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Ethers as Gasoline Additives –Toxicokinetics and Acute Effects in Humans

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Nog finns det mål och mening med vår färd -
men det är vägen som är mödan värd.

Ur "I rörelse" av Karin Boye

To my late grandmother, Ida Andersson.

List of Original Papers

This thesis is based on the following papers, which will be referred to by their Roman numerals. The publications are reproduced with the kind permission of the publishers.

I Nihlén A, Löf A, Johanson G. Experimental exposure to methyl *tertiary*-butyl ether: I. Toxicokinetics in humans. *Toxicol Appl Pharmacol* 1998;148:274-280.

II Nihlén A, Löf A, Johanson G. Controlled ethyl *tertiary*-butyl ether (ETBE) exposure of male volunteers: I. Toxicokinetics. *Toxicol Sci* 1998;46:1-10.

III Nihlén A, Sumner S, Löf A, Johanson G. ^{13}C-Labeled methyl *tertiary*-butyl ether ([1,2-$^{13}C_2$]-MTBE): Toxicokinetics and characterization of urinary metabolites in humans. *Submitted*.

IV Nihlén A, Löf A, Johanson G. Liquid/air partition coefficients of methyl and ethyl *t*-butyl ethers, *t*-amyl methyl ether, and *t*-butyl alcohol. *J Expos Anal Environ Epidemiol* 1995;5(4):573-582.

V Nihlén A, Johanson G. Physiologically based toxicokinetic modeling of inhaled ethyl *tertiary*-butyl ether in male volunteers. *Submitted*.

VI Nihlén A, Wålinder R, Löf A, Johanson G. Experimental exposure to methyl *tertiary*-butyl ether: II. Acute effects in humans. *Toxicol Appl Pharmacol* 1998;148:281-287.

VII Nihlén A, Löf A, Johanson G. Controlled ethyl *tertiary*-butyl ether (ETBE) exposure of male volunteers: II. Acute effects. *Toxicol Sci* 1998;46:143-150.

Abbreviations

ANOVA	analysis of variance
BW	body weight
CNS	central nervous system
CO	carbon monoxide
CV	coefficient of variation
ETBE	ethyl *tertiary*-butyl ether
FEV_1	forced expiratory volume in one second
GC	gas chromatography
HBA	α-hydroxyisobutyric acid
LC_{50}	the concentration when 50% of the tested animals dies
LD_{50}	the dose when 50% of the tested animals dies
MPD	2-methyl-1,2-propanediol
MTBE	methyl *tertiary*-butyl ether
NMR	nuclear magnetic resonance
OEL	occupational exposure limit
PBTK	physiologically based toxicokinetic
PEF	peak expiratory flow
ppm	parts per million
ppb	parts per billion
TAME	*tertiary*-amyl methyl ether
TBA	*tertiary*-butyl alcohol
TLco	transfer factor (diffusing capacity)
TWA	time-weighted average
^{13}C	carbon-13 labeled
D_2O	deuterium labeled water
95% CI	95% confidence interval
λ	partition coefficient

ACGIH	American Conference of Governmental Industrial Hygienists
IARC	International Agency for Research on Cancer
CIIT	Chemical Industry Institute of Toxicology, North Carolina, USA
IMM	Institute of Environmental Medicine, Karolinska Institute, Sweden.
NIