as measured by various indices on a test of vigilance performance designed for children • Deficiencies in both fine and gross motor control • Lower scores on the Wechsler Preschool and Primary Scale of Intelligence (WPPSI)
Age 7 years	• Decreased performance on a vigilance test, indicating attention deficits • Lower scores on the Wechsler Intelligence Scale for Children-Revised (WISC-R) • Lower scores on achievement tests for reading, spelling, and arithmetic
Age 14 years	• Deficient performance on a task requiring the sounding out of nonsense words • Deficient ability to carry out arithmetic problems presented verbally • Problems with attention, short-term memory, response inhibition, and learning of spatial relationships

[a] Data are from Streissguth et al. 1994 unless otherwise indicated.

Potential Health Effects of Oxygenates

Long-Term Effects

INTRODUCTION

This section reviews what is known about the long-term effects of exposure to fuel oxygenates, including the induction of nonneoplastic lesions, cancer, and other diseases. When human studies are available, they are evaluated; however, for the ether additives (e.g., MTBE, ETBE, and TAME), such information is not available. For these compounds, the current understanding of the potential hazards of prolonged exposure is based on the results of animal bioassays and, in some cases, in vitro studies. Whenever possible, information from the peer-reviewed literature is presented; however, for some compounds, only contractors' reports were available.

The scientific evidence pertaining to the toxicity and carcinogenicity of long-term exposure to the ether oxygenates, MTBE (and its metabolites TBA and formaldehyde), ETBE, and TAME, is reviewed first. Then putative mechanisms of carcinogenicity of these compounds and their relevance for humans exposed in ambient or occupational settings are discussed. Following the evaluation of the ethers, the effects of prolonged exposure to ethanol are considered. Finally, conclusions are presented.

The reader should note that although the following evaluation discusses each compound as an isolated entity, actual human exposures are much more complex. Humans are generally exposed to complex mixtures of gasoline and oxygenates, and little information is available on the toxicity of gasoline-oxygenate mixtures. Furthermore, gasoline emissions contain a large number of hydrocarbons, some of which, such as benzene and 1,3-butadiene, are known or suspected carcinogens. Adding oxygenates to gasoline at a level of 10% to 15% may decrease exposure to some of the hydrocarbons in gasoline (see Appendix A) while it increases exposure to the oxygenates and aldehydes.

ETHERS

MTBE

MTBE is the most common oxygenate that is blended with gasoline. Because of the possible long-term environmental exposure of humans to MTBE, it is critical to determine the potential health effects of long-term exposure to this fuel additive and its metabolites.

Human Studies

No epidemiologic studies of the health effects of prolonged exposures to MTBE have been conducted. Because of this lack of human data, the potential human cancer risk of MTBE exposure must be estimated using data from studies such as long-term animal bioassays and in vitro assays for genotoxicity.

Animal Studies

Three studies designed to examine the health effects of long-term exposure to MTBE in laboratory animals have been reported. These bioassays used laboratory rodents (i.e., rats or mice) and various exposure regimens. Two studies used inhalation as the route of exposure, and one used oral administration. Two studies used laboratory rats, but different strains (i.e., Sprague-Dawley and F344). The results of these studies are summarized in Table 31 and discussed below.

Carcinogenicity Bioassay by Intragastric Administration in Sprague-Dawley Rats Sprague-Dawley rats were repeatedly exposed to MTBE by oral gavage in a chronic bioassay conducted by Belpoggi and coworkers (1995). In this study, rats in control, low-dose, and high-dose groups were given MTBE (> 99% purity) at 0, 250, or 1,000 mg/kg body weight, respectively, in olive oil, once a day, 4 days/week, for 104 weeks. Each treatment group contained 60 male and 60 female, 8-week-old Sprague-Dawley rats at the beginning of the study. Rats were allowed to live until their natural deaths (i.e., there were no designated death time points). Complete necropsies were performed on all animals. Using histologic analysis, it was found that MTBE caused a dose-related increase in lymphohematopoietic tumors (i.e., lymphomas and leukemias) in female rats (2/60 in control rats, 6/60 in the low-dose group, and 12/60 in the high-dose group) (Table 32). The investigators did not describe the morphologic features of the induced lymphomas and leukemias, and combined the two lesions as one tumor type for statistical analysis, rather than maintaining two distinct neoplastic classifications. Increases in the incidence of lymphohematopoietic tumors related to MTBE were not evident in male rats. The major MTBE-related

nonneoplastic lesion was a dysplastic proliferation of lymphoreticular tissue in female rats exposed to MTBE. The authors did not report that this hyperplastic response in lymphoid tissues occurred in male rats exposed to MTBE.

Two important limitations of this report are that the investigators (1) did not identify the types of lymphohematopoietic tumors observed in the vascular and extravascular tissues, and (2) did not explain why the lymphomas and leukemias were analyzed as one neoplastic lesion. Concerns about how these tumor data were analyzed have been raised (Mennear 1995). Scientific guidelines and criteria for combining neoplasms when interpreting data from rodent carcinogenesis studies have been published by a panel of pathologists assembled by the National Toxicology Program (McConnell et al. 1986). They recommended that lymphomas and leukemias be analyzed separately. If, however, the neoplastic lesions in the present study were composed of similar cell types (e.g., mononuclear cells) and the lymphomas were interpreted as extravascular invasions of leukemic cells, as is commonly observed in F344 rats with mononuclear cell leukemia (Boorman et al. 1990), then combining the lymphomas and leukemias may have been an appropriate analytic approach. Important new information for understanding the pathogenesis of these tumors, however, would still have been gained by identifying the principal neoplastic cell types observed in the vascular and extravascular compartments of the affected organs, and by determining the incidence levels of animals with only leukemias, those with only lymphomas, and those with both lymphohematopoietic tumors.

Belpoggi and coworkers (1995) also found a greater incidence of interstitial cell tumors of the testes, compared with the control group, in male rats exposed to MTBE at 1,000 mg/kg body weight (2/60 control rats, 2/60 in the low-dose group, and 11/60 in the high-dose group) (Table 32). In addition, a possible increase in uterine sarcomas was evident in female rats in the low-dose group, but not in female rats in the high-dose group (1/60 in the control group, 5/60 in the low-dose group, and 0 in the high-dose group). Also noted were possible decreases in the incidence of fibromas and fibroadenomas of the mammary gland (40/60 in the control group, 27/60 in the low-dose group, and 16/60 in the high-dose group), pituitary gland adenomas (22/60 in the control group, 16/60 in the low-dose group, and 13/60 in the high-dose group), and adrenal gland pheochromocytomas (18/60 in the control group, 11/60 in the low-dose group, and 10/60 in the high-dose group) in treated female rats. All of these decreases in tumor incidence were thought to reflect an increase in early deaths in these groups.

Carcinogenicity Bioassay by Inhalation in F344 Rats An inhalation bioassay to determine the carcinogenicity of MTBE was conducted at the Bushy Run Research Center (Chun et al. 1992). The investigators divided F344 rats, 6 to 7 weeks of age at the start of the study, into filtered-air control, low-exposure, middle-exposure, and high-exposure groups (n = 50 males and 50 females per group) and exposed them to 0, 400, 3,000, or 8,000 ppm MTBE (99% purity), respectively, for 6 hours/day, 5 days/week, for 24 months. After death, complete necropsies and extensive histologic examinations were performed on these rats. The mean survival time of male rats was significantly reduced in all the MTBE-exposed groups compared with the control group (632 days for control rats, 617 days for the low-exposure group, 587 days for the middle-exposure group, and 516 days for the high-exposure group). In addition, the body weights of both male and female rats in the high-exposure groups were significantly lower than those of control animals.

Because of the excessive mortality observed in the animals exposed to 3,000 or 8,000 ppm MTBE, animals in these two experimental groups were killed before the end of 24 months of exposure (at 97 and 82 weeks, respectively).

The principal nonneoplastic (both gross and microscopic) changes associated with MTBE exposure were increased incidence and severity of chronic nephropathy. Increased incidence of nephropathy in male rats was evident at all levels of exposure. A compound-related increase in the incidence of this renal lesion was observed in female rats exposed to 8,000 ppm MTBE. In addition to the MTBE-related renal lesions, female rats exposed to 3,000 or 8,000 ppm MTBE had increased liver weights. Because no histopathologic findings were observed in the livers of these female rats, the increased liver weights were assumed to be due to a metabolic response (i.e., microsomal enzyme induction) to MTBE exposure.

The principal neoplastic findings in this study were MTBE-related increases in renal and testicular tumors in male rats (Tables 33 and 34, respectively). Chun and coworkers (1992) reported increased incidence of renal tumors (renal tubular adenomas and carcinomas combined) in the male rats exposed to MTBE compared with control animals (1/50 in the control group, 0/50 in the low-exposure group, 8/50 in the middle-exposure group, and 3/50 in the high-exposure group). Three of the eight renal tumors in the middle-exposure group were carcinomas. The investigators found no carcinomas in the other exposure groups. Two male rats in the low-exposure group were reported to have preneoplastic adenomatous hyperplasias in renal tubules. Interestingly, the investigators reported one renal tubular adenoma in the female rats exposed to 3,000 ppm MTBE. The significance of these renal tumors for human risk assessment will be discussed later in this section.

Chun and coworkers (1992) also found MTBE-related increases in the incidence of interstitial cell tumors of the testes (32/50 in the control group, 35/50 in the low-exposure group, 41/50 in the middle-exposure group, and 47/50 in the high-exposure group) (Table 34). The investigators noted that this type of testicular tumor has a high spontaneous rate of occurrence in aged F344 rats (Boorman et al. 1990). However, in this study the incidence of the testicular adenomas in the control rats was unusually low (64%) compared with that in other studies conducted in their laboratory (86% to 91%). Although the incidence in the group exposed to 8,000 ppm MTBE (94%) was higher than in the control group, it was within the range previously reported for aged F344 rats. Therefore, whether the increased incidence of testicular adenomas observed in this study was truly related to exposure is open to debate. In addition, the usefulness of compound-associated increases in the incidence of this specific rat neoplasm for predicting human carcinogenic responses has been questioned by some investigators (Prentice and Meikle 1995).

Carcinogenicity Bioassay by Inhalation in CD-1 Mice A long-term inhalation exposure of mice was conducted at the Bushy Run Research Center. Burleigh-Flayer and coworkers (1992) divided CD-1 mice, 6 to 7 weeks of age at the start of the study, into filtered-air control, low-exposure, middle-exposure, and high-exposure groups of 50 males and 50 females, and exposed the mice to 0, 400, 3,000, or 8,000 ppm MTBE (99% purity), respectively, for 6 hours/day, 5 days/week, for 18 months. At the end of the scheduled exposure period, they performed complete necropsies along with standard histologic analyses. In this study, only the male mice in the high-exposure group had an increased mortality with a decreased survival time compared with the control group (mean survival of 510 days for control animals and 438 days for mice exposed to 8,000 ppm MTBE). The only MTBE-related increased incidence of nonneoplastic lesions was hepatocellular hypertrophy in both male and female mice exposed to 8,000 ppm MTBE and in males exposed to 3,000 ppm. The principal neoplastic findings were: (1) an increased incidence of hepatocellular carcinomas in the male rats exposed to 8,000 ppm MTBE (2/49 in the control rats, 4/50 in the low-exposure group, 3/50 in the middle-exposure group, and 8/49 in the high-exposure group) (Table 35); and (2) an increased incidence of hepatocellular tumors (adenomas and carcinomas combined) in female animals exposed to 8,000 ppm MTBE (2/50 in the control group, 1/50 in the low-exposure group, 2/50 in the middle-exposure group, and 11/50 in the high-exposure group) (Table 36). Liver tumors in the female mice were predominantly adenomas, but did include one hepatocellular carcinoma in the low-exposure group (400 ppm MTBE) and one in the high-exposure group (8,000 ppm MTBE). An increased incidence of total hepatocellular tumors (adenomas and carcinomas combined) also occurred in male mice exposed to 8,000 ppm (12/50 control rats, 12/50 rats in the low-exposure group, 12/50 in the middle-exposure group, and 16/50 in the high-exposure group); however, this incidence was not significantly different from that in the control group when the data were analyzed using Fisher's Exact Test. It must be noted, however, that this method of statistical analysis does not adjust for differences in survival between control and exposure groups.

The duration of the exposure period in this long-term bioassay was 18 months, which is shorter than the 24-month period routinely used by the National Toxicology Program in its inhalation bioassays. Therefore, it is possible that additional, late-developing tumors may have been detected if this study had been continued through 24 months.

In Vitro Assays

MTBE has been tested for genotoxicity with generally negative results. MTBE was neither toxic nor mutagenic in studies using the *Salmonella* mutation (Ames) assay (Life Science Research 1989a; Cinelli et al. 1992), nor did exposing primary rat hepatocytes to MTBE in culture cause unscheduled DNA synthesis, an indicator of DNA damage (Life Science Research 1989b; Cinelli et al. 1992). As part of the inhalation carcinogenicity bioassay study conducted by Bushy Run Research Center (1989), bone marrow cells from male and female rats were analyzed for chromosomal aberrations. No MTBE-related chromosomal damage or increase in micronuclei was detected in these cells. Ward and coworkers (1995) also reported that oral administration of MTBE (1, 10, 100, or 1,000 mg/kg) to male and female CD-1 mice for 3 weeks did not produce mutations at the *hprt* locus of lymphocytes. In addition, MTBE did not induce sex-linked recessive lethal mutations in the fruit fly, *Drosophila melanogaster*, when administered at 0.03%, 0.15%, or 0.30% in its food (Hazelton Laboratories America 1989). The only report of MTBE-induced genotoxicity is an abstract indicating that it is mutagenic in an S9-activated mouse lymphoma assay (McGregor et al. 1988), a response that has been attributed to formaldehyde production (Garnier et al. 1993).

Summary of MTBE Studies

Evidence from animal bioassays demonstrates that long-term, high-level exposures to MTBE by either the oral or

inhalation routes of exposure cause cancer in rodents. Inhalation exposure to MTBE produced an increased incidence of renal and testicular tumors in male rats and hepatic tumors in mice. Oral administration of MTBE produced an increased incidence of lymphomas and leukemias in female rats and testicular tumors in male rats.

A number of concerns arise when extrapolating these findings to humans. First, the increased tumor incidences were observed at exposure levels of MTBE that were toxic. Second, each of the animal bioassays had notable technical limitations that may have biased the results. Third, some of the tumors are of questionable relevance because they may be a species-specific phenomenon involving cytotoxic responses to the high-dose exposure regimen. However, at the present time, the mechanism by which MTBE causes cancer in rodents is not known. Physiologically based pharmacokinetic models for MTBE and its metabolites have to be developed to estimate properly, from experimental data, the potential cancer risk of MTBE in humans.

METABOLITES OF MTBE: TBA AND FORMALDEHYDE

Chemically induced cancers result from the combined effects of the parent compounds and their associated metabolites. Evidence derived largely from animal and cellular studies indicates that MTBE is oxidatively demethylated to produce TBA and formaldehyde, which is ultimately converted to carbon dioxide and water. At present, we do not have a complete understanding of how MTBE is metabolized in humans, or of the rates at which MTBE metabolites are formed in different tissues. Both TBA and formaldehyde are potential carcinogens. Many publications discuss the association between formaldehyde and cancer; the literature on TBA carcinogenicity, however, is limited to one animal bioassay. The following discussion presents the key findings regarding the carcinogenicity of TBA and formaldehyde; the animal bioassay results are summarized in Table 31. There are other MTBE metabolites, for example, 2-methyl-1,2-propanediol and 2-hydroxyisobutyrate; however, no data are available regarding their carcinogenicity.

TBA

TBA is a major metabolite of MTBE and, in rats, is eliminated mainly via the lungs and the kidneys. Some studies indicate that it has a longer half-life than MTBE, which underscores the importance of considering TBA-induced responses when evaluating the potential carcinogenicity of MTBE.

Human Studies Although TBA is used in a variety of consumer products and is a major U.S. commodity, no information on its carcinogenicity in humans has been published.

Animal Studies Information on the carcinogenicity of TBA is derived largely from a National Toxicology Program bioassay in which two animal species were exposed to TBA in drinking water for 2 years (Cirvello et al. 1995; National Toxicology Program 1994).

In this study, rats were divided into control, low-dose, middle-dose, and high-dose groups; male F344/N rats received doses of 0, 1.25, 2.5, or 5 mg TBA/mL drinking water, respectively; female rats received 0, 2.5, 5, or 10 mg/mL, respectively. After 15 months of exposure, mineralization of the kidneys was evident in exposed males and nephropathy was evident in both males and females, although the males were more severely affected. These renal lesions are common in aged F344/N rats, and from the data presented by Cirvello and coworkers (1995), it seems that all of the animals, including the control group, showed evidence of nephropathy after 2 years on the experimental protocol. This lesion was classified as minimal to mild in the kidneys of control female rats and mild to moderate in TBA-exposed male animals. In addition, transitional epithelial hyperplasia of the kidney was associated with TBA exposure in both male and female rats. An increase in the incidence of kidney tumors (adenomas and carcinomas) was noted in male rats in the TBA-treated groups (13/50 in the low-dose group, 19/50 in the middle-dose group, and 13/50 in the high-dose group, compared with 8/50 in the control group). No neoplasms were observed in the female rats.

In the same study, male and female $B6C3F_1$ mice received TBA at 0, 5, 10, or 20 mg/mL drinking water. In mice, Cirvello and coworkers (1995) reported an increase in the incidence of follicular cell hyperplasia of the thyroid gland in males and females exposed to high concentrations of TBA. The authors considered this lesion to be a precursor to thyroid gland neoplasms, which were increased in female mice in the high-dose group (9/59 compared with 2/58 in the control group). The investigators also recorded a small, and not statistically significant, increase in the incidence of thyroid gland neoplasms in male mice in the middle-dose group (4/59 compared with 1/60 in the control group). TBA-related inflammation and hyperplasia of the urinary bladder also were reported in male and female mice exposed to 20 mg/mL drinking water, although the lesions were more severe in male animals.

In Vitro Assays The National Toxicology Program tested TBA for genetic toxicity (both with and without metabolic activation) and reported that it was not mutagenic in either

the *Salmonella* mutation (Ames) assay or the mouse lymphoma cell assay (National Toxicology Program 1994). Furthermore, it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells. In vivo testing showed no increase in the frequency of micronucleated erythrocytes in the blood of mice treated with TBA in drinking water for 13 weeks.

Formaldehyde

Current evidence indicates that MTBE is metabolized to formaldehyde, which is converted to formate and ultimately to carbon dioxide (see Figure 5 in the Metabolism and Disposition section). Formaldehyde, a simple but highly reactive compound, is both an air pollutant and a normal metabolic intermediate that is present in most human tissues. Some studies suggest that the rate at which formaldehyde is produced in the body is a critical factor in determining its toxicity and the body's carcinogenic response. Key factors in evaluating the potential carcinogenicity of formaldehyde in the context of MTBE exposure are the rates at which formaldehyde is formed from MTBE in different species and how this rate compares with the species' endogenous production.

Exposure to formaldehyde occurs in many settings, including occupational environments, outdoor and indoor air, and where cigarettes are smoked. The extensive literature on its potential toxicity and carcinogenicity includes evaluations by the EPA (Grindstaff et al. 1991) and the International Agency for Research on Cancer (IARC) (1982, 1987, 1995). Most of the scientific evidence is based on inhaled formaldehyde, and for this route of long-term exposure, the most notable effects in humans and animals are on the nasal mucosa. The following summary is based largely on the recent evaluation by IARC (1995) and reviews by McLaughlin (1994) and Partanen (1993).

Human Studies An IARC working group (1995) recently concluded that nasopharyngeal cancer was consistently associated with formaldehyde exposure in professional and industrial workers; this type of neoplasm increased in frequency in two of six cohort studies and in three of the four case-control studies. Some reviewers, after adjusting for confounding factors, concluded that no association between formaldehyde exposure and cancer can be found (McLaughlin 1994). However, the IARC (1995) noted that two metaanalyses of more than 30 studies (Blair et al. 1990; Partanen 1993) found an increase in the relative risk of nasopharyngeal cancers in workers exposed to formaldehyde. This relative risk was statistically significant and highest (2.1 to 2.7) in subjects classified as having "substantial" exposure to formaldehyde. The IARC working group noted that the observed associations "cannot reasonably be attributed to other occupational agents" and that "taken together, the epidemiological studies suggest a causal relationship between exposure to formaldehyde and nasopharyngeal cancer, although the conclusion is tempered by the small numbers of observed and expected cases in the cohort studies" (International Agency for Research on Cancer 1995).

Some studies also suggest an increased risk of cancer of the nasal cavities and paranasal sinuses in formaldehyde-exposed workers; however, the results of the cohort and case-control studies are not consistent. The International Agency for Research on Cancer also summarized the evidence of histopathologic abnormalities in nasal biopsy samples collected from people exposed to formaldehyde in residential or occupational settings, and noted that many of the findings were not statistically significant and were often confounded by concomitant exposure to other air pollutants. The epidemiologic studies do not suggest any excess risks of other cancer types (including lymphatic or hematopoietic cancers).

Animal Studies Cancer bioassays in which animals were exposed to formaldehyde by inhalation consistently show an increase, at exposures higher than 5.6 ppm, in the induction of squamous cell carcinomas in the nasal cavities of rats (Swenberg et al. 1980; Kerns et al. 1983; Morgan et al. 1986), but not of hamsters (Dalbey 1982). Studies of mice show only a weak response at comparable doses of formaldehyde (Kerns et al. 1983). Prominent features of the rat response are the nonlinear dose-response (Swenberg et al. 1985) and the concomitant increase in nasal cell proliferation and associated nonneoplastic lesions at the sites of tumor development (Monticello et al. 1993).

Nasal metabolism of formaldehyde appears to be closely related to the effects observed in long-term inhalation studies, including possible cancer induction. Swenberg and coworkers (1995) found that the dose-response of covalently bound formaldehyde was nonlinear. Heck and Casanova (1987) observed 4- to 7-fold fewer DNA-protein cross-links at low doses than at high doses, and concluded that cancer induction increased sharply at high doses of formaldehyde due to a saturated glutathione-dependent detoxification pathway. A 2.5-fold increase in formaldehyde dose resulted in a 50-fold increase in squamous cell carcinomas in the noses of rats, from a 1% increase in carcinomas at 5.6 ppm to a 50% increase at 15 ppm (no increase was noted at 2 ppm) (Kerns et al. 1983). These findings were confirmed by Monticello and coworkers (1993), who observed a correlation of cell proliferation and cancer induction at formaldehyde exposure concentrations between 5.6 and 15 ppm, but not between 2 and 5.6 ppm. These results are consistent with the hypothesis that the

main detoxification pathway for inhaled formaldehyde in this species becomes saturated at high exposure concentrations.

The IARC working group (1995) noted that "in rodents and monkeys, there is a no-observable-effect level (2 ppm) with respect to cell proliferation and tissue damage in otherwise undamaged nasal mucosa"; the group "considered these responses as contributing to subsequent development of cancer. Although these findings provide a basis for extrapolation to humans, conclusive data demonstrating that such cellular and biochemical changes occur in humans exposed to formaldehyde are not available."

Most studies of formaldehyde toxicity have focused on inhalation as the route of administration. Some information also is available on the oral toxicity of formaldehyde (reviewed by International Agency for Research on Cancer 1995; Restani and Galli 1991). When formaldehyde was administered in drinking water to rats, the most notable pathological changes were lesions of the forestomach and glandular stomach in animals that received formaldehyde at approximately 50 to 100 mg/kg body weight per day. At present, information is not available to assess the relevance of the bioassay data to formaldehyde production after inhalation of MTBE.

Although the carcinogenicity of inhaled formaldehyde (at concentrations greater than 5.6 ppm) has definitely been established in rats, there is less certainty about its effects in animals administered formaldehyde in drinking water (reviewed by Restani and Galli 1991, and International Agency for Research on Cancer 1995). Til and coworkers (1989) conducted a 2-year drinking water study of Wistar rats treated at dose levels of formaldehyde of 0, 1.2, 15, and 82 mg/kg/day for the male animals and 0, 1.8, 21, and 109 mg/kg/day for the females. The highest test dose caused damage to the gastric mucosa of both the forestomach and the glandular stomach but did not produce gastric tumors or tumors at other sites. Similar results were reported for a smaller study conducted by Tobe and coworkers (1989). They treated Wistar rats for 24 months with 0%, 0.02%, 0.10%, or 0.50% formaldehyde in their drinking water (estimated by IARC [1995] to correspond to approximately 0, 10, 50, and 300 mg/kg/day), and reported nonneoplastic gastric lesions in rats given the highest dose of formaldehyde.

In contrast, positive evidence of carcinogenicity was reported by Soffritti and coworkers (1989). The latter group of investigators administered formaldehyde (0, 10, 50, 100, 500, 1,000, and 1,500 mg/L) in drinking water to Sprague-Dawley rats; animals treated with either untreated water or water containing methyl alcohol (15 mg/L) served as controls. The investigators reported treatment-related increases in the incidence of lymphoblastic leukemias and lymphosarcomas at the three highest test doses and an increase in intestinal tumors at the highest dose. Concerns about this study have been noted by Feron and coworkers (1990) and the IARC working group (1995). The IARC working group reanalyzed the available data and reported a statistically significant linear dose-response relationship for both tumor types. They also reported that the incidence of leukemias in the treated groups differed significantly from that in the drinking water controls ($p \leq 0.01$), but not from that in the controls given methanol.

The results of the three drinking water studies are in agreement that repeated oral exposure to relatively large amounts of formaldehyde produces nonneoplastic changes in the gastric mucosa of rats. Two studies found no evidence of carcinogenicity; one study reported an increased incidence of gastrointestinal tumors and leukemias.

In Vitro Assays In contrast to MTBE and TBA, formaldehyde is genotoxic in a wide variety of experimental systems (International Agency for Research on Cancer 1995). It reacts in vitro with cellular macromolecules to produce DNA damage in animal tissues, DNA-protein cross-links, and DNA single-strand breaks in human cells. Other genotoxic effects in vitro include chromosomal aberrations, sister chromatid exchanges, and gene mutations. Under certain experimental conditions, formaldehyde also induces genotoxic effects in bacteria, yeast, fungi, and insects.

Key considerations in evaluating the potential carcinogenicity of formaldehyde as an MTBE metabolite are (1) whether comparable effects are produced in tissues other than the nasopharynx, (2) whether the rate of formaldehyde production following exposure to ambient levels of MTBE would be sufficiently high for this unstable metabolite to be toxicologically important, and (3) whether the rate at which humans metabolize MTBE to produce formaldehyde and then formate is faster or slower than that of rodents.

Summary of MTBE Metabolite Studies

MTBE is metabolized to at least two intermediates that have carcinogenic potential: TBA and formaldehyde. Scientists have not evaluated the carcinogenicity of TBA in any human studies. A limited number of tests indicate that it is not genotoxic. One carefully conducted National Toxicology Program bioassay demonstrated that exposure to high concentrations of TBA in drinking water produces tumors in two rodent species: kidney tumors in male rats, and follicular cell adenomas in the thyroid glands of mice. The mechanism of TBA-induced tumor formation in rodents has not been established. It is possible that these tumors may result from nongenotoxic mechanisms and may be secondary to the cytotoxic effects produced by the high-

dose regimen of the animal bioassay. However, at present, the experimental evidence to support this hypothesis is lacking.

Formaldehyde, another MTBE metabolite, is a normal metabolic intermediate as well as a ubiquitous air pollutant. It is genotoxic in a variety of experimental systems and, when inhaled, can be carcinogenic. Inhalation of high concentrations of formaldehyde (greater than 5.6 ppm) induces squamous cell carcinomas in the nasal cavities of rats, an effect that some scientists consider to be driven by formaldehyde-induced proliferative and cytotoxic responses. Epidemiologic studies have established an association between exposure to formaldehyde in the workplace and an increased incidence of certain types of nasal cancers. Both the epidemiologic and toxicologic data relate to formaldehyde exposures by inhalation and not to exposures due to endogenous formaldehyde production from MTBE metabolism. The impact of these differences is not well understood.

OTHER ETHERS

ETBE is one of the alternative ethers that may be added to gasoline to reduce motor vehicle emissions. No information is available regarding its potential carcinogenicity in either humans or animals. The IIT Research Institute (1991) conducted a 4-week inhalation toxicity study of ETBE. Sprague-Dawley rats were exposed by inhalation to 0, 500, 2,000, or 4,000 ppm ETBE vapors for 6 hours/day, 5 days/week. In the absence of any evidence of infection, a statistically significant increase in white blood cells was noted in female rats exposed to 2,000 ppm or 4,000 ppm ETBE. Kidney weights also increased in male rats exposed to 4,000 ppm ETBE; however, there was no histologic evidence of nephropathy (see Table 13). ETBE was negative in mutagenicity assays in bacteria and Chinese hamster ovary cells, in a bone marrow micronucleus test in mice, and in an in vitro chromosome aberration assay (Zeiger et al. 1992; Vergnes 1995; Vergnes and Kubena 1995a,b).

On the basis of structural considerations, acetaldehyde is predicted to be an ETBE metabolite. Acetaldehyde is genotoxic in bacterial and mammalian systems, and at sufficiently high concentrations, it produces cancer in the upper airways of rodents (reviewed by Leikauf 1992). In evaluating the relative potential carcinogenicity of MTBE and ETBE, acetaldehyde may be more of a concern than the MTBE metabolite formaldehyde because of its greater stability.

TAME is another oxygenate that is structurally similar to MTBE and may be used as a fuel additive. As with ETBE, the information available is not adequate to judge its potential carcinogenicity. A recent report on the acute toxicity of TAME indicates that it was not mutagenic in five standard *Salmonella* strains, either with or without metabolic activation, and it was negative in the micronucleus assay (Daughtrey and Bird 1995). Daughtrey and Bird (1995) also conducted a 28-day subchronic toxicity study using an oral gavage route of exposure in which Sprague-Dawley rats were exposed to TAME (in corn oil) at 125, 500, or 1,000 mg/kg body weight. Mean body weights were lower in male rats exposed to 1,000 mg/kg, and dose-related increases in adrenal gland and kidney weights were measured in the male animals. The investigators reported no treatment-related histopathologic changes (see Table 13).

Structure-activity models have predicted that ETBE, TAME, and MTBE, but not DIPE, would be carcinogenic in rodents (Y.P. Zhang, O.T. Macina, H.S. Rosenkranz, M.H. Karol, and D.R. Mattison, personal communication). These predictions for ETBE, TAME, and DIPE should be interpreted cautiously until they have been evaluated experimentally.

In summary, at present, the data available are not adequate to assess the toxic and carcinogenic effects of long-term exposure to ETBE, TAME, or DIPE.

POSSIBLE MECHANISMS OF MTBE-INDUCED CARCINOGENICITY

As discussed above, the animal studies clearly indicate that under certain experimental conditions MTBE and its metabolites, TBA and formaldehyde, are rodent carcinogens. At present, the applicability of the animal carcinogenicity results to human risk assessment is the subject of considerable debate (Lucier et al. 1995; Maltoni and Soffritti 1995; Medlin 1995; Rudo 1995). The questions at the core of the debate are what the mechanisms are by which oxygenates cause tumors in rodents under the conditions of the bioassays, and whether such mechanisms operate in humans under ambient exposure conditions.

Regulatory agencies are increasingly incorporating information on mechanisms of carcinogenesis into common risk assessments (Vainio et al. 1992; U.S. Environmental Protection Agency 1994a; National Academy of Sciences/National Research Council 1994). For example, some agents that increase tumor incidence in animals do so by mechanisms that may be species-specific or are secondary responses to the high-dose regimen used in the study. In either case, such agents may not pose a carcinogenic threat to humans because the mechanism does not operate in other species or does not occur unless the dose is sufficiently high to cause toxicity or other biological responses. Alternatively, mechanistic data might point to the carcinogenic potential of an agent even in the absence of epidemiologic

data or bioassay data. Although there is not yet a consensus on this issue, both the EPA and IARC are moving to include information on mechanisms in their evaluations of potential carcinogens in order to communicate the strength and the relevance of the experimental findings. The discussion below uses information derived from studies of other chemicals to make inferences about the possible mechanisms by which MTBE and TBA might cause tumors in rodents.

Genotoxic and Nongenotoxic Mechanisms

In considering possible mechanisms of carcinogenicity for any chemical, the first question is usually: Is the compound mutagenic? The in vitro and in vivo mutagenicity and clastogenicity tests that were performed with MTBE and its metabolite TBA provide no evidence of genotoxicity for either of the two compounds. However, the other major metabolite of MTBE, formaldehyde, is mutagenic, is carcinogenic in animals, and is possibly a human carcinogen. Therefore, it is possible that the carcinogenicity of MTBE in rodents involves both genotoxic and nongenotoxic mechanisms. Furthermore, it is conceivable that other, as yet unidentified genotoxic MTBE metabolites are formed in vivo (for further discussion see Metabolism and Disposition section).

The genotoxic mechanisms of action by which carcinogens effect changes, such as activating oncogenes and inactivating tumor suppressor genes, are the subject of intensive investigation. This topic has been amply reviewed in numerous articles (e.g., Vainio et al. 1992) and need not be further discussed here. Rather, possible nongenotoxic mechanisms that may be involved in MTBE- and TBA-induced rodent carcinogenesis are discussed.

No unifying theory has been advanced to date to explain the mechanism of action of nonmutagenic carcinogens. Most attempts to explain the carcinogenicity of nongenotoxic carcinogens are based on a great deal of conjecture and speculation. The lack of knowledge in this field is clearly expressed in the preface to the proceedings of a recent symposium entitled "Nongenotoxic Carcinogenesis" (Cockburn et al. 1994). In it the authors write:

> A nongenotoxic carcinogen can be defined as a compound which causes cancer, but which does not cause damage to DNA as its primary biological activity. This negative definition covers a range of carcinogens acting through a variety of mechanisms. Such chemicals often produce tumors only in a single organ and species, and there are few common locations which are affected most often. For example, in male rats, certain carcinogens bind to $\alpha_{2\mu}$-globulin to form a complex which accumulates in the kidney tubular cells, which is followed by necrosis and compensatory cell proliferation leading to neoplasia. Other common mechanisms include hormonal imbalance resulting in thyroid tumors or peroxisome proliferation resulting in liver cancer. . . . The majority of known human carcinogens are genotoxic, and, for this class of carcinogen, it is relatively straightforward to take steps to assure human safety. For nongenotoxic carcinogens, in contrast, much more information is needed such as species specificity, threshold effects, and mechanisms of action before rational, safe levels for humans can be set using appropriate safety margins.

The following discussion of the possible mechanisms involved in inducing cancer by exposing rodents to MTBE or TBA will, by necessity, be nonsystematic and rather speculative because the mechanism of action of such compounds is poorly understood. The question underlying this discussion is whether the particular tumorigenic response observed in the rodent studies is likely or unlikely to be relevant to humans and human exposure conditions, considering the probable or proposed mechanism of action of the compound in the particular species.

High-Dose Effects

In all of the studies with rodents, MTBE and TBA increased tumor incidence above that for control groups only at very high gastric or inhalation exposure concentrations (Burleigh-Flayer et al. 1992; Chun et al. 1992; Belpoggi et al. 1995), levels that would not be encountered by humans. For example, the exposure concentrations of MTBE that caused a statistically significant increase in neoplasms in the rodent inhalation bioassays were 3,000 to 4,000 ppm for 6 hr/day (Burleigh-Flayer et al. 1992; Chun et al. 1992). The general public is unlikely to be exposed to concentrations of MTBE any greater than 1 ppm (and possibly up to 10 ppm for short periods during refueling; see Exposure Assessment section). This is important to keep in mind in reviewing these studies because prolonged exposure to high concentrations of MTBE can directly or indirectly cause disturbances in the homeostasis and metabolism of organ systems; such disturbances would not occur at moderate or low exposure levels. The problem of extrapolating the effects seen at high doses to low doses is, of course, not unique to nonmutagenic carcinogens. However, it is a particularly puzzling problem with compounds like MTBE and TBA, for which the mechanism of toxicity is so poorly defined.

Tumor Responses by Organ Site

Exposing mice or rats to MTBE or its metabolite TBA has been shown to cause statistically significant tumor re-

sponses. After exposure to MTBE, renal and testicular tumors in male F344 rats (Chun et al. 1992), testicular tumors in male and lymphomas or leukemias in female Sprague-Dawley rats (Belpoggi et al. 1995), and liver tumors in male and female CD-1 mice (Burleigh-Flayer et al. 1992) have been documented. After exposure to TBA, renal tumors in male F344 rats and thyroid tumors in female $B6C3F_1$ female mice (Cirvello et al. 1995) have been reported.

Renal Tumors in Male F344 Rats The renal tumors induced by various chemicals in male F344 rats are thought to be secondary to $\alpha_{2\mu}$-globulin nephropathy, also called hyaline droplet nephropathy (for discussion, see U.S. Environmental Protection Agency 1991; Swenberg 1994). The $\alpha_{2\mu}$-globulin is produced in the liver under androgenic control (but low levels are also produced by female rats) and secreted by the kidney. The $\alpha_{2\mu}$-globulin accumulates in the lysozomes of the P2 segment of the nephron of male rats, where it can lead to necrosis, regenerative proliferation, and formation of granular casts. A large number of diverse chemicals have been shown to exaggerate the nephropathy and to bind reversibly to the $\alpha_{2\mu}$-globulin in the kidney, thus leading to delayed excretion. The accumulation of these chemical-$\alpha_{2\mu}$-globulin complexes in the kidney and the increased proliferation are thought to cause the renal tumor response that is dependent on species and gender (Swenberg 1994).

It is conceivable that the MTBE- and TBA-induced renal tumor response may involve similar pathogenetic mechanisms. However, not all the findings are consistent with this interpretation. First, an increased incidence in nephropathy also was observed in female F344 rats, and yet a significant excess of renal tumors did not develop (Chun et al. 1992). Furthermore, using $\alpha_{2\mu}$-globulin–specific antibodies developed in Dr. James Swenberg's laboratory, Fowler and Chun (1993) were unable to detect $\alpha_{2\mu}$-globulin immunoreactivity in the kidneys of male F344 rats exposed for 13 weeks to MTBE. It is possible that other proteins that are related to $\alpha_{2\mu}$-globulin, or have similar characteristics, might be involved. The point is that although it appears that the hyaline nephropathy in male F344 rats may be rightfully implicated as a potential factor in the pathogenesis of the renal tumor response, other factors also may be involved.

Testicular Tumors in Male F344 and Sprague-Dawley Rats Increased incidences of testicular tumors were observed following MTBE exposure in F344 rats (Chun et al. 1992), which have a high incidence of spontaneous testicular tumors, and in Sprague-Dawley rats (Belpoggi et al. 1995), which have a low spontaneous incidence of such tumors.

A large number of diverse mutagenic and nonmutagenic compounds induce testicular tumors in rats and mice (see Prahalda et al. 1994). Although the precise mechanisms are not clear and are likely to vary depending on the chemical or experimental manipulation in question, hormonal dysregulations seem to play an important role in many instances. In contrast to mice, estrogen administration does not produce testicular tumors in rats. The procedures known to cause testicular tumors in rats are (1) ligating the testicular blood supply; (2) administering cadmium salts, which cause acute damage to the testicular vasculature with testicular necrosis (Gunn et al. 1965); and (3) intrasplenically grafting testicular tissue into castrated recipients (which results in the pituitary gland secreting excessive amounts of gonadotropins). Several pharmacologic agents have been shown to cause Leydig cell hyperplasia or Leydig cell tumors in rats. Buserelin (Donaubauer et al. 1987), an analogue of the natural gonadotropin-releasing hormone, luteinizing hormone–releasing hormone, has caused hyperplasia, and the calcium channel blocker SDZ 200-110 (Roberts et al. 1989) has caused tumors, presumably either by increasing gonadotropins (which stimulate Leydig cells to grow) or by increasing the hormone receptors on Leydig cell membranes.

Because of the high levels of prostaglandin H_2 synthase in the testes of various species including rats (Christ and Van Dorp 1972), it is also possible that the increase in testicular tumor incidence following MTBE exposure results from local production of mutagenic metabolites, including formaldehyde. Cooxidation reactions catalyzed by PHS have been shown to result in the formation of mutagenic metabolites (Marnett and Eling 1983) (also see the Metabolism and Disposition section).

Liver Tumors in Female CD-1 Mice In the study of Burleigh-Flayer and coworkers (1992), in which the liver tumor incidence increased in female CD-1 mice exposed to MTBE, a concentration-dependent increase in liver weights also was noted in both males and females. This clearly indicates that the liver was a target organ for MTBE at the exposure concentrations used in that study. It has been suggested that the increase in liver weight, which also was observed in the rat studies with MTBE (Chun et al. 1992), may be related to cytochrome P-450 enzymes being induced, which seems to be possible with exposure to MTBE.

A diverse group of nongenotoxic chemicals have been found to induce liver tumors in rodents (for review, see Schulte-Hermann et al. 1994). Almost all of the nonmutagenic rodent hepatocarcinogens induce cell proliferation and hyperplasia in the liver, and many also induce drug-metabolizing enzymes, peroxisomal enzymes, or enzymes of other metabolic pathways (Lake 1994; Schulte-Hermann et al. 1994). The mechanistic relationship between induction of such enzymes and cell proliferation is poorly understood. Many of the nonmutagenic carcinogens that act on

rodent livers also have been shown to be classic tumor promoters, enhancing the development of neoplastic lesions from preneoplastic liver foci induced by low doses of a genotoxic carcinogen. Evidence suggests that this type of tumor promoter also may promote spontaneously initiated cells, which are common in mouse strains that have a high incidence of spontaneous liver tumors (Lake 1994; Drinkwater 1994). It also has been suggested that some of the nongenotoxic rodent liver carcinogens may act by producing free radicals (e.g., peroxisome proliferators), which cause DNA damage by indirect mechanisms.

Hepatocarcinogenesis in mice (spontaneous and induced) has been shown to be under genetic control of the "risk modifier genes" (Drinkwater 1994), and under strong hormonal control. For example, castrating male mice results in a decrease in liver tumors, even though ovariectomies in female mice cause an increase in hepatic tumors. These hormonal influences are thought to be mediated by hormones controlling the proliferation of preneoplastic foci. It is conceivable that exposing mice to high concentrations of MTBE for a prolonged time causes imbalances in the hormonal homeostasis and thus influences the development of preneoplastic and neoplastic liver foci.

Thyroid Tumors in Female B6C3F$_1$ Mice Administering TBA orally caused an increased incidence of thyroid tumors in female B6C3F$_1$ mice. This could be the result of direct effects of TBA on the thyroid gland. However, xenobiotics that interfere with different steps of thyroid hormone synthesis or catabolism also can affect the thyroid gland indirectly, causing marked changes in the regulation of growth, ultimately resulting in thyroid tumor formation (Hill et al. 1989; Thomas 1994). For example, it is known that administering phenobarbital (and other chemicals that induce microsomal liver enzymes) increases metabolism of triiodothyronine (T_3) and tetraiodothyronine (T_4) in the liver, which in turn causes increased production of thyroid-stimulating hormone (TSH) by the pituitary gland. TSH is the major regulator of cell proliferation in the thyroid gland, and excess levels of TSH in the circulation have been shown to lead to thyroid adenomas and carcinomas (for review, see Hill et al. 1989; Thomas 1994). Even though no overt signs of liver toxicity were observed in the mice exposed to TBA (significant increases in liver weights were, however, observed in male and female rats at all dose levels, see National Toxicology Program 1994), it is possible that more subtle changes in liver function occurred, which may have resulted in increased metabolism of T_3 and T_4. As a result, TSH production could have caused increased thyroid growth and tumor formation.

Lymphomas and Leukemias in Female Sprague-Dawley Rats The increased incidence of lymphomas and leukemias in female Sprague-Dawley rats that were exposed to MTBE by intragastric intubation (Belpoggi et al. 1995) is difficult to explain. To our knowledge, no precedent exists for nonmutagenic chemicals causing such tumors. It is conceivable that the route of administration resulted in high levels of a putative mutagenic metabolite (e.g., formaldehyde) accumulating in the hemopoietic and lymphopoietic tissues. (In contrast, lymphomas and leukemias were not seen in F344 rats exposed by inhalation to high concentrations of MTBE. This difference could be the result of strain differences in tissue-specific metabolism and tumor susceptibility.) The reported dysplastic proliferation in the lymphoreticular tissues of exposed Sprague-Dawley rats suggests the presence of precursor lesions. Because of the many unknowns, no readily supportable hypothesis can be offered to describe a possible mechanism of hemopoietic and lymphopoietic tumor induction by MTBE.

Summary of Possible Mechanisms of Carcinogenicity

The mechanisms by which exposure to high concentrations of MTBE or TBA causes tumor formation in different organ systems (kidney, testes, liver, thyroid, and hemopoietic tissues) of mice and rats are not understood. Several examples of nonmutagenic chemicals causing tumors in the same target tissues that are affected by MTBE or TBA have been reported. In some of these, possible pathogenetic mechanisms, often involving complex endocrine disturbances, have been proposed. Although similar mechanisms could be operating in tumor responses induced by MTBE or TBA, no experiments have been reported to date to elucidate the mechanisms of tumor induction by either compound.

In assessing the overall significance of the cumulative data produced by the studies investigating MTBE and TBA in rodents, the most disconcerting aspect of the findings is that the two chemicals produced tumors at five different organ sites in two strains of two species. Considering that the mechanisms of action of these and other nonmutagenic rodent carcinogens are poorly understood, it would seem imprudent to dismiss these results as irrelevant to the human condition. The fact, however, that increased tumor incidences have been found only in groups of animals exposed to toxic concentrations of the test chemicals, often resulting in severe morbidity and premature death, complicates interpretation of the data and assessment of their implications for possible risks to human health.

ETHANOL

NONNEOPLASTIC EFFECTS

Chronic ingestion of large quantities of alcohol is associated with degenerative changes in many organs of the body. In the pathogenesis of such lesions, nutritional disturbances as well as direct toxicity are commonly involved (Table 37).

Effects on the Liver

Disturbances in metabolism and nutrition resulting from repeated ingestion of large quantities of alcohol can lead to degenerative changes in the liver. Although these are generally reversible after short-term ingestion of alcohol, the degenerative changes are likely to become progressive and severe with prolonged daily ingestion of ethanol in quantities of 160 g or more (Carithers 1992). Any of the following three overlapping forms of liver disease may result from excessive drinking: hepatic steatosis ("fatty liver"), alcoholic hepatitis, and cirrhosis.

Hepatic steatosis is characterized by lipid droplets accumulating within liver cells. If allowed to progress, it may cause the liver to become enlarged, and may ultimately impair liver function. *Alcoholic hepatitis* typically appears after a period of heavy drinking. It may be asymptomatic or may produce fulminant liver failure. When severe, it carries a 10% to 20% probability of death (Carithers 1992). *Alcoholic cirrhosis* is characterized by severe fibrotic scarring of the liver, with impairment of liver function, and is commonly the last stage of prolonged liver injury induced by excessive use of alcohol. It occurs in 10% to 15% of subjects with alcoholism, and may result in fatal hepatic failure, gastrointestinal hemorrhage, intercurrent infection, or liver cancer (Carithers 1992).

Effects on the Heart

Alcohol is toxic to the myocardium; chronic alcoholism usually is associated with thiamine deficiency and other nutritional disorders, the combined effects of which may produce degenerative and inflammatory changes in the heart (alcoholic myocarditis) that resemble those of beri beri heart disease (Cotran et al. 1994).

Effects on the Central Nervous System

Of the various changes seen in the central nervous system, Wernicke's encephalopathy characterized by focal degeneration, demyelinization, and hemorrhages, is the most commonly encountered. Its symptoms are typically ataxia, global confusion, ophthalmoplegia (paralysis of the eye muscles), and nystagmus (rapid movement of the eyeball). After treatment with thiamine, it is followed in some subjects by the Korsakoff syndrome, which is characterized by severe memory loss.

Dose-Effect Relationships

The hepatic and cardiac changes described above have been observed only after ingestion of ethanol in large quantities over a prolonged period. It is doubtful that they could result from inhaling ethanol at the atmospheric concentrations associated with its use in fuels.

NEOPLASTIC EFFECTS

In virtually all of the human populations studied to date, the frequencies of cancers of the oral cavity, pharynx, larynx, esophagus, and liver have increased with the consumption of alcoholic beverages (National Academy of Sciences/National Research Council 1982; International Agency for Research on Cancer 1988). Although known or suspected carcinogens other than alcohol are frequently present in such beverages, it is the content of ethanol that has been implicated in the observed cancer increases (International Agency for Research on Cancer 1988). Furthermore, the risks associated with alcohol, which are amply evident in nonsmokers and smokers alike, seem to be synergistic with the risks of smoking to produce cancers of the oral cavity, pharynx, and esophagus (International Agency for Research on Cancer 1988; Franceschi and La Vecchia 1992). On the basis of evidence that alcohol may cause 2% to 4% of all cancers in the U.S. population, it has been ranked as one of the major avoidable causes of cancer (Doll and Peto 1981).

By itself, ethanol has not proved to be carcinogenic in most animal models, nor has it proved to be genotoxic in most assay systems (International Agency for Research on Cancer 1988). Growing evidence, however, indicates that it can enhance the action of certain genotoxic carcinogens. Although these effects are incompletely understood, they seem to involve a variety of mechanisms, depending on the particular carcinogens and exposure conditions in question (Anderson 1992; Watson 1992; Aze et al. 1993).

Many publications on the association between ethanol and cancer, including extensive summaries elsewhere (e.g., International Agency for Research on Cancer 1988; Watson 1992), are readily available. Hence only salient aspects of the subject are summarized here.

Human Studies

Cancers of the Oral Cavity, Pharynx, and Hypopharynx
The results of numerous cohort studies and case-control studies consistently show that cancers of the oral cavity,

pharynx, and hypopharynx increase in frequency with alcohol consumption (International Agency for Research on Cancer 1988; Franceschi and La Vecchia 1992). The risks of these malignancies are appreciably higher in smokers than in nonsmokers, and the combined effects of smoking and drinking on the risks of these diseases appear to be multiplicative rather than merely additive.

Cancer of the Larynx Cohort and case-control studies demonstrate a similar dose-dependent relationship between the frequency of laryngeal cancer and the intake of alcohol (International Agency for Research on Cancer 1988). As with the aforementioned cancers, the risks of laryngeal cancer are higher in smokers than in nonsmokers, and the combined effects of drinking and smoking on the risk of the disease appear to be multiplicative.

Cancer of the Esophagus With cancer of the esophagus, likewise, cohort and case-control studies show that the risk increases with alcohol consumption (International Agency for Research on Cancer 1988; Franceschi and La Vecchia 1992), and the joint effects of smoking and drinking on the risk of the disease are multiplicative in nature.

Cancer of the Liver In numerous cohort and case-control studies, primary cancer of the liver has increased in frequency with the intake of alcohol (International Agency for Research on Cancer 1988). The data also suggest that alcohol may interact with tobacco, or hepatitis B virus, or both to have carcinogenic effects on the liver (International Agency for Research on Cancer 1988; Ohnishi 1992; Villa et al. 1992).

Cancers of Other Organ Sites Cancers of the stomach, colon, rectum, pancreas, breast, and lung have been reported to increase in frequency with alcohol consumption, but the data have not been consistent enough thus far to establish a causative relationship (International Agency for Research on Cancer 1988; Seitz and Simanowski 1992). Cancers of the urinary tract, ovary, prostate gland, lymphoid organs, and hemopoietic system have shown no association with alcohol intake (International Agency for Research on Cancer 1988). The data on other cancers are too sparse to enable a definitive evaluation (International Agency for Research on Cancer 1988).

Animal Studies

Ethanol alone has not been shown to be carcinogenic when administered orally, percutaneously, or transplacentally to laboratory animals (International Agency for Research on Cancer 1988). When administered orally in combination with the following carcinogens, however, it has enhanced their carcinogenic effects in rodents, depending on the conditions of treatment: *n*-nitrosodimethylamine, *n*-nitrosodiethylamine, *n*-nitrosopyrrolidine, *n*-nitrosomethylethylamine, *n*-nitrosomethylbenzylamine, *n*-nitrosonornicotine, *n*-nitrosodipropylamine, acetoxymethyl-methylnitrosamine, diethylnitrosamine, methylamylnitrosamine, azomethane, dimethylhydrazine, azoxymethane, vinyl chloride, and urethane (International Agency for Research on Cancer 1988; Anderson 1992; Anderson et al. 1992; Seitz and Simanowski 1992; Aze et al. 1993; Mirvish et al. 1994).

Administering ethanol to rats has been reported to increase the amount, or the persistence, or both of O^6-methylguanine formed in the cells by *n*-nitrosodimethylamine (Mufti 1992) or by *n*-nitrosomethylbenzylamine (Kouros et al. 1983), and to increase the activation of *n*-nitrosopyrrolidine by hepatic, esophageal, and pulmonary microsomes (Wynder and Bross 1961). Alcohol administered to mice also has increased the genotoxicity of *n*-nitrosonornicotine, 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone, and *n*-nitrosopyrrolidine (Knasmuller et al. 1994).

The major intermediary metabolite of ethanol is acetaldehyde, the chronic inhalation of which has been observed to increase the incidence of respiratory tract tumors in rats and hamsters (International Agency for Research on Cancer 1988). Acetaldehyde also has been reported to increase the carcinogenicity of intratracheally instilled benzo[*a*]pyrene in hamsters (International Agency for Research on Cancer 1985, 1987). On the basis of the available data, IARC working groups have concluded that there is *sufficient evidence* that acetaldehyde is carcinogenic in laboratory animals. The U.S. Environmental Protection Agency (1993c) classified acetaldehyde as a probable human carcinogen (B2).

In Vitro Assays

Exposure to ethanol has been reported to increase the frequency of transformed foci in cultured mouse C3H/10T1/2 cells (Abernathy et al. 1982), but not in primary cultured Syrian golden hamster cells (Bokkenheuser et al. 1983), and to inhibit intercellular communication in Chinese hamster V79 cells (Chen et al. 1984).

In the absence of an exogenous metabolic system, ethanol has not been observed to induce DNA damage in cultured rodent cells; in the presence of such a system, however, it has been reported to increase the frequency of chromosomal aberrations in Chinese hamster ovary cells (de Raat et al. 1983; Takehisa and Kanaya 1983; Darroudi and Natarajan 1987; International Agency for Research on Cancer 1988).

Ethanol has proved to be nonmutagenic to bacterial cells in most of the assay systems used; however, it has been observed to increase the frequency of revertants in *S. typhimurium* strain TA102 exposed in the presence of an exogenous metabolic system (De Flora et al. 1984; International Agency for Research on Cancer 1988). In assays

employing plant, fungal, or insect cells, ethanol has occasionally been observed to increase the frequency of aberrations in micronuclei, chromosomes, or both.

Possible Mechanisms of Ethanol-Induced Carcinogenicity

Although the mechanisms of the observed carcinogenic effects of ethanol are still to be defined in detail, various hypothetical mechanisms have been proposed. These include (1) enhanced metabolic activation of carcinogens through the induction of activating enzymes, such as cytochrome P-450 2E1; (2) interference with carcinogen detoxification through competitive inhibition of detoxifying enzymes, such as *n*-nitrosodimethylamine demethylase; (3) facilitation of the entry of carcinogens into cells through effects on the cell membrane; (4) stimulation of DNA synthesis through cytotoxicity-induced regenerative hyperplasia; (5) interference with DNA repair; (6) modulation of the immune system; (7) production of oxygen free radicals; (8) increased lipid peroxidation; and (9) adverse effects on nutrition leading to deficiencies in vitamin A and other nutrients (Anderson 1992; Klygis and Barch 1992; Mufti 1992; Odeleye et al. 1992).

Summary

Existing evidence demonstrates unequivocally that ingestion of ethanol can increase the risks of certain forms of human cancer, depending on the conditions of exposure. Ethanol itself has not proved to be carcinogenic to laboratory animals, but it has been found to enhance the carcinogenicity of other agents under appropriate experimental conditions. The carcinogenic effects of ethanol remain to be elucidated in full, but a number of putative mechanisms for them have been postulated. Apart from effects on cultured cells, however, the carcinogenic effects of ethanol have been observed only after ingestion of the substance in relatively large quantities. It is doubtful that comparable effects could result from inhaling alcohol at the low concentrations found when using ethanol in fuels.

CONCLUSIONS

ETHERS

Although information on the long-term effects of MTBE exposure in humans does not exist, exposure of animals to high concentrations results in increased incidences of renal, testicular, and lymphohematopoietic neoplasms in rats, and liver tumors in mice.

One of MTBE's major metabolites, formaldehyde, is genotoxic. When inhaled at high concentrations (greater than 5.6 ppm), it causes nasal tumors in rodents. Epidemiologic data suggest that it may also cause nasopharyngeal tumors in humans. TBA, the other major metabolite of MTBE, has been observed to increase the incidence of kidney tumors in male rats and thyroid tumors in female mice exposed to high concentrations of this compound in drinking water. The mechanisms of tumor induction by TBA are unknown. No data exist on its carcinogenicity in humans. Apart from the possibility that the tumors induced by MTBE may involve the genotoxic metabolite formaldehyde (or others), the mechanism of MTBE-induced carcinogenesis remains to be defined.

For ethers other than MTBE, limited information indicates that they are not genotoxic in most short-term bioassays. No information is available on their tumorigenicity in animals or humans.

With respect to tumor causation by MTBE or TBA, multiple tumors induced in two animal species is a cause for concern. However, the tumors have been observed only at high exposure concentrations (3,000 ppm for inhalation studies and 1,000 mg/kg body-weight by gavage), each of the available bioassays has technical limitations, and the mechanisms of tumor induction are unknown. For these reasons, the implications of the animal carcinogenesis data for human cancer risk are uncertain.

ETHANOL

Dose-dependent increases of certain forms of cancer are associated with the consumption of alcoholic beverages, providing strong evidence that ethanol is carcinogenic for humans. Further evidence of the carcinogenicity of ethanol for humans is provided by the interaction between ethanol consumption and cigarette smoking, the combined effects of the two being more nearly multiplicative than additive. Although prolonged ingestion of ethanol itself has not proved to be tumorigenic in laboratory animals, it has been observed to enhance the tumorigenicity of other agents in rodents.

Ethanol is not genotoxic in most assay systems, implying that its tumorigenic effects may involve nongenotoxic mechanisms; however, genotoxicity related to oxidative processes operating in vivo cannot be precluded.

The tumorigenic effects of ethanol have been observed only after prolonged ingestion of the substance in relatively large amounts. Data on the long-term effects of inhalation exposure are lacking.

It is unlikely that carcinogenic effects would result from inhalation exposure to ethanol at the low ambient air concentrations associated with its use in fuels.

Table 31. Summary of the Rodent Carcinogenicity Data for MTBE and Its Metabolites

Compound and References	Species	Route of Exposure	Gender	Tumors Showing a Statistically Significant Increase in Incidence						
				Kidney	Testes	Liver	Lymphomas and Leukemias	Thyroid	Nasal Cavity	Gastro-intestinal Tract
MTBE										
Chun et al. 1992	Rat (F344)	Inhalation	M F	+	+					
Belpoggi et al. 1995	Rat (Sprague-Dawley)	Gavage (in oil)	M F		+		+			
Burleigh-Flayer et al. 1992	Mouse (CD-1)	Inhalation	M F			+ +				
TBA										
Cirvello et al. 1995	Rat (F344)	Oral	M F	+						
	Mouse ($B6C3F_1$)	Oral	M F					+		
Formaldehyde										
Several studies reviewed by IARC 1995	Rat	Inhalation	M F						+ +	
Three studies reviewed by IARC 1995[a]	Rat	Oral	M & F				±			±

[a] A ± in this row indicates that both positive and negative data were presented in the studies reviewed.

Table 32. Incidence of Tumors in Sprague-Dawley Rats Exposed to MTBE by Ingestion[a]

Dose (mg/kg)	Males Testicular Interstitial Cell Adenomas		Females Lymphomas or Leukemias	
0	2/60[b] (3%)	2/26[c] (8%)	2/60[b] (3%)	2/58[c] (3%)
250	2/60 (3%)	2/25 (8%)	6/60 (10%)	6/51 (12%)
1,000	11/60 (18%)	11/32 (34%)[d]	12/60 (20%)	12/47 (26%)[e]

[a] Belpoggi et al. 1995.

[b] Data in this column reflect the number of animals with tumors/the number of animals at the start of the study.

[c] Data in this column reflect the number of animals with tumors/the number of animals surviving at the time when the first tumor of this type appeared.

[d] Significantly different from the 0-mg/kg group ($p < 0.05$).

[e] Significantly different from the 0-mg/kg group ($p < 0.01$).

Table 33. Incidence of Kidney Tumors in Male F344 Rats Exposed to MTBE by Inhalation[a]

Dose (ppm)	Adenoma[b]	Carcinoma[b]	Adenoma or Carcinoma[b]
0	1/50	0/50	1/50
400	0/50	0/50	0/50
3,000	5/50	3/50	8/50
8,000	3/50	0/50	3/50

[a] Chun et al. 1992.

[b] Number of animals with tumors/number of animals in the exposure group.

Table 34. Incidence of Testicular Interstitial Cell Neoplasms in Male F344 Rats Exposed to MTBE by Inhalation[a]

Dose (ppm)	Adenoma[b]
0	32/50
400	35/50
3,000	41/50
8,000	47/50

[a] Chun et al. 1992.

[b] Number of animals with tumors/number of animals in the exposure group.

Table 35. Incidence of Liver Tumors in Male CD-1 Mice Exposed to MTBE by Inhalation[a]

Dose (ppm)	Adenoma[b]	Carcinoma[b]	Adenoma or Carcinoma[b]
0	11/49	2/49	12/49
400	11/50	4/50	12/50
3,000	9/50	3/50	12/50
8,000	12/49	8/49	16/49

[a] Burleigh-Flayer et al. 1992.

[b] Number of animals with tumors/number of animals in the exposure group.

Table 36. Incidence of Liver Tumors in Female CD-1 Mice Exposed to MTBE by Inhalation[a]

Dose (ppm)	Adenoma[b]	Carcinoma[b]	Adenoma or Carcinoma[b]
0	2/50	0/50	2/50
400	1/50	1/50	2/50
3,000	2/50	0/50	2/50
8,000	10/50	1/50	11/50

[a] Burleigh-Flayer et al. 1992.

[b] Number of animals with tumors/number of animals in the exposure group.

Table 37. Major Nonneoplastic Lesions Associated with Chronic Alcoholism[a]

Site	Lesion	Mechanism
Liver	Steatosis Acute hepatitis Cirrhosis	Direct toxicity Direct toxicity Direct toxicity
Heart	Myocarditis	Direct toxicity
Central nervous system	Wernicke encephalopathy Korsakoff syndrome Cerebellar degeneration	Thiamine deficiency Direct toxicity and thiamine deficiency Nutritional deficiency
Peripheral nerves	Neuropathy	Thiamine deficiency
Pancreas	Acute pancreatitis	Unclear
Skeletal muscle	Fiber degeneration	Direct toxicity
Testes	Atrophy	Unclear

[a] Adapted from Cotran et al. 1994.

Potential Health Effects of Oxygenates

Summary

This section summarizes the information presented in earlier sections of the report on health effects of oxygenates added to gasoline, with an emphasis on MTBE and ethanol because of the small amount of information available on other ethers.

METABOLISM AND DISPOSITION

When MTBE is inhaled by humans, a portion (about 32% to 42%) is rapidly absorbed into the blood and distributed to tissues. MTBE is eliminated by either exhalation or metabolism. The first step in the metabolism of MTBE, which occurs primarily in the liver, is oxidative demethylation by the cytochrome P-450 enzymes; this process yields formaldehyde and TBA. To a lesser extent, metabolism may also occur in the respiratory tract, where P-450 enzymes also are found. MTBE has a half-life in blood of 0.5 to 1.5 hours in rodents and humans. A slower component (with a half-life on the order of 20 hours) has been detected in human exposure studies. TBA is metabolized more slowly than MTBE and has a longer half-life in blood. Because MTBE is not produced by endogenous metabolic pathways, no background level of MTBE exists in blood or tissues of unexposed animals or humans.

As with MTBE, when ethanol is inhaled, a portion (about 60%) is rapidly taken up into the blood stream and distributed to tissues. Ethanol is metabolized primarily by the liver, but some metabolism also may occur in the upper respiratory tract. Metabolism of ethanol at low concentrations is catalyzed primarily by alcohol dehydrogenase, and results in the formation of acetaldehyde. In contrast to MTBE, ethanol is a product of many catabolic pathways and is present in blood even in the absence of ingested ethanol. Ethanol is found in many foods and alcoholic beverages. Limited studies, which sometimes have estimated blood ethanol levels from breath samples, have reported endogenous levels in humans that span a range of 100-fold (0.2 to 30 mg/L). Levels at the lower end of the range were measured in fasting people. The incremental ethanol burden that results from exposure to ethanol from fuel is expected to be very small relative to the endogenous levels. A 3-minute exposure to 1 ppm during refueling is estimated to result in an incremental increase in blood ethanol of about 1 μg/L in a person weighing 70 kg and breathing at a ventilation rate of 14 L/min (see section on Metabolism and Disposition). In this typical refueling scenario, the amount of ethanol added to the blood is 300-fold less than the lowest endogenous level cited above. An extreme exposure scenario of 10 ppm for 15 minutes would translate to 40 μg/L in blood, which is 7.5-fold lower than the lowest endogenous level noted above. For comparison, one half-ounce of alcohol in a beverage would elevate blood levels of ethanol by about 350 mg/L, which is 4 orders of magnitude more than the example of extreme exposure given above, and more than 5 orders of magnitude greater than the typical refueling scenario. In conclusion, the incremental ethanol burden that results from exposure to ethanol in fuel is predicted to be lower than endogenous blood levels.

SHORT-TERM EFFECTS

ETHERS

After oxyfuel containing MTBE was introduced in Fairbanks, AK, in the fall of 1992, residents complained about the odor of MTBE and the increased cost of gasoline. They also reported a variety of symptoms, including headache, eye irritation, a burning sensation in the nose and throat, cough, dizziness, a sense of disorientation, and nausea. Similar constellations of symptoms were reported to varying degrees in some other areas when oxyfuel was introduced, and two years later when RFG was introduced in Wisconsin and some other areas. Community studies were carried out to evaluate acute responses in Fairbanks and several other areas using oxyfuel or RFG, as well as in areas not using oxygenates in gasoline for comparison. Results of these studies are discussed in the section on Short-Term Effects.

The community studies were planned quickly and conducted with limited resources. Exposure assessment in some studies was based on presumed differences in MTBE exposure related to a person's occupation or activities. Measurements of exposure, including air levels and blood levels, were carried out in only a few studies. Similar community studies have not been conducted with the other ethers, which have had much more limited use in gasoline. The community studies do not provide a consistent picture

of the effects of MTBE exposure. Some of the community studies provided evidence that people with higher levels of exposure had a higher prevalence of symptoms, but other studies did not. Differences in sensitivity could obscure a dose-response relationship unless sensitive groups are identified. Also, the data do not establish whether the reported symptoms were direct toxicologic effects of MTBE exposure or indirect psychophysiological reactions induced by exposure to the odor of MTBE. A difference in the detection threshold or in how unpleasant the odor seems could affect the perceived symptoms. The publicity surrounding the introduction of oxygenates in some areas, which may have affected the reporting of symptoms, complicates interpretation.

The Alaska studies seem to provide the strongest evidence of an effect of MTBE. It has been suggested that the uniquely cold climate and the topography of Fairbanks could influence both exposure and effects (U.S. Environmental Protection Agency 1994b). In addition, odor threshold testing suggests that the Alaska fuel blend with MBTE produced the greatest increase in odor. Finally, the intense publicity surrounding both the health effects and other aspects of the fuel may have influenced the reporting of symptoms there. These unique features of the Alaska experience suggest that it is not necessarily applicable to other locations.

Three controlled human exposure studies investigated whether exposing healthy subjects to MTBE in the absence of gasoline vapors causes an increase in objective measurements of effects on the eyes, nose, and central nervous system, in addition to the symptoms investigated in the community studies. Exposure levels in two of these studies were similar (1.4 or 1.7 ppm for 1 hour); in the other study, three higher MTBE levels were used (5, 17, or 50 ppm for 2 hours). In none of these studies did subjects exposed to MTBE report symptoms more frequently than those not exposed to MTBE, even though they were able to detect the odor. Mean blood levels in the two studies with the lower exposure levels were 9 and 18 μg/L, compared with an upper quartile level of 3.8 μg/L in workers in the Stamford study who had a higher prevalence of one or more symptoms. In the third study, blood levels reached 148 μg/L at the highest exposure level. Although these studies resulted in high blood levels of MTBE compared with the community studies, elevated levels of symptoms were not observed after MTBE exposure. However, the controlled human exposure studies did not replicate the conditions of exposure that occur in real-life situations, including exposure to MTBE as part of a complex mixture of constituents of gasoline vapors and exhaust emissions. Also, each study involved only a small number of healthy subjects.

The results from animal studies with high levels of exposure suggest that MTBE produces acute, reversible neurotoxic effects. The endpoints evaluated in studies after acute exposure to MTBE—motor activity and functional observational batteries—are considered to be useful in hazard evaluation, but are usually less sensitive indicators of neurotoxicity than measurements of more complex central nervous system functions. In a 6-hour exposure study, the motor activity of male rats increased at 800 ppm, but decreased at 8,000 ppm MTBE. Ataxic gait and a phenomenon termed "duck-walk" were observed at the two higher exposure levels, and at the highest exposure concentration additional effects were observed, including lacrimation, labored respiration, decreased muscle tone, hind limb splay, and decreased performance on a treadmill. Because a NOAEL was not found in this study, and because the endpoints likely to be more sensitive were not evaluated, the results leave uncertainties about the potential for neurotoxic effects. Central nervous system effects also were investigated in two of the three controlled human exposure studies mentioned earlier. No effects were observed, but the interpretation of these findings is limited because the kinds of endpoints used are not considered to be particularly sensitive.

Little investigation of the potential neurotoxicity of other ethers that might be used as oxygenates in gasoline has been carried out. For ETBE, minor effects in neurotoxicity tests were seen in rats exposed to 4,000 ppm for28 days, the highest concentration evaluated, but not in rats exposed to 2,000 ppm; however, activity, a more sensitive endpoint for MTBE, was not measured, nor were measures of complex central nervous system functions.

In summary, based on the studies conducted, it appears that most people do not experience unusual symptoms or significant medical consequences in response to short-term exposure to MTBE in gasoline. Evidence from some of the community studies suggests that MTBE in gasoline may cause acute symptoms in some people or under some circumstances. However, the data are not sufficient to attribute or to not attribute these effects directly to MTBE exposure. Animal studies indicate that MTBE is neurotoxic at a relatively high concentration (800 ppm) in rats using rather insensitive assays. These findings raise the possibility that effects might be seen at lower concentrations if more complex functions were assessed.

ETHANOL

Introduction of ethanol as an oxygenate in gasoline was not accompanied by the same negative publicity as was associated with the introduction of MTBE. A community study was conducted in Anchorage, AK, in the winter of

1994/1995 when ethanol was used in gasoline. This study was designed to assess the same symptoms as were reported by residents when MTBE was being used, although it is not known whether ethanol exposure would trigger the same set of symptoms. The results of this study were that symptom prevalence was similar when ethanol was in the fuel, when it was being phased out, and when it was no longer in the fuel.

Ethanol has been shown to be neurotoxic at high levels in both animal and human studies. The most sensitive functional outcome of acute exposure is impaired performance. After oral doses of ethanol in humans, performance fell on a variety of neurobehavioral tests, including finger tapping, target tracking, and hand steadiness at the higher level of exposure (which gave peak blood levels of 630 mg/L), but not after a lower level of exposure (which gave peak blood levels of about 300 mg/L). For comparison, a half-ounce of ethanol in a beverage corresponds to a peak blood ethanol level of approximately 350 mg/L. Vigilance and monitoring are considered to be the most sensitive endpoints for the neurotoxic effects of ethanol. It has been proposed that blood levels as low as about 100 mg/L may induce performance degradation under conditions that are not optimal such as low illumination, which is representative of nighttime driving. For comparison, a typical refueling scenario (1 ppm for three minutes) is predicted to produce blood ethanol levels of 1.0 μg/L, and an extreme scenario (10 ppm for 15 minutes) to produce concentrations of 40 μg/L. Thus, exposure to ethanol from its use in fuel is not expected to cause neurotoxic effects.

REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

ETHERS

No human studies have been conducted on reproductive or developmental effects of MTBE or other ethers added to gasoline. Such effects have been investigated in rats, mice, and rabbits exposed to MTBE levels as high as 8,000 ppm (see section on Reproductive and Developmental Effects). Maternal toxicity has been observed in rats exposed for extended periods to 3,000 or 8,000 ppm MTBE, and in mice and rabbits exposed to 4,000 or 8,000 ppm.

Developmental effects in offspring were observed when parents were exposed to MTBE concentrations as low as 1,300 ppm in rats and 3,000 ppm in mice. No effects were observed on rabbit fetuses, even when parents were exposed to 8,000 ppm MTBE. Developmental effects noted in rats include (1) small decreases in the viability of offspring (at 1,300 and 3,400 ppm, but not 300 ppm); (2) decrease in body weight during part of the lactation period (at 3,000 and 8,000 ppm, but not 400 ppm); and (3) central nervous system effects (hypoactivity and reduced startle reflex) in second generation offspring after weaning (at 3,000 and 8,000 ppm, but not 400 ppm). In these rat studies the NOAELs were 300 and 400 ppm. In another rat study no developmental effects were observed, even at 2,500 ppm. However, in that study exposure was limited to the second and third weeks of gestation and only gross malformations were assessed. In mice, skeletal malformations were noted at 4,000 and 8,000 ppm, but not at 1,000 ppm. The concentrations of MTBE that produced developmental effects are approximately 3 orders of magnitude greater than levels in usual refueling scenarios; NOAELs in rat studies that report effects were more than 2 orders of magnitude higher than these levels. In addition, the short periods of exposure during refueling (1 to 3 minutes) would elevate blood levels much less than exposure for 6 hours each day, as in the animal studies.

ETHANOL

It is well documented that embryonic exposure to ethanol by maternal drinking can result in serious developmental effects. The Fetal Alcohol Syndrome, which includes characteristic malformations and functional deficits, results from alcohol abuse during pregnancy, particularly from binge drinking. Lower levels of maternal ethanol consumption result in Fetal Alcohol Effects, characterized by functional deficits that result from brain damage. Although a statistically definable threshold is elusive, some investigators have proposed an apparent threshold of about one drink (one-half ounce alcohol) per day, corresponding to a peak blood level of about 350 mg/L. Periodic exposures to ethanol in refueling situations or other exposures to fuel containing ethanol are predicted to produce ethanol levels at least 4 orders of magnitude lower than this proposed threshold and thus should not contribute to developmental effects.

LONG-TERM EFFECTS

ETHERS

Increased frequencies of the following tumors have been observed in rodents exposed to high doses of MTBE for prolonged periods of time: (1) lymphomas and leukemias in female Sprague-Dawley rats exposed orally; (2) testicular tumors in male Sprague-Dawley rats exposed orally and in male F344 rats exposed by inhalation; (3) kidney tumors in

male F344 rats exposed by inhalation; and (4) liver tumors in male and female CD-1 mice exposed by inhalation. Pending clarification of the dose-response relationships and mechanisms of these effects, their significance for human cancer risks remains uncertain. It is noteworthy that the two primary metabolites of MTBE—TBA and formaldehyde—have been reported to be tumorigenic. TBA has been reported to cause kidney tumors in male F344 rats and thyroid tumors in female $B6C3F_1$ mice when administered orally. Formaldehyde has been reported to cause leukemias, lymphomas, and tumors of the gastrointestinal tract in rats when administered orally, cancers of the nasal cavity in rats when administered by inhalation, and cancers of the nasal cavity and nasopharynx in occupationally exposed workers.

In view of the multiplicity of different types of tumors that have increased in rodents exposed to high levels of MTBE, the occurrence of tumors in males and females of different species and strains, and the tumorigenicity of MTBE's two major metabolites, the possibility that ambient levels of MTBE may pose some risk of carcinogenic effects in human populations cannot be excluded.

ETHANOL

Chronic ingestion of large amounts of ethanol is associated with degenerative processes in the liver, heart, and central nervous system. Nutritional disturbances and direct toxicity are commonly involved in the pathogenesis of these changes. Inhalation exposure to ethanol from its use in fuel are not expected to cause such effects.

A dose-dependent excess of several types of cancer is also associated with the heavy consumption of alcoholic beverages, providing strong evidence that ethanol is carcinogenic for humans. Sites at increased risk for cancer are the oral cavity, pharynx, hypopharynx, larynx, esophagus, and the liver. For cancers of the oral cavity, pharynx, and esophagus, risks associated with alcohol are synergistic with those of smoking. In laboratory animals, prolonged ingestion of ethanol is not tumorigenic, but has been observed to enhance the tumorigenicity of other agents. Although the mechanisms of the tumorigenic effects of ethanol remain to be established, it is unlikely that tumors would result from inhalation exposure to ethanol at the concentrations associated with its use in oxygenated fuels, which are not predicted to increase blood concentrations of ethanol significantly.

Potential Health Effects of Other Pollutants

INTRODUCTION

The main reason for using oxyfuel in the wintertime is to decrease the emission of carbon monoxide, a pollutant hazardous to sensitive subjects with coronary artery disease. In addition, adding oxygenates to fuel affects the emission of some air toxics. Oxygenates are also used year round in RFG, which is intended to reduce emissions of ozone-forming hydrocarbons and air toxics. The minimum concentration of oxygen required is lower in RFG than in oxyfuel (2.0% vs 2.7% by weight). For RFG, additional requirements apply to the content of the fuel and emissions.

Although the rationale for adding oxygenates to gasoline is understandable, the possibility that human health may be adversely affected by the oxygenates themselves or by changes in pollutants resulting from their use continues to raise concern. This section compares the potential health effects of pollutants (CO and several air toxics) that may increase or decrease in emissions when oxygenates are used in fuel with the potential health effects of the oxygenates themselves. In addition, it provides some perspective on the health effects of other constituents of gasoline vapors or motor vehicle emissions, and summarizes the health effects of ozone, a pollutant formed from precursors in exhaust reacting in the presence of sunlight. Although ozone-forming hydrocarbons in emissions are probably not affected significantly by oxygenates themselves, they do appear to be reduced by using RFG. Thus, a complete assessment of fuels containing oxygenates would include ozone.

This report qualitatively summarizes these potential health effects. A more comprehensive evaluation of the overall effects of oxyfuel and RFG on air quality is one of the major objectives of the review that is being conducted by the White House Office of Science and Technology Policy, which also will evaluate effects on fuel economy, engine performance, and groundwater contamination.

To simplify this discussion, the following assumption has been made: In most regions, changes in ambient levels of pollutants tend to parallel changes in motor vehicle emissions, particularly when motor vehicle emissions are a major source of the pollutants in question. Adding oxygenates to gasoline is projected to decrease emission of CO and benzene, but increase emission of formaldehyde or acetaldehyde, depending on the oxygenates used; in addition to all these changes, using RFG may decrease emission of 1,3-butadiene and precursors of ozone (Table 38). However, the ambient level of a given pollutant will depend on the amount of the pollutant that is contributed from all sources combined, as well as on the atmospheric conditions and the characteristics of the motor vehicle fleet within the region in question. Also, evaluating the health implications of any changes in the ambient levels is complicated by the uncertainties inherent in estimating the levels of personal dose by extrapolating from the measured ambient exposure levels.

Many organs may be affected adversely by pollutants derived from automotive emissions. Some inhaled pollutants affect the respiratory tract directly, whereas others affect the heart, nervous system, other organs, or the developing embryo (Utell 1994). Constituents of conventional gasoline that affect the nervous system, for example, include toluene, xylene, and benzene, which may give rise to dizziness, weakness, euphoria, headaches, nausea, staggering, tightness in the chest, and other symptoms (Burbacher 1993; Reese and Kimbrough 1993). Other constituents, including benzene, toluene, and 1,3-butadiene, can have developmental effects. Many known or potential carcinogens also are present in exhaust and evaporative emissions, including benzene, 1,3-butadiene, aldehydes, and polycyclic aromatic hydrocarbons; aldehydes are respiratory irritants as well. Hence, to determine the net effects of adding oxygenates to gasoline requires weighing diverse types of health effects, encompassing common complaints, such as headaches, and less common but more serious disorders, such as cancers or developmental effects. Such an assessment is further complicated by uncertainties about the dose-response relationships for many of the effects in question and by uncertainties related to possible interactive effects with other components of the complex mixture formed from gasoline vapors and motor vehicle emissions.

CARBON MONOXIDE

Insofar as decreasing the emission of CO is the major objective of adding oxygenates to gasoline, the resulting net benefit will depend on the ratio between reducing the negative health effects of CO and increasing deleterious effects by adding oxygenates (this includes effects of the

oxygenates and other pollutants that are affected). All oxygenates used or under consideration, including MTBE, ETBE, TAME, DIPE, and ethanol, have reduced emission of CO by about 10% to more than 20% (Hochhauser et al. 1991; Reuter et al. 1992; Koehl et al. 1993; Noorman 1993; Auto/Oil AQIRP 1995; Kirchstetter et al. 1996; see Appendix A for a summary of emissions data). Projecting the actual changes in ambient levels of CO from emissions studies is very complex because the motor vehicle fleet continuously evolves, the number of miles driven increases in most areas, and weather conditions vary from year to year. Nevertheless, considerable evidence demonstrates that the number of exceedences of the CO standard has dropped in many areas since oxyfuel was introduced (see the Exposure Assessment section). However, the Oxygenates Evaluation Committee did not analyze how much the use of oxyfuel may have contributed to this reduction.

When inhaled into the lungs, CO diffuses across the alveolar-capillary barrier into the plasma and through the red blood cell membrane. Inside the red blood cell, CO reacts rapidly with hemoglobin to form carboxyhemoglobin (COHb). The reaction of CO with hemoglobin decreases the amount of hemoglobin available to combine with oxygen in the blood, and also causes oxygen to be less readily released from hemoglobin; the result is that the amount of oxygen delivered to peripheral tissues is reduced, and a state of relative hypoxia is produced in the tissues (Roughton and Darling 1944). Although high levels of COHb are lethal because the tissues are deprived of oxygen, low levels that result from ambient exposures are generally not associated with adverse effects in healthy individuals. However, conditions that limit oxygen delivery to the tissues can make individuals more susceptible to the adverse effects of CO exposure. These include chronic lung disease, which causes hypoxemia because of gas-exchange deficiencies, and coronary artery disease, in which blood flow to the myocardium (or heart muscle) is limited. At this time, coronary artery disease is the susceptibility factor of greatest concern because individuals with this condition have experienced negative effects at lower COHb levels (2.0%) than have caused other effects (Allred et al. 1989a,b). Neurotoxic effects of CO have been demonstrated at COHb levels as low as about 5% in healthy individuals (U.S. Environmental Protection Agency 1992b). Of these, effects on vigilance or coordination could be of particular concern with respect to safety while driving in dense traffic, where ambient CO concentrations may be relatively high.

People with coronary artery disease have a decreased ability to increase their coronary blood flow in response to an increased consumption of myocardial oxygen during exercise. When myocardial blood flow is not sufficient to meet this oxygen demand, the myocardium becomes ischemic, resulting in chest pain (angina pectoris), electrocardiographic changes, or both. Indicators of myocardial ischemia occur in individuals with coronary artery disease at specific levels of exercise and may limit their exercise capacity. Several studies have reported a decrease in the time to onset of myocardial ischemia during exercise after CO exposure that produced COHb levels of about 2% to 3% (Anderson et al. 1973; Aronow 1981; Aronow and Isbell 1973; Allred et al. 1989a,b, 1991; Kleinman et al. 1989). In a study conducted at three clinical centers (Allred et al. 1989a,b, 1991), subjects experienced a mean 5.1% decrease in the time to electrocardiographic changes indicative of myocardial ischemia, and a mean 4.2% decrease in the time to angina at 2.0% COHb, compared with air exposure, during a progressively increasing exercise test; greater decreases in the time to onset of myocardial ischemia occurred at 3.9% COHb. The data from this study demonstrate a significant dose-response relationship for the individual differences in time to the onset of electrocardiographic changes. For the range of actual, individual COHb levels in the study (0.2% to 5.1%), the time to ST segment decreased 3.9% endpoint for every 1% increase in COHb, with no sign of a threshold. The time at which myocardial ischemia was noted in this study can be associated roughly with a level of exercise equivalent to light or moderate activity, such as climbing two flights of stairs or walking on level ground at a pace of two to three miles per hour for a distance of one-half to one mile (Allred et al. 1989a). Chest pain clearly involves discomfort and interferes with activities. However, some people with coronary artery disease do not develop chest pain in conjunction with myocardial ischemia and thus may be at greater risk than those who do. Although the production of myocardial ischemia during exercise is not necessarily related to major cardiac events, such as myocardial infarction, it is generally agreed that myocardial ischemia is detrimental and may have cumulative effects (Allred et al. 1989a; Environmental Protection Agency 1992b).

Of potential importance are reports of increased hospital admissions for cardiovascular disease during episodes of air pollution. Recently, it was reported that, in seven cities, ambient CO levels were positively associated with hospital admissions for congestive heart failure among elderly people (Morris et al. 1995). Congestive heart failure involves the inability of the heart to adequately perfuse peripheral tissues. In this study, the association between hospital admissions and CO persisted after adjusting for seasonal effects, temperature, and several pollutants (nitrogen dioxide, sulfur dioxide, and ozone). Schwartz (1995) pointed out that fine particles, which were not included in these

analyses, might be responsible for the effects rather than CO. The estimated relative risk of hospital admissions for congestive heart failure associated with an increase of 10 ppm in CO ranges from 1.10 in New York City, to 1.37 in Los Angeles (Morris et al. 1995).

Individuals take CO into the blood at different rates when exposed to the same concentrations in the air. The best way to assess as individual's exposure is by measuring the amount of COHb in the blood. An exposure to 35 ppm CO, a level allowed by the 1-hour NAAQS for CO, is projected to produce blood COHb levels up to 2.2% in 1 hour under conditions of moderate exercise (U.S. Environmental Protection Agency 1992b). Thus, individuals with coronary artery disease could be adversely affected by ambient CO levels near the current standard if they were exercising while being exposed. However, it is the 8-hour (9 ppm) standard, rather than the 1-hour standard, that has been exceeded in areas out of compliance with the NAAQS for CO. A 1-hour exposure at the level of the 8-hour standard would not result in a COHb level of 2%. Nonetheless, because the CO levels in areas near traffic are likely to be higher than those recorded by ambient monitoring, people may experience levels of COHb higher than 2%, even if the 1-hour or 8-hour standard is not exceeded. Evidence of this is provided by the study of Wallace and Ziegenfus (1985), who found that CO levels at monitoring stations in 20 U.S. cities did not correlate well with COHb levels measured in the blood of residents. Although many individuals had COHb levels greater than 2%, only one of the 36 monitoring stations reported a mean level above the 8-hour standard of 9 ppm.

Carboxyhemoglobin levels in the blood of people with heart disease in Denver were estimated for "as-is" air quality and "just attain" air quality (U.S. Environmental Protection Agency 1992b). Just attaining the 8-hour ambient air quality standard was projected to result in a decrease in the number of "heart disease person-days" from 42,600 to 100, assuming that COHb levels greater than or equal to 2.1% are of concern, and from 17,700 to 20, assuming that COHb levels greater than or equal to 2.3% are of concern. Although these projections seem reassuring because they suggest that lowering CO levels would have a beneficial effect on the CO exposure of sensitive subjects, many uncertainties remain about the number of sensitive individuals with coronary artery disease and their personal activity and exposure patterns. Because this group is sensitive to low levels of CO exposure only when exercising, understanding their activity patterns in areas with high CO levels is essential for interpreting whether lowering CO levels actually provides health benefits for them.

AIR TOXICS AND CARCINOGENICITY

Evaporative and exhaust emissions of motor vehicles are known to contain many compounds that have been classified as carcinogens and many others that are potentially carcinogenic. These include substances in gasoline vapors and gasoline particulate matter, as well as the compounds designated in the Clean Air Act Amendments of 1990 as mobile-source air toxics whose emission levels need to be reduced—formaldehyde, acetaldehyde, benzene, 1,3-butadiene, and polycyclic organic matter (POM). To consider possible changes in carcinogenicity, this discussion focuses on the air toxics targeted by the CAAA of 1990 (benzene, 1,3-butadiene, formaldehyde, and acetaldehyde). In addition, it includes information about other carcinogenic constituents of gasoline or emissions to provide further perspective for interpreting the possible carcinogenicity of MTBE or other oxygenates. The main concern about air toxics has been their carcinogenicity because, if it is assumed that there is no threshold dose, then even low levels are of concern. It is important to note that these air toxics have other effects of potential concern, as mentioned earlier, but those generally occur at levels above those in ambient air. However, the EPA has noted that benzene may produce developmental effects even at exposure levels as low as 1 ppm (U.S. Environmental Protection Agency 1994b).

Although reducing air toxics is not an explicit goal of the oxyfuel program, adding oxygenates does tend to reduce the total mass of air toxics (see Appendix A for emissions data). Reducing air toxics, as a whole, is a goal of the reformulated gasoline program. Studies summarized in Appendix A indicate that levels of benzene decrease in emissions with the use of oxygenates (Gorse et al. 1991; Reuter et al. 1992; Koehl et al. 1993; Noorman 1993; Auto/Oil AQIRP 1995). Emission of 1,3-butadiene may decrease somewhat with the use of RFG (containing a variety of oxygenates), but little or not at all with oxyfuel (Gorse et al. 1991; Reuter et al. 1992; Koehl et al. 1993; Noorman 1993; Auto/Oil AQIRP 1995). However, using oxygenates in gasoline may lead to increased emission of formaldehyde or acetaldehyde. Specifically, using ETBE, DIPE, or ethanol as oxygenates cause acetaldehyde emissions to increase, whereas use of MTBE or TAME increases formaldehyde emissions (Gorse et al. 1991; Reuter et al. 1992; Koehl et al. 1993; Noorman 1993; Auto/Oil AQIRP 1993). In older fleets, no increase or less of an increase in formaldehyde emissions is observed with the use of MTBE because formaldehyde is already emitted at higher levels. Of course, for all of these air toxics, the amount that their emissions increase or decrease is a product not only of the composition of the gasoline but also of the engine and emissions control technology, and will be

affected by the atmospheric conditions and contributions from other sources.

In order to consider the relative carcinogenicity between exposure to emissions from conventional gasoline and emissions from oxyfuel and RFG, as a first step, one can look at the projected risks of the air toxics that decrease and compare them with those that increase. These air toxics are present at different levels in the air, are affected differently by changing the gasoline composition, and have different projected potencies. Because 1,3-butadiene has such a high projected unit risk, a small decrease in its emission could offset a larger increase for most other carcinogens. However, the projected risk from 1,3-butadiene is uncertain because both its potency and the spectrum of tumors induced in mice are very different from those in rats (Health Effects Institute 1993; U.S. Environmental Protection Agency 1993c). The EPA (1993c) presented 17 different unit risk numbers for 1,3-butadiene developed by several organizations and individuals based on different studies (rat or mouse) and different types of tumors; these unit risk numbers vary by about 4,000-fold in potency. It is not clear which animal model may be more relevant to the human situation (Melnick and Kohn 1995; Bond et al. 1995). Human data are just now emerging, that will help in this assessment.

Table 39 illustrates the uncertainty in potencies of four air toxics: acetaldehyde, benzene, 1,3-butadiene, and formaldehyde. It provides estimates summarized by the EPA (1993c) of the projected number of cancer cases per year in the United States attributed to these four air toxics using different unit risk estimates (the highest, lowest, and the official EPA unit risk estimates). The projected cancer incidence for each air toxic is presented because it combines the unit risk and exposure estimates for each. The exposure estimates take into account the requirements of the Clean Air Act (including introducing oxyfuel, RFG, and more stringent vehicle emissions standards), but the uncertainties in the exposure estimates are not considered in Table 39. With EPA's official unit risk numbers and estimates of exposure, the number of projected cancer cases for the four air toxics combined decreases by 33% (from 423 to 282) between 1990 and 1995 and by 45% (from 423 to 235) between 1990 and 2000. The uncertainties in the unit risk estimates are illustrated by comparing the cancer cases projected using different risk estimates. Reflecting the range in unit risks, the difference between estimates of cancer cases for total air toxics is about 400-fold. This range would be increased greatly by factoring in the uncertainty in each of the exposure estimates.

Besides varying in projected potency for humans, the air toxics also vary in the likelihood that they will be carcinogenic to humans, which must be kept in mind when interpreting risk assessments. Based on the strength of the evidence, including whether evidence from human studies is available, substances have been classified for their carcinogenicity by the EPA, and similarly by other agencies, as follows (U.S. Environmental Protection Agency 1994b): (A) a known human carcinogen, based on sufficient evidence from human studies; (B1) a probable human carcinogen, based on limited evidence from epidemiologic studies, with or without animal studies; (B2) a probable human carcinogen, based on animals studies with inadequate (or no) data from epidemiologic studies; and (C) a possible human carcinogen, based on limited evidence from animal studies. In this scheme, benzene is the only mobile-source air toxic thus far classified as a known human carcinogen (A) because of its ability to cause leukemia in humans at relatively high exposure levels. Three air toxics are classified as probable human carcinogens: formaldehyde (B1), acetaldehyde (B2), and 1,3-butadiene (B2). The EPA (1994b) tentatively classified MTBE as a possible human carcinogen (C) on the basis of two inhalation studies, and intended to consider changing the classification to a probable carcinogen (B2) after publication of the gavage study (Belpoggi et al. 1995).

The remaining discussion addresses other potentially carcinogenic constituents of gasoline or emissions that are important to consider in putting the possible carcinogenicity of MTBE in perspective. These include polycyclic organic matter, gasoline particulate matter, and gasoline vapors. The levels of some of these in emissions may change when oxygenates are added to gasoline, but the Oxygenates Evaluation Committee has not evaluated information on that.

Polycyclic organic matter is defined in the CAAA of 1990 as a class of organic compounds having more than one benzene ring and a boiling point of 100°C or higher. The class includes polycyclic aromatic hydrocarbons (PAHs), substituted PAHs such as nitro-PAHs and alkyl-PAHs, heterocyclic compounds such as aza-arenes and thioarenes, and other subclasses such as lactones (Health Effects Institute 1993). Polycyclic organic matter compounds with five or more benzene rings are usually associated with particles, whereas those having four or fewer rings are semivolatile. Many POM compounds are mutagenic and some are carcinogenic. Evidence demonstrates that the incidence of lung cancer may increase as a result of high-level occupational exposures to inhaled POM (International Agency for Research on Cancer 1984a,b,c, 1985, 1989). Thus, POM compounds may add to the carcinogenicity of motor vehicle emissions in proportions as yet unknown.

Gasoline particulate matter consists of a carbon core and a soluble organic fraction that includes some POM compounds, sulfates, and trace elements. It is another constituent of motor vehicle emissions that is likely to contribute to its carcinogenicity. Extracts of gasoline particulate matter have been tested by skin painting, subcutaneous injection, intratracheal instillation, and lung implantation in mice, rats, and Syrian hamsters; these studies have resulted in excess skin tumors, lung tumors, and tumors at the injection site in such tests (U.S. Environmental Protection Agency 1993c). An unofficial unit risk based on the comparative potency method, which utilizes epidemiologic data from other emissions (coke oven, roofing tar, and cigarette smoke) along with mutagenicity data from extracts of gasoline particulate matter, has been calculated by the EPA; but this type of risk assessment is difficult to interpret, and no official unit risk and no EPA classification have been established. The IARC (1989) classified gasoline engine exhaust as a possible human carcinogen based on testing of the extracts. Because particulate matter is emitted at such low levels from gasoline engines, it is difficult to measure.

Another category of emissions to consider in putting the possible carcinogenicity of MTBE into perspective is gasoline vapors. Conventional gasoline is composed of alkanes (66% to 69%), aromatics (including benzene) (24% to 27%), and alkenes (6% to 8%) that distill from crude oil between 100°F and 400°F (U.S. Environmental Protection Agency 1993c). These components are present in different relative amounts in gasoline vapors than in gasoline because they vary in their volatility. Gasoline vapors consist mainly of alkanes (84% to 92%); alkenes range from 2% to 8%, and aromatics from 1% to 5%, based on different studies (U.S. Environmental Protection Agency 1993c). Gasoline vapors also contain oxygenates when they have been added to the fuel. Results of a wide variety of mutagenicity tests of unleaded gasoline have generally been negative (U.S. Environmental Protection Agency 1993c), but long-term exposure to wholly vaporized gasoline has increased the incidence of kidney tumors in male F344 rats and liver tumors in female $B6C3F_1$ mice (MacFarland et al. 1984). When considering their applicability to possible effects in people exposed to gasoline vapors in the air, two factors must be considered (Health Effects Institute 1988). First, wholly vaporized gasoline is not representative of the mixture of compounds that evaporates in ambient situations. The larger hydrocarbon molecules are less volatile than smaller ones and, therefore, are present in lower proportions in evaporative emissions than in wholly vaporized gasoline. These hydrocarbons include the compounds that appear to be responsible for the nephrotoxicity seen in male rats that may be related to the development of kidney tumors (Health Effects Institute 1988; Raabe 1993). Second, questions about the relevance of the rat kidney tumors and the mouse liver tumors to human cancer remain unanswered. The $B6C3F_1$ mouse strain has a high incidence of spontaneous liver tumors, and an increased incidence of such tumors has been observed in humans after $B6C3F_1$ mice have been treated with a broad variety of chemicals, including many that are nongenotoxic in humans, which suggests that this response may be nonspecific, initiated via mechanisms such as stimulation of cell proliferation or altered gene expression. Whether such effects in animal strains with a high incidence of spontaneous tumors are relevant to assessing the risk of human cancer is a matter of debate. Furthermore, the male rat is unusually susceptible to renal toxicity induced by hydrocarbons, which is postulated to involve a male urinary protein, $\alpha_{2\mu}$-globulin, that has not been detected in other species, including humans. This protein is thought to trigger pathologic events that result in nephropathy and ultimately renal tumors (Health Effects Institute 1988; Swenberg 1993). Recent and updated information from epidemiologic studies of oil refinery and distribution workers have not provided a definitive answer about the relationship between gasoline exposure and kidney cancer (Enterline 1993; Poole et al. 1993; Rushton 1993; Schattner et al. 1993; Wong et al. 1993).

In conclusion, many uncertainties complicate assessing the carcinogenicity of motor vehicle emissions produced from conventional gasoline, oxyfuel, and RFG. These include the relative levels of exposure to each of the carcinogens when different types of gasoline are used, and the carcinogenic potency of each compound in humans, which could range from zero to levels exceeding the highest unit risk estimate that has been calculated if the carcinogen is more potent in humans than in laboratory animals. At present, too little is known about the relevant potency and exposure levels to allow a firm conclusion as to whether one fuel mixture is more carcinogenic than another; when possibile changes in the emission levels of carcinogenic compounds other than oxygenates is considered, arriving at a meaningful conclusion is even more difficult.

OZONE

Reformulated gasoline is intended to decrease emissions of ozone-forming hydrocarbons, and some evidence indicates that it does (see the section on Exposure Assessment and Appendix A). However, the effect of RFG on exposure to ozone in ambient air has not been evaluated in this review. Reformulated gasoline is used in areas of the country with the most serious violations of the NAAQS for ozone. These areas have "severe" or "serious" levels of

ozone and populations of 250,000 or more. The current NAAQS for ozone is met when the number of days per calendar year with maximum hourly average concentrations above 0.12 ppm is equal to or less than one.

Acute ozone exposures of human subjects at ambient levels cause reductions in lung function and increases in respiratory symptoms, airway reactivity, airway permeability, and airway inflammation. Reversible decreases in lung function have been found in healthy, young men exposed for 6.6 hours at levels as low as 0.08 ppm ozone while exercising for 5 hours in an exposure chamber (Horstman et al. 1990). Subjects exposed to ozone have a wide range of response (McDonnell et al. 1983). As the ozone concentration is increased to 0.4 ppm, the percentage of subjects who respond increases. Studies of children in summer camps (Spektor et al. 1988; 1991) showed that daily exposure to ozone levels at or near the NAAQS were associated with reversible decrements of lung function. Changes in lung function appear to be largely due to involuntary inhibition of inspiration (Hazucha et al. 1989). The long-term health significance of these transient lung function changes is not known. Although these effects may be serious in people with severely compromised lung function, they are generally thought not to have serious consequences for healthy individuals.

Short-term ozone exposure also induces upper and lower airway inflammatory responses that do not correlate with lung function responses in the individuals but may be of greater concern. Seltzer and coworkers (1986) exposed healthy human subjects to 0.4 or 0.6 ppm ozone for 2 hours. They reported that the percentage of neutrophils increased nine-fold in samples of bronchoalveolar lavage fluid recovered from the subjects exposed to ozone compared with controls. Increased levels of neutrophils and other markers of inflammation were found in bronchoalveolar lavage samples of exercising subjects after they were exposed either for 2 hours to 0.4 ppm ozone (Koren et al. 1989) or for 6.6 hours to concentrations as low as 0.10 or 0.08 ppm ozone (Devlin et al. 1991). It is possible that repeated inflammatory episodes lead to production of excess connective tissue and ultimately to the development of chronic pulmonary disease. However, at present, human data are lacking to either prove or disprove this hypothesis.

Recently, a comprehensive set of studies, designed to investigate the noncancer effects of long-term exposure of rats to ozone, was completed (Collaborative Ozone Project Group 1995). F344 rats were exposed for 6 hours/day, 5 days/week, for 20 months to ozone at concentrations of 0.12, 0.5, or 1.0 ppm. The most striking effects in this study were found in the nose, where the epithelium lining the nasal cavity was structurally altered and the flow of mucus was impaired at the two highest ozone concentrations (Harkema et al. 1994). Also, at 0.5 and 1.0 ppm ozone, structural effects were seen in other regions of the respiratory tract, particularly in the centriacinar region, and small biochemical changes in connective tissue. Small alterations in epithelial cells in the centriacinar region were reported in particular areas after exposure to 0.12 ppm ozone. No effects on pulmonary function were observed, however. Although the rats developed some fibrotic lesions in the centriacinar region of the lungs, they did not develop diffuse pulmonary fibrosis analogous to the human condition. Several aspects of the study design limit the ability to extrapolate the findings to humans: (1) the exposure pattern did not simulate the human ambient exposure pattern, which is intermittent and includes extended periods during which peak ozone levels are below 0.12 ppm; (2) it was conducted using healthy animals; and (3) it involved exposure to ozone in the absence of other air pollutants.

Some evidence indicates that ozone may exacerbate the effects of other pollutants in ambient air. For example, Molfino and coworkers (1991) found that exposing asthmatics subjects with seasonal symptoms to 0.12 ppm ozone for 1 hour enhanced their reactivity to an inhaled allergen. Ozone, along with acidic aerosols and sulfur dioxide, has been associated with an increase in hospital admissions for asthma (Bates and Sizto 1987, 1989).

In summary, effects of concern from ozone include (1) transient impairment of pulmonary function affecting activity and exercise performance in some people, but which may have more serious effects in those individuals with already compromised lung function; (2) inflammatory effects of short-term exposure that may eventually lead to chronic pulmonary disease, although this relationship has not been clearly demonstrated; (3) possible exacerbation of asthma in conjunction with other air pollutants; and (4) chronic exposure may compromise the upper respiratory tract functions that normally protect the distal airways.

CONCLUSIONS

This section has provided information on the potential health effects of several pollutants that increase or decrease in emissions from oxyfuel or RFG to provide a qualitative comparison with the potential effects of exposure to MTBE and other oxygenates. The main conclusions are:

- Gasoline vapors and motor vehicle exhaust are complex mixtures with many toxic constituents. The potential effects of concern from exposure to MTBE and other oxygenates—headaches and other symptoms,

neurotoxicity, and cancer—are similar to the effects of some other constituents of gasoline and motor vehicle emissions.

- On the basis of effects observed in emissions, likely changes in exposure from adding oxygenates to gasoline include decreased exposure to CO, which was the main goal of the oxyfuel program. Of various potentially sensitive groups that have been studied, individuals with coronary artery disease have been found to be sensitive to the lowest levels of CO. They experience myocardial ischemia, sometimes causing chest pain (angina pectoris), when they exercise in the presence of CO at levels that elevate their COHb to 2%. No evidence of a threshold for these effects has been found. However, the information on personal exposure to CO for individuals with coronary artery disease and on the number of sensitive individuals is insufficient to determine the health benefit of the decrease in CO emissions.
- Adding oxygenates to gasoline also affects the emission of several air toxics. It decreases emission of benzene and may slightly decrease, or not affect, the emission of 1,3-butadiene; but it increases emission of aldehydes, which are partial combustion products of the oxygenates. The knowledge about the carcinogenic potency in humans of these air toxics, MTBE, and other carcinogens in gasoline and emissions, and about personal exposure to them, is too uncertain to allow a meaningful assessment of the relative carcinogenic risk of conventional gasoline, oxyfuel and RFG.
- Reformulated gasoline may decrease emission of ozone-forming compounds, but its effects on ambient ozone were not evaluated in this review. (Oxygenates do not contribute significantly to this effect on ozone-forming compounds.) Ambient ozone levels near or exceeding the current standard can cause transient decrements in pulmonary function that affect exercise performance in some people, and may have more serious effects in those with already compromised pulmonary function; they also cause inflammatory effects, which may contribute to the development of chronic pulmonary disease, although this has not been demonstrated.

Table 38. Potential Health Effects of Low-Level Exposure to Various Pollutants and the Projected Direction of Change in Exposure Levels for Each Pollutant When Oxyfuel or RFG Containing MTBE or Ethanol Is Used

Pollutant	Potential Health Effects	Projected Change in Exposure Level From That of Conventional Gasoline			
		MTBE Oxyfuel	Ethanol Oxyfuel	MTBE RFG	Ethanol RFG
Oxygenates MTBE	• Symptoms (headache, eye irritation, disorientation) • Neurotoxicity • Cancer in animals	↑	0	↑	0
Ethanol	• Effects unlikely when inhaled at low levels (cancer and developmental effects seen with high-level ingestion exposure)	0	↑	0	↑
Air Toxics Formaldehyde	• Irritation • Cancer (probable human carcinogen)	↑	0	↑	0
Acetaldehyde	• Cancer (probable human carcinogen)	0	↑	0	↑
Benzene	• Cancer (known human carcinogen) • Developmental effects	↓	↓	↓	↓
1,3-Butadiene	• Cancer (probable human carcinogen)	0 ?	0 ?	↓	↓
Carbon Monoxide	• Myocardial ischemia (including angina) during exercise • Decreased exercise capacity	↓	↓	↓	↓
Ozone	• Respiratory symptoms • Lung function decrements • Decreased exercise capacity • Chronic lung injury?	0	0	↓ ?	↓ ?

Table 39. Projected Cancer Incidence[a]

Unit Risk Estimate Used[b]	Projected Cancer Incidence[c]		
	1990	1995	2000
Benzene			
Low	< 1	< 1	< 1
EPA	70	43	35
High	438	269	219
1,3-Butadiene			
Low	< 1	< 1	< 1
EPA	304	209	176
High	3,691	2,538	2,137
Acetaldehyde			
EPA (Low)	5.3	3.6	2.8
High	6.5	4.4	3.4
Formaldehyde			
Low	2	1	1
EPA (High)	44	28	21
Total			
Low	9	7	6
EPA	423	282	235
High	4,180	2,839	2,380
High:Low	464	406	397

[a] Adapted from U.S. Environmental Protection Agency 1993c.

[b] More than one unit risk estimate for cancer has been calculated for each of these air toxics. In this table, "low" represents the lowest unit risk included in the EPA's summary (U.S. Environmental Protection Agency 1993c), "high" represents the highest unit risk, and "EPA" represents the unit risk currently approved by the EPA. For some of the air toxics, a more recent EPA unit risk has been calculated but not approved.

[c] Cancer incidence (number of cases of cancer in the U.S. per year) projected by the EPA using the indicated unit risks and estimates of exposure to the air toxics, assuming the requirements in the CAAA of 1990 are carried out. For benzene, values indicate the projected incidence of death, rather than cancer.

Overall Assessment and Conclusions

The conclusions of the HEI Oxygenates Evaluation Committee about the potential health effects of oxygenates added to gasoline are summarized in this section, and qualitatively compared with the health effects that could result from (1) projected changes in the levels of other toxic substances in motor vehicle emissions, and (2) exposure to conventional gasoline and exhaust.

OXYGENATES

EXPOSURE

More than 70 million people in the United States live in areas in which oxygenates are used in fuel and, therefore, may be exposed to oxygenates, mainly MTBE and ethanol. The major route of exposure for most people appears to be through inhaling oxygenates in the evaporative emissions of gasoline. However, MTBE and other oxygenates can move into groundwater from contaminated soil, and limited measurements of MTBE in shallow wells suggest that some contamination has occurred. Thus, ingestion and skin absorption are also routes of exposure of potential concern for MTBE and possibly for other oxygenates in gasoline. Dermal exposure also could occur during refueling and other activities in which gasoline containing oxygenates is handled.

Air concentrations of MTBE have been measured in a variety of locations to assess human exposure. These studies indicate that the general public is likely to encounter the highest air concentrations during refueling of cars, with median exposure levels of about 0.2 to 1.5 ppm and peak levels occasionally exceeding 10 ppm. Most people would experience these exposure levels for no more than a few minutes every few days. Commuters may be exposed to MTBE concentrations of several parts per billion (up to 17 ppb in the studies summarized in this review) inside their cars for one or two hours per day. Workers handling the oxygenates or fuels containing them are exposed to higher concentrations of MTBE and for longer periods of time than consumers. Service station attendants are exposed repeatedly for 6 to 8 hours per day to the same concentrations of MTBE described above for the general public during refueling. The highest levels of exposure are experienced by workers involved in transporting MTBE or fuels containing it. In a large study of occupational exposure to MTBE, the median value for short-term (30-minute) measurements during the transport of neat MTBE was 13.8 ppm, with a range of 0.3 to 1,050 ppm; during transport of fuel mixtures, the median was 2.4 ppm, with a range of 1 ppb to 508 ppm.

Information on exposure to ethanol is much more limited than what is available on exposure to MTBE. Measurements of ethanol during refueling at service stations were generally under the minimal detectable concentration of about 1 ppm, but occasionally high levels, up to 46 ppm, were recorded. Few measurements have been taken of air concentrations of ETBE or TAME, which also have been used in fuels. Although exposure information is much more extensive for MTBE than for the other oxygenates and provides a rough estimate of exposure ranges associated with various activities for the general population, the frequency and distribution of these activities and the amount of exposure by dermal and oral routes is uncertain. Because of these limitations, using these data to calculate a cumulative exposure for use in risk assessment is not appropriate.

HEALTH EFFECTS

Review of the literature on the toxicity and health effects of MTBE has indicated three areas of uncertainty and potential concern: (1) symptoms reported after short-term exposure in a number of communities using fuel containing oxygenates; (2) neurotoxicity based on effects on motor activity observed in studies of rats exposed to MTBE; and (3) tumors observed in rodents after exposure to MTBE.

Symptoms in response to short-term exposure, including noticeable odor, headaches, eye irritation, dizziness, and a burning sensation in the nose and throat, were reported in various communities after MTBE was introduced. Based on the studies conducted, it appears that most people do not experience unusual symptoms or significant medical consequences in response to short-term exposure to MTBE in gasoline. However, MTBE in gasoline may cause acute symptoms in some people or under some circumstances. The Alaska studies provide the strongest evidence of an effect of MTBE; unique factors there, such as the cold, dry climate or the greater increase in odor compared with the fuels in other areas, may have contributed to these effects. The existing body of data does not allow us to conclude

whether the symptoms reported can be attributed directly to MTBE.

The observation of increased levels of motor activity in rats exposed to 800 ppm MTBE raises concerns about neurotoxic effects of MTBE. In the absence of (1) studies of motor activity in rats at lower exposure concentrations, (2) evaluation of neurotoxic endpoints in sensitive human subjects, and (3) animal or human studies evaluating more complex central nervous system functions that are often more sensitive indicators of central nervous system effects, the possibility of neurotoxic effects at lower levels of exposure must be considered.

Researchers have observed an increase in the frequency of tumors in several organs of rats and mice exposed to high, toxic levels of MTBE via two routes of exposure (inhalation and oral administration). In addition, the two primary metabolites of MTBE, TBA and formaldehyde, have been reported to cause tumors in rodents. On the basis of these studies, it is difficult to project what the likelihood is that MTBE would cause tumors in people exposed to levels 3 orders of magnitude lower, where toxicity would not occur. Nevertheless, the observation of tumors at several sites and in two species is of some concern.

Not enough information is available on the toxicity of ETBE and TAME to evaluate their potential health effects, but more research is being planned. No information is available on the toxicity of DIPE.

It is well known that ethanol is neurotoxic upon acute exposure by ingestion. Prolonged intake of moderate to large amounts of ethanol is associated with degenerative processes in the liver, heart, and nervous system. Nutritional disturbances and direct toxicity are commonly involved in the pathogenesis of these changes. Ingesting high levels of ethanol over long periods of time is associated with cancer in several organs. Maternal ingestion of moderate to high levels of ethanol during gestation can cause prenatal developmental effects. A threshold dose below which effects would not occur has not been identified for either developmental or cancer effects. Nonetheless, adverse effects are not expected to result from the levels to which most people would be exposed from ethanol used in gasoline. Such low exposure levels are not predicted to increase blood levels of ethanol, which is a normal metabolic product found in the blood.

OTHER POLLUTANTS

EXPOSURE

Adding oxygenates to fuel reduces emissions of certain pollutants and increases others. The main goal of adding oxygenates to fuel is to reduce CO. Emissions data summarized in this review indicate that, when oxygenates are added to fuels, CO emissions decrease by 10% to more than 20%, depending on the fuel formulation and fleet used in the emissions studies. The Oxygenates Evaluation Committee noted that the number of exceedances of the 8-hour NAAQS had decreased substantially in many areas since oxyfuel was introduced, but did not attempt to evaluate the contribution of using oxyfuel to the overall reduction of ambient CO levels.

Although adding oxygenates appears to cause an overall reduction in the emission of air toxics, levels of some air toxics increase. Older fleet vehicles have higher emissions of all air toxics than current fleet vehicles. Total emissions of air toxics from gasoline-fueled vehicles are dominated by benzene, which represents 65% to 80% of the total, and benzene levels are generally decreased by using oxygenates. In the emissions studies considered in this review, concentrations of 1,3-butadiene were reduced very slightly or not at all in exhaust emissions from vehicles using fuel containing oxygenates, but more consistently reduced by using RFG. Formaldehyde emissions tend to increase when MTBE or TAME is added to fuel; this effect is seen mainly in newer vehicles that produce low formaldehyde emissions. Acetaldehyde emissions are increased when ethanol, ETBE, or DIPE is added to fuel.

These changes in emissions when oxygenates are added to fuel are expected to be reflected to varying degrees in ambient exposures, particularly in high traffic areas. The extent to which this would be true in any region depends on many factors, including the fuels used, the composition of the fleet of vehicles, the number of vehicle miles traveled, the contributions of pollutants from other sources, and local atmospheric conditions. This review did not evaluate information on ambient levels of CO, air toxics, or ozone. The effects on air quality of using oxygenates will be evaluated in a broader review of the oxyfuel and RFG programs by the White House Office of Science and Technology Policy.

HEALTH EFFECTS

To place the potential health effects of adding oxygenates to gasoline in current perspective, one must consider how their presence may change the levels of other pollutants that affect human health, namely CO and some air toxics, as discussed above. It is also important to keep in mind that these pollutants are part of complex mixtures of gasoline vapors and motor vehicle exhaust that contain many toxic constituents, some of which are teratogenic, carcinogenic, or neurotoxic. The short-term symptoms reported with using MTBE are not unlike those sometimes reported after

exposure to gasoline vapors or motor vehicle emissions from conventional gasoline.

People with coronary artery disease have been shown to experience adverse effects when exposed to a lower dose of CO (measured by the percentage of hemoglobin that combined with CO in the blood) than that to which any other sensitive groups have responded. In controlled human exposure studies, levels of COHb as low as 2% have been shown to accelerate the time to myocardial ischemia, sometimes causing chest pain (angina pectoris), in some individuals with coronary artery disease during exercise, when oxygen demand to the heart is increased. The health benefit of reducing CO is uncertain because of severe limitations in the information about the number of sensitive individuals with coronary artery disease, their personal exposure to CO, and their activity patterns.

Our current understanding of the human effects from various carcinogens in motor vehicle emissions (including MTBE and those air toxics that have increased or decreased levels when oxygenates are used) is not sufficient to make confident predictions about whether using MTBE will cause any overall increase or decrease in the total carcinogenicity of emissions compared with using conventional gasoline. The knowledge about both potency of the carcinogens in people and exposure to them is too uncertain to conclude whether one fuel mixture is more carcinogenic than another, especially considering the possible changes in emissions of other carcinogenic compounds, and the possible interactions between oxygenates and other emissions. However, a substantial increase in carcinogenic risk from using gasoline containing MTBE or ethanol is not expected.

CONCLUSIONS

This review of the potential health effects of oxygenates added to gasoline, mainly to decrease emission of CO, has noted several areas of concern about the potential health effects of oxygenates. After the HEI Oxygenates Evaluation Committee carefully considered the data on MTBE, concerns have remained about symptoms reported by people exposed to MTBE in gasoline, neurotoxicity observed in rats exposed to high levels of MTBE, and an increased frequency of cancers at multiple sites in MTBE-exposed rats and mice. On the other hand, even though exposure to moderate to high levels of ethanol causes neurotoxic, developmental, and cancer effects, the Committee concluded that health effects from exposure to ambient levels of ethanol are unlikely because endogenous blood ethanol levels are not predicted to increase significantly from inhaling ethanol in fuel.Evidence shows that adding oxygenates to gasoline reduces the emission of CO, benzene, and possibly 1,3-butadiene from motor vehicles, and thereby may lower certain health risks to exposed persons. On the other hand, adding oxygenates to fuel is expected to increase exposure to aldehydes as well to the oxygenates, which themselves may pose some health risks. Possible health effects from exposure to oxygenates—headaches and other symptoms, neurotoxicity, and cancer—are of a similar nature to effects associated with some constituents of conventional gasoline and its emissions.

Looking qualitatively at the whole picture, it is unlikely that using fuel containing oxygenates would substantially increase the overall health risk from fuel used in motor vehicles. Therefore, the HEI Oxygenates Evaluation Committee did not find that the questions about potential health risks are sufficient to warrant an immediate reduction in oxygenate use. It has concluded, however, that a number of important research needs must be met if there is to be continued widespread use of oxygenates over the long term. These research needs, which are summarized in the next section of this review, are directed toward decreasing the uncertainty in projecting risk to the public from exposure to oxygenates. The Committee recommends that in the future any such new use of a substance be preceded by a sufficiently comprehensive research and testing program and be accompanied by exposure assessment and epidemiologic studies.

The EPA requested that HEI review its two documents evaluating the potential health effects of oxygenates, *Assessment of Potential Health Risks of Gasoline Oxygenated with Methyl Tertiary Butyl Ether (MTBE)* (1993a) and *Health Risk Perspectives on Fuel Oxygenates* (1994b). In general, the HEI Oxygenates Evaluation Committee found that EPA had conducted a thorough and credible review of the evidence available. The HEI Committee's review of the EPA's conclusions, including areas where it concurred and differed with the EPA, is summarized in Appendix B.

Research Priorities for Oxygenates

This review has identified gaps in information that have limited what the HEI Oxygenates Evaluation Committee could conclude about the health effects of oxygenates added to gasoline. The specific research needs in each of the areas evaluated are outlined below. Those that the Committee thought to be of the highest priority for resolving questions about health effects of oxygenates are marked with three asterisks (***), those with moderate priority with two asterisks (**), and those of lower priority with one (*).

A number of studies to investigate further the effects of MTBE and to characterize the toxicity of other ethers are already planned or ongoing (see U.S. Environmental Protection Agency 1995). These are indicated in the appropriate categories below. In addition, testing for fuel registration, mandated under Section 211 (b) of the CAAA of 1990, will begin soon. The current requirement consists of evaluating the evaporative and combustion emissions from fuels containing oxygenates. The tests to be conducted on the emissions include a 90-day subchronic inhalation toxicity study, reproductive and developmental studies and neurotoxicity assessment and possibly a 2-year carcinogenicity study. The EPA has indicated its interest in modifying these requirements to ask for a more appropriate assessment of the emissions' toxicity. The Oxygenates Evaluation Committee encourages the EPA and industry to consider the following research priorities in developing alternative testing requirements.

Testing of the individual oxygenates falls under the Toxic Substances Control Act. The Interagency Testing Committee designated ETBE and TAME to be tested (Federal Register 1994). As a result of a consent agreement between the EPA and theAPI, testing of TAME started in 1995 (Federal Register 1995). The research plan includes pharmacokinetic studies, studies of subchronic exposure in two species, reproductive and developmental toxicity, mutagenicity, and neurotoxicity. At this time, a consent agreement has not been agreed upon for ETBE testing. However, ARCO has indicated a commitment to conduct toxicity studies in rats and mice.

EXPOSURE ASSESSMENT

*** A comprehensive set of studies needs to be undertaken to determine levels of personal exposure to oxygenates using standardized protocols. Although more information on MTBE is needed, the need is particularly great for assessing exposure to ethanol, ETBE, and TAME because these compounds are currently in use, or may be soon, and the resulting exposures have not been adequately assessed. These factors should be considered in planning such studies:

- Using standardized methods for collecting samples (including the sampler's flow rate, sampling time, analytical methods, and calibration procedures); applying quality control procedures consistently across studies;
- Assessing exposures in microenvironments where consumers have the highest-level exposures such as in refueling vehicles, and in occupational settings where significant exposure is likely to occur;
- Measuring gasoline components other than oxygenates that might serve as markers for the complex mixture in the ambient air;
- Measuring levels of oxygenates and their metabolites (as biomarkers) in blood;
- Collecting data at different times of the year, and in areas with different climatic conditions, including extremely low and high temperatures and humidity; and
- Identifying sensitive populations and measuring their exposures.

* Environmental sampling data are needed to assess the fate and distribution of atmospheric transformation products of MTBE and other oxygenates such as *tert*-butyl formate.

* The extent of MTBE contamination of drinking water needs to be analyzed.

METABOLISM AND DISPOSITION

** Further studies of the metabolism of MTBE would be of great value in assessing the health risks from expo-

sure to MTBE and in understanding the importance of differences in the metabolic process in determining sensitivity in individuals. Studies involving exposure to oxygenates as parts of complex mixtures that represent gasoline vapors and motor vehicle exhaust should be conducted to determine the potential interactive effects among gasoline components. (Some research in this area is under way at the Chemical Industry Institute of Toxicology [CIIT], funded by the Oxygenated Fuels Association [OFA], and research will be funded by HEI this year from its recent RFA on "Comparative Metabolism and Health Effects of Ethers Added to Gasoline to Increase Oxygen Content.") Areas to be investigated include:

- The kinetics of TBA, formate, and formaldehyde formation and the role of the cytochrome P-450 enzymes in metabolizing MTBE and TBA;
- The metabolic fate of TBA in response to concerns about the potential toxicity of possible metabolites and of free radicals produced during oxidative metabolism.

** Pharmacokinetic studies need to be extended to the other ethers, especially ETBE and TAME. (HEI is planning to fund studies to compare MTBE with other ethers. Also, for TAME, pharmacokinetic studies are being conducted to comply with regulations specified in the Toxic Substances Control Act.)

* Studies that compare inhalation and oral exposure should be conducted to determine the kinetics of uptake and disposition of ethanol in human subjects at concentrations expected to be encountered in ambient air. This information would enhance confidence in the current conclusion that ambient air exposures would not result in a significant increase in blood levels of ethanol.

SHORT-TERM EFFECTS

*** Controlled human exposure studies should be conducted to assess the short-term effects of MTBE, other ethers, and ethanol in a hydrocarbon mixture that is representative of gasoline, and compare subjects' symptomatic reactions to that mixture with reactions to the hydrocarbons alone.

- Studies should include potentially sensitive subjects, such as individuals who have reported symptomatic responses to exposure to oxyfuel, as well as other groups hypothesized to be sensitive, perhaps individuals who have allergies or who are elderly. The effects of exercise on responses should be assessed. (Studies of individuals who have reported a sensitivity to MTBE are under way or planned at the EPA and the Environmental and Occupational Health Sciences Institute.)
- Blood levels of the oxygenates and pertinent metabolites should be measured in these studies to understand the relationships among exposure, dose, and effects and to compare with levels measured in real-life situations.
- For MTBE, these studies should also evaluate possible neurotoxic effects at several exposure levels using sensitive tests to measure complex central nervous system functions.

** Epidemiologic studies should be conducted to evaluate in the general population the short-term effects of MTBE, other ethers, and ethanol as gasoline additives. The limitations of the currently available information on the short-term effects of MTBE have been discussed in depth in the previous sections. The community-based studies provide an indication of what symptoms might be encountered and insights concerning hypotheses to be tested. Future studies should aim at providing information on the relations between activities and exposure, exposure and biomarkers of dose, and dose and health outcomes. Several types of efforts would be informative concerning potential health consequences of MTBE:

- Longitudinal studies are needed that prospectively collect daily symptom reports before and after oxygenates are added to fuel in various geographical areas;
- Protocols should be developed for studies of symptom outbreaks, including standardized questionnaires for symptoms and for assessing factors that may predispose some individuals to these symptoms;
- Study designs should be developed to assess what factors define susceptibility and to identify susceptible subgroups;
- Occupational studies of workers involved in producing, handling, or transporting MTBE would provide useful information about a broader range of exposures and situations than those encountered by the general population;
- Consideration should be given to studies of outcomes other than symptoms, including neurobehavioral effects (such as reaction times, attention, and vigilance) and immunologic effects (such as T-cell counts).
- Hybrid protocol designs that bring individuals from the community into laboratory investigations

involving controlled exposure also may be informative.

** Animal studies at relevant exposure levels also may be helpful in investigating the neurotoxic and other effects of MTBE and as a screening tool for other ethers. Behavioral tests that explore a broad range of complex motor, sensory, cognitive, and motivational measurements should be used. These studies should include measuring blood levels of MTBE and reporting, for dose-response relationships, a measurement such as a 10% change in performance, which would then be the precursor to a benchmark dose calculation.

LONG-TERM EFFECTS

*** Epidemiologic studies of workers who have been exposed to MTBE since the early 1970s should be conducted to determine whether the frequency of some types of tumors is increased in this population, as has been reported in animal studies.

*** To determine the potential neoplastic and nonneoplastic effects of MTBE as part of a complex fuel mixture, studies involving long-term exposure to MTBE in gasoline should be conducted in rats and mice.

*** To interpret the carcinogenic results from studies of MTBE in animals and extrapolate them to assess human risk, the following studies are needed:

- Studies should be conducted to investigate whether significant amounts of genotoxic metabolites are formed in organs in which tumors were observed in studies of long-term exposure to MTBE. Particular attention should be paid to formaldehyde, metabolites of TBA, and their putative macromolecular adducts.
- Studies should investigate whether the MTBE-induced tumorigenic responses can be explained by any of the mechanisms that have been suggested. For example, it has been argued that some of the tumors in the liver, testis, and thyroid induced with nonmutagenic carcinogens may result from endocrine disturbances caused by high doses of the test compounds or, in the case of the kidney, from a species- and gender-specific mechanism that is not relevant to humans. (Some of these studies are being conducted at CIIT, funded by OFA).

DEVELOPMENTAL EFFECTS

* Although the effects of MTBE on developmental processes seem to occur only at high doses at which maternal toxicity also is observed, studies of developmental effects of MTBE have not included extensive behavioral testing. Behavioral assays on the offspring of pregnant rodents exposed to MTBE by inhalation, or on preweanling newborns exposed to MTBE, should be conducted. They should explore a broad range of complex motor, sensory, cognitive, and motivational measures. (Developmental studies of neat TAME are currently being conducted as part of the TSCA requirements.)

HEALTH EFFECTS RESEARCH ON ETHERS OTHER THAN MTBE

*** A comprehensive plan including, but not limited to, the types of studies listed under the various areas of research should be developed for investigating the health effects of other ethers. They should be based on the current knowledge of the effects of MTBE and on the results of pharmacokinetic studies of MTBE and other ethers. (Toxicity testing of TAME is in process under TSCA, and some work on ETBE in rats and mice will be funded by ARCO [90-day subchronic study, neurotoxicity screening].)

Appendix A. The Effects of Using Fuel Containing Oxygenates on the Emission of Other Pollutants

INTRODUCTION

The Clean Air Act Amendments (CAAA) of 1990 require that, starting in 1992, oxyfuel be used in areas of the United States with high carbon monoxide (CO) levels and that, starting in 1995, reformulated gasoline (RFG) containing oxygenates be used in areas with high ozone levels. The main goal of using oxyfuel, which is conventional gasoline to which at least 2.7% oxygen by weight has been added, is to reduce emission of CO. Reformulated gasoline, which must contain at least 2.0% oxygen by weight, also has reduced levels of benzene and aromatics, and using it must decrease emission of ozone-forming hydrocarbons and toxic air pollutants while not increasing levels of NO_x.

To evaluate the effectiveness of these fuels, it is necessary to measure not only the levels of the emissions they were expected to reduce, but als· the levels of other emissions that may change; this is particularly true for formaldehyde and acetaldehyde, which are partial-combustion products of some of the oxygenates. This appendix summarizes a portion of the emissions information developed to assess the effects of adding oxygenates and of other changes to fuel. The goal of this brief summary is to provide a general understanding of how these alterations in fuels affect emissions, rather than to provide a complete review of the literature or to come to conclusions about possible changes in exposure to various pollutants from using different fuels. In this appendix we compare the effects of using different oxygenates, including ethanol and the ethers MTBE, ETBE, TAME, and DIPE, on levels of other pollutants in emissions.

Although one would expect changes in ambient levels of pollutants to reflect to some degree the changes in related emissions, the extent that this is true in any region depends on a large number of factors. These include the actual fuels used, the composition of the fleet of vehicles, the number of miles traveled, the contribution of pollutants from other sources, and local atmospheric conditions such as the ratio of hydrocarbons to NO_x in the air (which affects ozone formation). Determining if and how oxyfuels and RFG actually change exposure to pollutants is an extremely complex task, which involves complex modeling procedures.

EFFECTS OF CHANGES IN FUELS ON EMISSIONS

Three U.S. automotive companies and 14 oil companies organized a cooperative research and testing program to develop data that would aid in understanding the effects of fuel components, engine technology, and emission control devices on vehicle emissions and air quality. Phase I of this Auto/Oil Air Quality Improvement Research Program (AQIRP) evaluated emissions from 20 current-fleet vehicles (1989 models) and 14 older-fleet vehicles (1983-1985 models) using special fuel formulations, which varied in content of oxygenates, aromatics, olefins, and the temperature at which 90% of the gasoline could be distilled (T_{90}) (Gorse et al. 1991; Hochhauser et al. 1991). The fuels were not RFG as defined by the CAAA of 1990; rather important components were varied to determine each of their effects on emissions. Benzene content was 1.5% by volume, the industry average level, which is higher than the level allowed in RFG (1.0%). These four factors were varied in the fuels for testing purposes:

- MTBE was used at 0% or 15% by volume (2.7% oxygen by weight); this is the level required in oxyfuel, and higher than the minimum level required in RFG (2.0% oxygen by weight).
- Aromatics were used at 45% or 20%. The current federal requirement for RFG limits aromatics to 25%; the industry average gasoline used for reference contained 32% aromatics.
- Content of olefins was 20% or 5%; these represent the 90th and 10th percentiles, respectively, for commercially available summertime fuels. The industry average reference gasoline was 12%.
- The T_{90} level was 360°F or 280°F; these represent the 90th and 10th percentiles, respectively, for commercially available summertime fuels. The T_{90} for the industry average gasoline was 335°F.

Sixteen formulations of fuels representing all combinations of both levels of each of the four fuel variables were tested. In addition, one fuel representing industry average levels of aromatics, olefins, and T_{90}, and another as an emission certification test fuel were tested as reference fuels. Regression analyses determined the major effects of

the four factors that had been varied. Effects of variations in the four factors on important constituents of exhaust and evaporative emissions in the current and older fleets are presented in Table A.1. Although Table A.1 includes results of changing T_{90} and olefins, some of which are striking, the summary below is limited to effects of adding oxygenates and of reducing aromatics, because of their relevance to oxyfuel or RFG as defined in the CAAA of 1990.

Effects of ETBE and ethanol were compared with the effects of MTBE in a 10-vehicle subset of the current fleet used in the Phase I Auto/Oil AQIRP (Reuter et al. 1992). Results of this study are shown in Table A.2. Comparisons are somewhat complicated because the fuels were not all the same. For example, the four fuels containing ethanol were splash blended, which means that ethanol was added to fuels after they were blended, whereas the ETBE and MTBE fuels were fully blended to certain specifications. Also, MTBE and ETBE were 2.7% oxygen by weight in the fuel and ethanol was 3.5%. Another study compared TAME and MTBE at 2.0% oxygen by weight blended to represent federal emission certification fuel in ten 1989 vehicles from the current fleet of the Auto/Oil AQIRP (Koehl et al. 1993).

Emissions of MTBE, DIPE, and TAME fuels were evaluated in seven 1989–1991 vehicles selected to represent a mixture of pollution control technologies and engine sizes that would likely be used in future model years (Noorman 1993). Combinations of the oxygenates also were used: half MTBE and half TAME; half MTBE and half DIPE; and one-third each of MTBE, TAME, and DIPE. The information in this study is of particular interest because the fuels used were designed to meet RFG standards set forth in the CAAA of 1990. They contained 1% benzene and had a vapor pressure of 7.5 psi. The fuels' oxygenate content was 2.7% oxygen by weight, as required for oxyfuel, rather than 2.0%, the minimum level required for RFG. The reference fuel had properties similar to the 1990 industry average fuel. Compared with the test fuels, it had higher benzene (1.4% by volume) and aromatic (35% by volume compared with about 25% by volume) content, and a higher RVP (8.4 psi). The changes in emissions in some of the test fuels compared with the reference fuel are summarized in Table A.3. Because these test fuels meet RFG standards, their actual emissions are of interest and are summarized in Table A.4.

Exhaust emissions of a gasoline that meets the California Phase 2 regulatory requirements, which contained 11% MTBE by volume (2.0% oxygen by weight), were compared with emissions from the Auto/Oil AQIRP industry average gasoline for 1988 in three fleets of vehicles designed to have progressively lower emission standards (Auto/Oil AQIRP 1995). The Auto/Oil AQIRP older fleet (1983–1985) and current fleet (1989) were used, as well as a fleet designed to meet the 1994 Federal Tier 1 standards. The results are summarized in Table A.5.

EXHAUST EMISSIONS

This section summarizes the major effects of varying fuel components on the exhaust emissions of interest in the studies described above.

Carbon Monoxide

Reducing CO emissions was the goal of using oxyfuel, as mandated by the CAAA of 1990. In all studies summarized in this appendix, which involved a variety of fuels and fleets, adding oxygenates always decreased CO emissions. In some of the studies, however, the fuels tested differed in more ways than the oxygen content. For example, sometimes reformulated fuels were compared with industry average gasoline. The oxygenates tested included MTBE, ETBE, TAME, DIPE, and ethanol.

In the Auto/Oil AQIRP set of fuel formulations, MTBE (2.7% oxygen by weight) lowered CO emissions by 11% in the current fleet and 14% in the older fleet (Table A.1) (Hochhauser et al. 1991). Lowering the content of aromatics from 45% to 20% (compared with 25% required in current RFG) resulted in a significant reduction in CO emissions in the current fleet but not in the older fleet. In a different set of fuels tested in the current fleet, ethanol, MTBE, and ETBE each produced a significant reduction in CO in exhaust (Table A.2) (Reuter et al. 1992). Although the results indicated different reductions (ethanol reduced CO by 13.4%, MTBE by 9.3%, and ETBE by 14.6%), these values were not found to be significantly different from each other. In a study designed to compare TAME with MTBE, TAME reduced CO emissions by about the same amount as MTBE (Koehl et al. 1993). In fuels that met federal RFG requirements, using MTBE, DIPE, or TAME at 2.7% oxygen by weight reduced CO emissions by about 20% compared with the reference industry average fuel (Table A.3) (Noorman 1993). Emissions of CO from vehicles using California Phase 2 RFG containing MTBE (2.0% oxygen by weight) were reduced by more than 20% in all three of the fleets used compared with industry average gasoline (Table A.5) (Auto/Oil AQIRP 1995).

Total Hydrocarbons and Nonmethane Hydrocarbons

As shown in Table A.1, in the Auto/Oil AQIRP set of fuel formulations, adding MTBE or lowering the aromatics resulted in lower emissions of both total hydrocarbons and nonmethane hydrocarbons in the current fleet (Hochhauser et al. 1991). In the older fleet, adding MTBE reduced total

and nonmethane hydrocarbons even more than in the current fleet. However, in the older fleet, lowering aromatic content of the fuel increased the level of total and nonmethane hydrocarbons by 14% and 11%, respectively. In a different set of fuels, adding ethanol, MTBE, or ETBE to gasoline each resulted in a statistically significant 5% reduction in hydrocarbons (Table A.2) (Reuter et al. 1992). Each of these oxygenates also reduced nonmethane hydrocarbons to a similar degree. In a different set of fuels, adding TAME also was found to reduce hydrocarbons and nonmethane hydrocarbons by an amount similar to that achieved with MTBE (Koehl et al. 1993), and DIPE was found to have effects on hydrocarbon emissions similar to those of MTBE and TAME (Table A.3) (Noorman 1993) in RFG. Emissions from vehicles using California Phase 2 RFG containing MTBE (2.0% oxygen by weight) had reduced nonmethane hydrocarbons compared to with the industry average gasoline, with greater reduction in the newer fleets (Table A.5) (Auto/Oil AQIRP 1995).

Nitrogen Oxides

One of the requirements for RFG is that NO_x emissions do not increase. In both the current and older fleets, using the Auto/Oil AQIRP set of fuel formulations, adding MTBE did not affect NO_x levels in emissions (Hochhauser et al. 1991). However, there was a significant interaction between aromatics and MTBE; when aromatics were low, as in RFG, adding MTBE raised NO_x. Aromatics alone decreased NO_x in the older fleet but not in the current fleet. Using a different set of fuels, NO_x levels were increased by adding ethanol, MTBE, or ETBE to gasoline in the current fleet (Table A.2) (Reuter et al. 1992). The authors pointed out that the results were significant only with ethanol because a smaller number of data points had been obtained for MTBE and ETBE fuels. However, the three oxygenates did not differ significantly in their effects on NO_x. In another study, TAME showed no significant differences from MTBE for NO_x emissions (Koehl et al. 1993). No significant differences in NO_x emissions were observed among fuels containing MTBE, DIPE, or TAME alone or in combination in RFG (Table A.3) (Noorman 1993). Emissions of NO_x from vehicles using California Phase 2 RFG containing MTBE (2.0% oxygen by weight) were not reduced significantly in the older fleet, but were reduced in the current fleet and in the Federal Tier 1 fleet compared with the industry average gasoline (Table A.5) (Auto/Oil AQIRP 1995).

Air Toxics

Benzene As shown in Table A.1 (Gorse et al. 1991), in the Auto/Oil AQIRP set of fuel formulations, reducing fuel aromatic content from 45% to 20% lowered benzene exhaust emissions by 42% in the current fleet and 31% in the older fleet. Adding MTBE did not affect benzene emissions significantly in the current fleet but did lower them by 11% in the older fleet. In a study using a different set of fuels (see Table A.2) (Reuter et al. 1992), ethanol, ETBE, and MTBE added to gasoline each resulted in a significant reduction in benzene exhaust emissions in the current fleet, in part due to the dilution of gasoline. The three oxygenates did not differ significantly in their effects. The average effect was about 10%. In a separate study, fuels with TAME and MTBE at 2.0% oxygen by weight (Koehl et al. 1993) had similar benzene emissions. Using fuels meeting RFG standards, no significant differences in benzene levels in exhaust emissions were seen among fuels containing MTBE, TAME, or DIPE alone or in combination at 2.7% oxygen by weight (Table A.3) (Noorman 1993). Benzene emissions from vehicles using California Phase 2 RFG containing MTBE (2.0% oxygen by weight) were reduced significantly in all fleets compared with the industry average gasoline (Table A.5) (Auto/Oil AQIRP 1995). Reduction was 34% in the older fleet, 43% in the current fleet, and 47% in the Federal Tier 1 fleet.

1,3-Butadiene In the Auto/Oil AQIRP set of fuel formulations, adding MTBE lowered 1,3-butadiene by 9% in the current fleet; reducing the aromatic content decreased the 1,3-butadiene emissions by 11% (Gorse et al. 1991). In the older fleet, neither lowering aromatics nor adding MTBE affected 1,3-butadiene emissions. An interaction between MTBE and aromatics was noted, such that when aromatics were reduced, 1,3-butadiene was increased only in the presence of MTBE; thus MTBE lowered 1,3-butadiene only when aromatics were at the higher level. In another study, in which the effects of ethanol, MTBE, and ETBE were evaluated in fuels that were not totally equivalent (Table A.2) (Reuter et al. 1992), each oxygenate reduced 1,3-butadiene emissions, but the effect was only significant with ethanol. Blended at 2.0% oxygen by weight to federal emission certification test fuel specifications, TAME and MTBE had similar effects on 1,3-butadiene emissions (Koehl et al. 1993). Reductions in 1,3-butadiene emissions achieved by using RFG containing MTBE, TAME, DIPE, or combinations of them (at 2.7% oxygen by weight) were similar, and bordered on being statistically significant (Table A.3) when compared with reference fuel (Noorman 1993). Levels of 1,3-butadiene emissions from vehicles using California Phase 2 RFG containing MTBE (2.0% oxygen by weight) were reduced significantly in all fleets compared with the industry average gasoline (Table A.5) (Auto/Oil AQIRP 1995). Reduction was 19% in the older fleet, 30% in the current fleet, and 34% in the Federal Tier 1 fleet.

Formaldehyde Formaldehyde is a partial combustion product of MTBE and TAME and would be expected to increase in emissions when they are added to fuel but not when other oxygenates are used. Using the Auto/Oil AQIRP set of fuel formulations in the current fleet, adding MTBE increased formaldehyde emissions by 27%; reducing aromatics increased formaldehyde by 19% (Table A.1) (Gorse et al. 1991). Reducing aromatic content raised formaldehyde levels only when MTBE was added, and adding MTBE raised formaldehyde only at the low level of aromatics. Adding MTBE did not affect formaldehyde levels in the older fleet. In a comparative study of ethanol, MTBE, and ETBE using fuels that were not entirely equivalent, no significant effects on formaldehyde emissions were noted for any of the oxygenates tested (Reuter et al. 1992). This is as expected for ethanol and ETBE. The explanation for why MTBE failed to cause an increase in formaldehyde emissions was that the data set for this study was not as large as the one for the study showing the increase. In a study comparing TAME and MTBE (2.0% oxygen by weight) (Koehl et al. 1993), using TAME resulted in 28% higher formaldehyde levels in emissions than using MTBE. Koehl and coworkers commented that the increase in fleet average formaldehyde with the TAME fuel occurs primarily in Bag 1 and may be due to relatively poorer combustion of TAME than of MTBE, which is more volatile, under cold-start conditions. No significant effects on formaldehyde levels in exhaust emissions were noted among fuels containing MTBE, TAME, DIPE, or combinations of them, at 2.7% oxygen by weight compared with the reference fuel (Table A.3) (Noorman 1993). Levels of formaldehyde emissions from vehicles using California Phase 2 RFG containing MTBE (2.0% oxygen by weight) were increased in all fleets compared with the industry average gasoline, but the results were significant only in the current fleet, in which the increase was 21% (Table A.5) (Auto/Oil AQIRP 1995).

Acetaldehyde Acetaldehyde is a partial combustion product of ETBE, DIPE, and ethanol, and would be expected to increase in emissions when they are added to fuel, but not when MTBE and TAME are used. Acetaldehyde emissions were not affected by adding MTBE in the AQIRP set of fuel formulations in either the current or older fleet (Table A.1) (Gorse et al. 1991). Reducing the level of aromatics increased acetaldehyde 20% in the current fleet and 33% in the older fleet. In another study, ethanol and ETBE increased acetaldehyde exhaust emissions significantly, but MTBE did not (Table A.2) (Reuter et al. 1992), as was expected. Blended at 2.0% oxygen by weight to meet federal emission certification test fuel specifications, TAME and MTBE affected acetaldehyde emissions similarly (Koehl et al. 1993). Acetaldehyde emissions were higher with DIPE in RFG than with MTBE or TAME, as expected (Table A.3) (Noorman 1993). Acetaldehyde emissions increased with increasing content of DIPE (1.1 mg/mi for a fuel containing DIPE, TAME, and MTBE in equal parts, 1.3 mg/mi for a fuel containing half DIPE and half MTBE, and 1.4 mg/mi for a fuel containing DIPE only). Without DIPE, the acetaldehyde emissions ranged from 0.8 to 1.0 mg/mi. However, acetaldehyde makes up less than 12% of the air toxics emitted, and no significant differences in total toxic emissions were found among the different test fuels. Levels of acetaldehyde emissions from vehicles using California Phase 2 RFG containing MTBE (2.0% oxygen by weight) were decreased in all fleets compared with the industry average gasoline, but the results were significant only in the current fleet, in which the decrease was 16% (Table A.5) (Auto/Oil AQIRP 1995).

Total Air Toxics In the current fleet of gasoline-fueled vehicles, total emissions of air toxics are dominated by benzene which represents 65% to 80% of the total (Gorse et al. 1991). In older-fleet vehicles operated on MTBE fuels, the contribution of the oxidation catalyst trucks tends to increase formaldehyde emissions to levels comparable to those of benzene. Older-fleet vehicles have higher emissions of all air toxics than do current-fleet vehicles. Decreasing fuel benzene levels may be an effective way to meet the toxic pollutant reductions that the CAAA of 1990 requires: 15% by 1995 and 25% by 2000 (Gorse et al. 1991). In actual RFGs containing DIPE, TAME, MTBE, or combinations of these oxygenates, levels of total toxics were reduced by 23% to 36% compared with the reference industry average fuel (Noorman 1993). The differences among the oxygenates were not statistically significant. With California RFG containing MTBE (2.0% oxygen by weight), total air toxic levels were reduced significantly in all fleets. In the current and Federal Tier 1 fleets, reductions were greater than the 25% that the CAAA of 1990 require by the year 2000.

Reactivity

A major goal of using RFG is to reduce ozone precursors. Therefore, an important goal of the Auto/Oil AQIRP was to model speciated data to predict the impact of fuel and vehicle changes on tropospheric ozone. Samples were analyzed for individual hydrocarbon species, and the hydrocarbon profiles were used to calculate reactivities (Hochhauser et al. 1992). Because no single reactivity scale is accurate over the possible range of atmospheric conditions, two types of factors were assessed: maximal incremental reactivity (MIR), an estimate of the ozone-forming reactivity when ozone formation is limited by the availability of hydrocarbons (low ratios of hydrocarbons to NO_x);

and maximal ozone reactivity (MOR), which represents ozone-forming reactivity under atmospheric conditions when both hydrocarbons and NO_x are important to ozone formation (moderate ratios of hydrocarbons to NO_x) and when the total ozone formation is at a maximum. Both specific reactivity (SR), expressed in grams of ozone per gram of nonmethane organic gas, and ozone-forming potential (OFP), expressed in grams of ozone per mile, were calculated for each of these factors. Comprehensive ozone modeling would be needed to estimate atmospheric ozone levels.

In the Auto/Oil AQIRP set of fuel formulations, adding MTBE resulted in higher SR in both the current and older fleets; both SR-MIR and SR-MOR were increased (Table A.1). Adding MTBE decreased OFP-MOR but had no effects on OFP-MIR, in both fleets. Reducing aromatics had no effect on SR-MIR in the current fleet, but lowered it in the older fleet; reducing aromatics increased SR-MOR in both fleets (by 12% in the current fleet) and decreased both the OFP-MIR and the OFP-MOR in the older fleet (but to differing degrees) and had no effect in the current fleet.

In the study by Reuter and coworkers (1992) (Table A.2), MTBE (2.7% oxygen by weight) resulted in a small increase in the specific reactivity for both the MIR and MOR scales, although ETBE (2.7% oxygen by weight) caused a much greater increase, especially on the MOR scale. Ethanol (3.5% oxygen by weight) did not have a significant effect on SR. None of these oxygenates had a significant effect on OFP in this study. However, when the nonsignificant reductions for the three oxygenates are combined, significant reductions (of about 4%) result for both OFP-MIR and OFP-MOR. TAME fuel was similar to MTBE fuel in estimated ozone-forming reactivity of exhaust emissions (Burns et al. 1992).

No significant differences were observed in the OFPs of RFG containing DIPE, TAME, or MTBE (Table A.3) (Noorman 1993). The OFPs of the emissions were found to be from 20% to 25% lower than that of the reference fuel (non-RFG with no oxygenates). California Phase 2 RFG containing MTBE (2.0% oxygen by weight) reduced reactivity-weighted emissions significantly in all three fleets compared with the industry average gasoline, but lowered the SR of emissions significantly only in the older and current fleets (Table A.5) (Auto/Oil AQIRP 1995).

EVAPORATIVE EMISSIONS

Diurnal and Hot Soak Emissions

In the Auto/Oil AQIRP set of fuel formulations, total evaporative emissions were reduced by 24% in the current fleet and 26% in the older fleet when aromatics in fuel were lowered (Table A.1) (Burns et al. 1992). In this study, adding MTBE had no significant effect on total evaporative emissions of either fleet. These results are a combination of effects on diurnal and hot soak emissions, which give similar pictures.

In another study with different fuels (Reuter et al. 1992), ethanol was found to increase diurnal hydrocarbon emissions by 30% (Table A.2), probably due to the increased RVP of the splash-blended fuels that were used for ethanol. Diurnal emissions of benzene were also increased (by 28%). MTBE and ETBE did not have significant effects on diurnal hydrocarbon or benzene emissions. Ethanol increased hot soak hydrocarbon emissions by 50% and benzene emissions by 45%. MTBE increased hot soak hydrocarbon emissions by 1 total evaporative emissions of. 3%, but ETBE did not have a significant effect on them. Burns and coworkers (1992) found the effect of MTBE on hot soak hydrocarbons to be difficult to understand. They stated that the hot soak emissions from fuel-injected vehicles would be created as function expansions in the vapor space of fuel tanks, but that there is no evidence that MTBE fuels generate more fuel tank vapor than nonoxygenated fuels at the same Reid vapor pressure. In a study comparing TAME with MTBE (Koehl et al. 1993), the only significant difference in evaporative emissions was that diurnal hydrocarbon emissions were 24% lower with TAME than with MTBE; diurnal benzene emissions were not significantly different, nor were hot soak benzene or hydrocarbon emissions. In a study comparing RFG with ethers added to an industry average fuel without oxygenates, DIPE, MTBE, and TAME were found to have similar effects on evaporative emissions (Table A.3) (Noorman 1993). Although decreases in total evaporative emissions and benzene emissions occurred for all diurnal, hot soak, and sealed housing for evaporative determination (SHED) measurements, these were often not statistically significant.

Reactivity

In the Auto/Oil AQIRP set of fuel formulations, the mass and ozone reactivity of evaporative emissions from vehicles were evaluated by Burns and coworkers (1992). Samples from all evaporative emissions tests were analyzed for individual hydrocarbon species. The hydrocarbon profiles were used to calculate reactivities (see Table A.1). The only significant effect of MTBE on SR was a decrease in hot soak SR (MOR) in both fleets. No significant effects on the OFP were found from adding MTBE. In the current fleet, reducing aromatics decreased the hot soak OFP by more than 20% for both MIR and MOR, but had no significant effect on the diurnal OFP. In the older fleet, aromatics decreased the OFP (both MIR and MOR) for both diurnal and hot soak emis-

sions by 20% to 30%. The effects on SR of reducing aromatics were quite different. Reducing aromatics had no significant effect on either the diurnal or hot soak MIR and resulted in a small (5% to 6%) increase in MOR in both diurnal and hot soak emissions. In a study comparing ethanol, MTBE, and ETBE (Reuter et al. 1992), ethanol decreased SR (SR-MIR and SR-MOR), but increased OFP (both OFP-MIR and OFP-MOR) by about 50%. ETBE had no effect on SR or OFP on either scale. MTBE had no effect on SR but increased OFP (OFP-MIR and OFP-MOR) by about 20%. Compared with MTBE, TAME fuel was about 24% lower in estimated diurnal reactivity-weighted emissions, a consequence of its lower mass emissions (Koehl et al. 1993). In a study comparing RFG with oxygenates to an industry average fuel without, decreases in OFP of evaporative emissions were not statistically significant for MTBE, DIPE, or TAME fuels (Noorman 1993). Reduction in total OFP ranged from 25% to 31% in different blends compared with the industry average fuel, but these differences were not statistically significant.

Table A.1. Changes in Exhaust and Evaporative Emissions with Changes in Fuel in Current and Older Fleets[a]

Component or Characteristic of Emissions	Change in Fuel							
	MTBE Increased (from 0% to 15%)		Aromatic Compounds Reduced (from 45% to 20%)		T_{90} Reduced (from 360°F to 280°F)		Olefins Reduced (from 20% to 5%)	
	Current	Older	Current	Older	Current	Older	Current	Older
EXHAUST EMISSIONS								
Mass Emissions[b]								
Total HC	↓6	↓9	↓7	↑14	↓22	↓6	↑6	↑6
Nonmethane HC	↓6	↓9	↓12	↑11	↓23	↓4	↑7	↑17
CO	↓11	↓14	↓13	↓2.5	NS	↑14	NS	NS
NO_x	NS	NS	↑2	↓11	↑5	NS	↓6	↓7
Air Toxics[c]								
Benzene	NS	↓11	↓42	↓31	↓11	NS	NS	NS
1,3-Butadiene	↓9	NS	↑11	NS	↓37	↓37	↓32	↓31
Formaldehyde	↑24	NS	↑27	↑19	↓26	NS	NS	NS
Acetaldehyde	NS	NS	↑20	↑33	↓24	NS	NS	NS
Specific Reactivity[b]								
SR-MIR	↑7	↑8	NS	↓7	↓18	↓15	↓8	↓10
SR-MOR	↑5	↑5	↑12	↑4	↓16	↓13	↓8	↓9
OFP-MIR	NS	NS	↓12	NS	↓30	↓15	NS	NS
OFP-MOR	↓5	↓8	↓5	NS	↓24	↓9	NS	NS
EVAPORATIVE EMISSIONS[d]								
Diurnal	NS	NS	↓22	↓31	↑30	NS	NS	↓28[e]
Hot Soak	NS	NS	↓27	↓27	↑31	↑45	NS	NS
Total Evaporative	NS	NS	↓24	↓26	↑30	↑38	NS	↓23[e]
Running Loss[f]	NS	—	NS	—	NS	—	NS	—
Ozone-Forming Potential								
Diurnal OFP-MIR	NS	NS	NS	↓34	↑51	↑34	↓18	↓45
Diurnal OFP-MOR	NS	NS	NS	↓31	↑44	↑33	NS	↓42
Hot soak OFP-MIR	NS	NS	↓25	↓30	↑28	↑32	↓16	↓29
Hot soak OFP-MOR	NS	NS	↓21	↓23	↑26	↑31	↓17	↓31
Specific Reactivity								
Diurnal SR-MIR	NS	NS	NS	NS	NS	NS	↓31	↓26
Diurnal SR-MOR	NS	NS	NS	↑6	NS	NS	↓26	↓22
Hot soak SR-MIR	NS	NS	NS	NS	↓13	↓9	↓24	↓16
Hot soak SR-MOR	↓9	↓5	NS	↑5.5	↓14	↓11	↓24	↓18
Running loss SR-MIR	NS	—	NS	—	NS	—	NS	—
Running loss SR-MOR	NS	—	NS	—	NS	—	NS	—

[a] Values are presented as percentages of change in emissions. Current fleet includes 20 1989 vehicles; older fleet includes 14 1983–1985 vehicles. NS = no significant effect; all numbers presented were significant at a 95% confidence level.

[b] Data adapted from Hochhauser et al. 1991.

[c] Data adapted from Gorse et al. 1991.

[d] Data adapted from Burns et al. 1992.

[e] Results highly influenced by one vehicle.

[f] The values given for the older fleet were single measurements, and are therefore not reported.

Table A.2. Percentages of Change in Exhaust and Evaporative Emissions with Addition of Different Oxygenates to the Fuel[a]

Component or Characteristic of Emissions	MTBE (0% to 15% by volume)	Ethanol (0% to 10% by volume)	ETBE (0% to 17% by volume)
EXHAUST EMISSIONS			
Total HC	↓7	↓5	↓5
Nonmethane HC	↓6	↓6	↓6
CO	↓9	↓13	↓15
NO_x	NS	↑5	NS
Air Toxics			
Benzene	↓11	↓12	↓8
1,3-Butadiene	NS	↓6	NS
Formaldehyde	NS	NS	NS
Acetaldehyde	NS	↑159	↑254
Total air toxics	NS	NS	NS
Specific Reactivity			
SR-MIR	↑4	NS	↑11
SR-MOR	↑3	NS	↑50
OFP-MIR	NS	NS	NS
OFP-MOR	NS	NS	NS
EVAPORATIVE EMISSIONS			
Diurnal HC	NS	↑30	NS
Diurnal Benzene	NS	↑28	NS
Hot Soak HC	↑13	↑50	NS
Hot Soak Benzene	NS	NS	NS
Ozone-Forming Potential			
Diurnal OFP-MIR	↑12	NS	NS
Diurnal OFP-MOR	↑9	NS	NS
Hot soak OFP-MIR	↑21	↑46	NS
Hot soak OFP-MOR	↑24	↑55	NS
Specific Reactivity			
Diurnal SR-MIR	NS	↑30	NS
Diurnal SR-MOR	NS	↑30	NS
Hot soak SR-MIR	NS	↓19	NS
Hot soak SR-MOR	NS	↓13	NS

[a] Adapted from Reuter et al. 1992. Numbers indicate significant changes at the 95% confidence level; NS = no significant change. Twenty current (1989) vehicles were used; these were the same as the current fleet referred to in Table A.1. Eleven fuels were evaluated: four with no oxygenates added; four splash-blended ethanol fuels (10% by volume); two fully blended MTBE fuels (15% by volume); and one fully blended ETBE fuel (17% by volume).

Table A.3. Changes in Exhaust and Evaporative Emissions with Addition of Various Ethers to Reformulated Gasoline[a]

Component or Characteristic of Emissions	Percentage of Change from Reference Fuel (Not RFG)			
	RFG with MTBE	RFG with DIPE	RFG with TAME	95% CI (±)
EXHAUST EMISSIONS				
Total HC	−20	−18	−18	10
CO	−26	−28	−24	18
NO_x	2	1	0	12
Air Toxics				
Benzene	−42	−38	−36	18
1,3-Butadiene	−26	−26	−27	26
Formaldehyde	−9	2	3	44
Acetaldehyde	−8	61	−2	30
Total air toxics	−36	−26	−28	16
Ozone-Forming Potential	−25	−23	−20	12
EVAPORATIVE EMISSIONS				
Total				
Diurnal	−24	−20	−25	18
Hot soak	−11	−8	−12	14
SHED	−16	−14	−18	12
Benzene				
Diurnal	−20	−20	−10	32
Hot soak	−24	−26	−12	20
SHED	−24	−30	−14	18
Ozone-Forming Potential				
Diurnal	−22	−19	−25	28
Hot soak	−17	−8	−9	20
SHED	−18	−12	−16	20

[a] From Noorman 1993. Seven 1989–1991 vehicles were selected to represent technologies that would likely be used in future model years and comprised a mix of pollution control technologies and engine sizes. The test fuels met the CAAA of 1990 RFG standards. They contained 1% benzene by volume and had a vapor pressure of 7.5 psi. The fuel oxygenate content was 2.7% by weight as required for oxyfuel, rather than the 2.0% minimum level required in RFG. Values are presented as the percentage of change from the reference fuel, which had properties similar to the 1990 industry average fuel. Compared with the test fuels it had higher benzene (1.4 vol%), higher aromatic content (35 vol% compared with about 25 vol%), and higher RVP (8.4 psi compared with about 7.4 psi).

Table A.4. Levels of Various Pollutants in Exhaust and Evaporative Emissions with Addition of Various Ethers to Reformulated Gasoline[a]

Component or Characteristic of Emissions	Reference Fuel	Ether			95% CI (±)
		MTBE	DIPE	TAME	
EXHAUST EMISSIONS					
Total HC (g/mi)	0.3	0.2	0.2	0.2	0.02
CO (g/mi)	4.3	3.2	3.1	3.3	0.41
NO_x (g/mi)	0.8	0.8	0.8	0.8	0.05
Air Toxics (mg/mi)					
Benzene	12.3	7.1	7.6	7.9	1.1
1,3-Butadiene	1.1	0.8	0.8	0.8	0.1
Formaldehyde	2.4	2.2	2.4	2.5	0.5
Acetaldehyde	0.9	0.8	1.4	0.8	0.1
Total air toxics	16.7	10.7	12.3	12.0	1.3
Oxygenate (mg/mi)					
MTBE	—	15.0	0.0	0.0	1.7
DIPE	—	0.0	12.5	0.0	0.8
TAME	—	0.0	0.0	8.8	0.8
Ozone-Forming Potential (g O_3/mi)	0.6	0.4	0.5	0.5	0.04
EVAPORATIVE EMISSIONS					
Total (g/test)					
Diurnal	0.2	0.2	0.2	0.2	0.02
Hot soak	0.3	0.2	0.2	0.2	0.02
SHED	0.4	0.4	0.4	0.4	0.03
Benzene (mg/test)					
Diurnal	6.4	5.1	5.1	5.7	1.0
Hot soak	11.2	8.5	8.3	9.9	1.1
SHED	18.0	13.6	12.5	15.4	1.6
Ozone-Forming Potential (g O_3/test)					
Diurnal	0.4	0.3	0.3	0.3	0.06
Hot soak	0.6	0.5	0.5	0.5	0.06
SHED	1.0	0.8	0.8	0.8	0.09

[a] From Noorman 1993. Seven 1989–1991 vehicles were selected to represent technologies that would likely be used in future model years and comprised a mix of pollution control technologies and engine sizes. The test fuels met CAAA of 1990 RFG standards. They contained 1% benzene by volume and had a vapor pressure of 7.5 psi. The fuel oxygenate content was 2.7% by weight as required for oxyfuel, rather than the 2.0% minimum level required in RFG. The reference fuel had properties similar to the 1990 industry average fuel. Compared with the test fuels it had higher benzene (1.4 vol%), higher aromatic content (35 vol% compared with about 25 vol%), and higher RVP (8.4 psi compared with about 7.4 psi).

Table A.5. Percentage of Change in Emissions Using California Reformulated Gasoline Containing MTBE Compared with the Industry Average Gasoline for 1988[a]

Component or Characteristic of Emissions	Older Fleet[b]	Current Fleet[c]	Federal Tier 1 Fleet[d]
Nonmethane HC	−12[e]	−22[e]	−27[e]
CO	−23[e]	−21[e]	−28[e]
NO_x	−9	−7[e]	−16[e]
Air Toxics			
Benzene	−34[e]	−43[e]	−47[e]
1,3-Butadiene	−19[e]	−30[e]	−34[e]
Formaldehyde	+16	+21[e]	+11
Acetaldehyde	−6	−16[e]	−12
Total air toxics	−9[e]	−27[e]	−32[e]
Reactivity-Weighted Emissions	−16[e]	−26[e]	−30[e]
Specific Reactivity	−9[e]	−6[e]	−3

[a] From Auto/Oil AQIRP 1995. The fuels were blended to represent the 1988 national industry average gasoline and fuel that would meet California Phase 2 1996 regulatory requirements. The RFG contained 11% MTBE by volume, which is 2.0% oxygen by weight.

[b] The older fleet consisted of seven 1983–1985–model vehicles, including five passenger cars and two light-duty trucks. These vehicles were selected from the seven pairs of vehicles used in earlier Auto/Oil AQIRP work. The vehicles had accumulated 46,000 to 79,000 miles at the time of testing in this study.

[c] The current fleet consisted of eight 1989-model passenger cars and two 1989 light-duty trucks. All but one were equipped with fuel injection and all but one had three-way catalysts without oxidation catalysts. These 10 vehicles represented the 10 pairs of vehicles in the current fleet used in earlier Auto/Oil AQIRP studies. These vehicles had accumulated 16,000 to 33,000 miles at the time of testing in this study.

[d] The Federal Tier 1 fleet consisted of five 1994-model passenger cars and one 1994 light-duty truck. The cars were designed to meet 1994 Federal Tier 1 standards of emissions. The truck was designed to meet Class 2 standards. All six vehicles were equipped with fuel injection and three-way catalysts. All of the vehicles had accumulated at least 4,000 miles before testing, and oxygen sensors had been subject to 50,000 miles of simulated aging. For all vehicles, exhaust emissions were measured according to Federal Test Procedures.

[e] Significant at $p < 0.05$.

Appendix B. Comparison of the Health Effects Institute's Conclusions with the Earlier Conclusions of the U.S. Environmental Protection Agency

The Health Effects Institute was asked to review two of the U.S. Environmental Protection Agency documents in which the potential health effects of oxygenates are evaluated: *Assessment of Potential Health Risks of Gasoline Oxygenated with Methyl Tertiary Butyl Ether* (1993a) and *Health Risk Perspectives on Fuel Oxygenates* (1994b). In this appendix, the conclusions of these assessments are compared with the Oxygenates Evaluation Committee's findings from its literature review.

EXPOSURE ESTIMATES

Referring to MTBE, the EPA 1993 document stated that "the data on air quality and microenvironments (e.g., during refueling, inside cars, in personal garages) are too limited for a quantitative estimate of population exposures." Nevertheless, the EPA did use these air quality measurements to calculate annual average human exposures likely to be associated with different types of settings and activities. The HEI Oxygenates Evaluation Committee also concluded that the exposure data are too limited to calculate a cumulative measure of exposure for use in risk assessment, but that the data do provide a useful, rough indication of exposure ranges for various activities.

EFFECTS OF SHORT-TERM EXPOSURE TO MTBE

In its 1994 document, the EPA considered the findings of epidemiologic studies conducted in areas using oxygenated fuels to be equivocal or to show no effects. The authors also added that the "possibilities of a subpopulation susceptible to pollutant mixtures related to the use of MTBE-fuels and of unique circumstances in Fairbanks cannot be ruled out." HEI's review, which included a study in Milwaukee, the rest of Wisconsin, and Chicago that was conducted after the EPA's review was published, came to similar conclusions: the evidence of an effect is not strong, but the occurrence of effects in some people or under some conditions cannot be ruled out. The EPA's suggestion (1994b) that the cold, dry climate and topography of Fairbanks could affect exposure and health effects is certainly plausible.

In its 1993 document, the EPA concluded, on the basis of two controlled human exposure studies, that "acute (1-hour) exposure to typically encountered high ambient levels of 'pure' MTBE does not appear to cause health symptoms, eye or nose irritation, or behavior changes in young, healthy adults under room temperature conditions . . ." but that "it is possible that there are more sensitive members of the population who would respond and that higher concentrations than those used in the human clinical studies could cause effects." After EPA's assessment was written, information on a third controlled human exposure study of MTBE became available (Johanson et al. 1995). Even though this study used much higher concentrations of MTBE (5, 25, and 50 ppm compared with 1.4 and 1.7 ppm) and a longer exposure duration (2 hours) than the other two studies (1 hour), no effects were reported, as discussed in this HEI review. The question about sensitive individuals raised by the EPA is an important one. Another important question relates to the potential difference in effects from exposure to MTBE alone and as part of a mixture of constituents of gasoline and exhaust emissions.

The Oxygenates Evaluation Committee differed somewhat from the EPA in its interpretation of studies of neurotoxicity. The HEI Committee concluded that the results of limited studies of neurotoxicity in animals suggest that acute, reversible neurotoxic effects are a possible result of short-term exposure to MTBE. This conclusion was based mainly on the results of a study of rats exposed to MTBE for 6 hours at concentrations of 800, 4,000, and 8,000 ppm. The HEI Committee considered the effects on motor activity after exposure to 800 ppm MTBE to be of concern because, for some neurotoxicants, effects are seen on complex central nervous system functions at much lower exposure levels than effects on motor activity. In its 1993 document, the EPA interpreted the effects only at the two higher concentrations (decreased muscle tone and staggered walking) to be "effects on the nervous system" and concluded that short-term exposure to "environmentally unrealistic levels of MTBE can cause reversible effects on the nervous system."

REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF MTBE

In its 1993 document, the EPA reported a preliminary estimate, with uncertainty spanning at least 1 order of

magnitude, of a level at which no adverse developmental toxicity is likely to occur in humans (48 mg/m^3 or 13 ppm). This calculation was based on a two-generation study of rats (Neeper-Bradley 1991) in which effects on body weight during lactation were seen at 3,000 ppm, but not at 400 ppm. First-generation pups from parents exposed to 3,000 ppm had significantly reduced body weight on lactational day 14; in second-generation pups, reductions in body weight were observed on lactational days 14 through 28. Two uncertainty factors were applied in calculating the no-effects level for humans: a factor of 3 for extrapolating from rats to humans, and a factor of 10 to account for sensitive human subpopulations. In its 1994 document, the EPA commented that "only some unusually high and infrequent human exposure scenarios (i.e., some refuelings) are of concern." The Oxygenates Evaluation Committee considered the EPA's conclusion to be reasonable in that 13 ppm is an unusually high exposure level for most people who are not occupationally exposed to MTBE, although this level is not uncommon in some occupations and could occur very rarely during refueling. Because of lack of information on the window of sensitivity for developmental effects, the EPA assumed that even a short exposure has the potential to result in developmental toxicity if the exposure concentration is sufficiently high. The HEI Committee agreed with that conclusion, but would point out that the period of exposure during refueling is much shorter (1 to 3 minutes) than the daily period of exposure (6 hours) in the rat study. A brief exposure would elevate blood levels much less, at any exposure level, than a prolonged exposure. It should also be noted that the NOAEL for developmental effects was 300 ppm in another rat study, where a small decrease in the viability of offspring was seen at 1,300 ppm. Developmental effects were noted in mice at 4,000 ppm, but not at 1,000 ppm. Thus, the NOAEL in mice is higher than in rats.

LONG-TERM EFFECTS OF MTBE

CANCER EFFECTS

The EPA's 1994 assessment concluded, as has HEI's review, that the carcinogenic effects of MTBE observed in laboratory animals are of concern with respect to the potential health risks of human exposure. The EPA's report states that the two inhalation studies of MTBE support classifying it in Group C as a possible human carcinogen, and that an additional study, with exposure by gavage, may provide enough evidence, "if the anecdotal reports are sustained after publication and a fuller evaluation," to classify MTBE as a Group B2 probable human carcinogen (U.S. Environmental Protection Agency 1994b). The results of the study referred to have now been published (Belpoggi et al. 1995) and are included in the results discussed in this HEI review. The Oxygenates Evaluation Committee agrees that the results of this study add to the weight of evidence of carcinogenicity of MTBE in animals and increase concern about its potential carcinogenicity in humans.

In addressing the issue of quantitative risk, the EPA's 1994 report commented that "for the MTBE cancer inhalation studies, the circumstances of the bioassays make the estimation of a quantitative unit risk very problematical. With three different tumor types (male rat kidney and testicular tumors and mouse liver tumors), the theoretical worst-case risks span a 25-fold range." The 1994 document also stated that MTBE may have a cancer unit risk about the same or somewhat lower than benzene, half that of formaldehyde, and about 50-fold less than that of 1,3-butadiene; and concluded that "from an individual chemical viewpoint, MTBE's carcinogenic properties are not that different from those of components already present in traditional gasoline emissions." HEI's review, although not attempting to calculate unit risks or to comment on them, presents a similar view of uncertainty. The Oxygenates Evaluation Committee concluded that large uncertainties remain in projecting human risk of cancer from MTBE, air toxics, and other components of evaporative and exhaust emissions of conventional gasoline, the emission levels of which change when fuel is oxygenated. The HEI Committee thinks that the band of uncertainty around any calculation of unit risks is wide, and that such risk estimates must be interpreted with caution.

In 1994, the EPA concluded that "the amount of carcinogenic compounds in emissions changes somewhat with MTBE, but the overall hazard potential probably has not changed . . ." and that "a good quantitative estimate of population risk for various gasolines, with and without oxygenates, is essentially impossible to make because of the crudeness and variability of exposure data." The Oxygenates Evaluation Committee agrees with the latter conclusion. Although the HEI Committee does not agree with the first conclusion because of the uncertainties in the exposure and risk estimates for all of the carcinogens, it does not expect that a substantial increase in the overall hazard potential will be found.

NONCANCER EFFECTS

The EPA concluded that "it does not appear that there is a significant risk for MTBE alone to cause chronic noncancer effects" (U.S. Environmental Protection Agency 1994b). The Oxygenates Evaluation Committee agrees that the data on noncancer effects of MTBE support this conclu-

sion, but considered the evaluation of noncancer endpoints at moderate levels of exposure to have been limited. The EPA 1994 document states that "0.83 ppm MTBE should not cause adverse (noncancer) effects even in susceptible people exposed for a lifetime." This reference concentration was derived from a study with rats exposed for a lifetime to various concentrations of MTBE, including a NOAEL of 1,450 mg/m^3 (400 ppm), a lowest-observed adverse-effects level (LOAEL) of 10,800 mg/m^3 (3,000 ppm), and the highest level of 28,800 mg/m^3 (8,000 ppm). To arrive at the reference concentration of 0.83 ppm MTBE, the NOAEL from the rat study was adjusted to approximate an equivalent continuous exposure level in humans, and then divided by 100, which represents a factor of 10 for sensitive individuals and a factor of 10 for interspecies extrapolation and lack of information (U.S. Environmental Protection Agency 1993a). At the LOAEL exposure concentration in the rat study, effects observed included increased liver and kidney weights, increased severity of spontaneous kidney lesions, increased incidence of prostration in female rats, and swollen periocular tissue in male and female rats. A similar study was conducted in which mice underwent long-term exposure to the same concentrations as those used in the rat study. Only the highest level of exposure (28,800 mg/m^3) produced effects in mice, including mortality, increased frequency of kidney disease, and ataxia. Thus, the NOAEL is higher in mice than in rats.

EFFECTS OF OTHER ETHERS AND ETHANOL

In 1994, the EPA concluded that the health risks from TAME, ETBE, and DIPE are virtually unknown because no major studies of exposure and toxicity had been completed. Information on these ethers is still very limited, although some studies of TAME and ETBE have now been completed.

At the same time, the EPA concluded that "insufficient evidence exists to determine whether, or at what levels of exposure, inhaled ethanol would produce significant adverse effects" because "the vast majority of research on ethanol health effects has been concerned with ingested ethanol." The Oxygenates Evaluation Committee has reviewed information on ethanol, and concluded that health effects for the public from using ethanol in gasoline are unlikely. The incremental ethanol burden that would result from exposure to ethanol during refueling is predicted to be lower than endogenous blood levels.

OVERALL EFFECTS

Looking at the overall effects of using oxygenates, in 1994 the EPA concluded that there is "no basis to expect that the use of MTBE-oxygenated gasoline or MTBE-reformulated gasoline will pose a greater public health risk than traditional gasoline." The Oxygenates Evaluation Committee has concluded that it is unlikely that using oxygenated fuel would substantially change the overall health risk associated with using conventional fuel in motor vehicles, but that a number of important research questions must be addressed if oxygenates will continue in widespread use over the long term.

ADDITIONAL COMMENTS

This review has not attempted to evaluate quantitative estimates made by the EPA, such as the assumptions used in calculating reference concentrations from no-effects levels in animal studies, estimates of quantitative risk, or calculations of population exposure.

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Abbreviations and Conversion Factors

ABBREVIATIONS

ADH	alcohol dehydrogenase
API	American Petroleum Institute
Auto/Oil AQIRP	Auto/Oil Air Quality Improvement Research Program
^{14}C	carbon-14
CAAA of 1990	Clear Air Act Amendments of 1990
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CNS	central nervous system
CO	carbon monoxide
CO_2	carbon dioxide
DIPE	diisopropyl ether
EPA	U.S. Environmental Protection Agency
ETBE	ethyl *tert*-butyl ether
FAS	fetal alcohol syndrome
GC (FID)	gas chromatography with flame ionization detection
GC/MS	gas chromatography with mass spectrometry
HC	hydrocarbons
IARC	International Agency for Research on Cancer
K_m	Michaelis constant
LC_{50} and LD_{50}	the [lethal] concentration or dose that produces death in 50% of the animals in a study
MDC	minimal detectable concentration
MTBE	methyl *tert*-butyl ether
mg%	mg/100 mL of blood; see Conversion Factors
MIR	minimal incremental reactivity
MOR	maximal ozone reactivity
MW	molecular weight
NAAQS	National Ambient Air Quality Standard
NTP	National Toxicology Program
NIOSH	National Institute of Occupational Safety and Health
NO_2	nitrogen dioxide
NOAEL	no observable adverse effect level
NO_x	oxides of nitrogen
OFP	ozone-forming potential
OH	hydroxyl group
OSTP	the White House Office of Science and Technology Policy
oxyfuel	gasoline containing 2.7% oxygen by weight
PMNs	polymorphonuclear neutrophilic leukocytes
ppb	parts per billion
ppm	parts per million
psi	pounds per square inch
RFG	reformulated gasoline
RVP	Reid vapor pressure
SHED	sealed housing for evaporative determination
SR	specific reactivity
T_3	triiodothyronine
T_4	tetraiodothyronine
T_{90}	temperature at which 90% of the gasoline can be distilled
TAME	*tert*-amyl methyl ether
TBA	*tert*-butyl alcohol
THF	tetrahydrofolic acid
TLV	threshold limit value
TSH	thyroid-stimulating hormone
TWA	time-weighted average
V_{max}	maximum velocity
VOC	volatile organic compound

CONVERSION FACTORS

mg MTBE/m^3 air = ppm MTBE × 3.6

mg ethanol/m^3 air = ppm ethanol × 1.9

1 mg% blood ethanol = 1 mg ethanol/0.1 L blood = 10 mg ethanol/L blood

µg MTBE/L blood = (µmol MTBE/L blood) × 88, which is the MW of MTBE

µg MTBE/L blood = (µL MTBE/L blood [or 1 ppm]) × 0.74, which is the density of MTBE

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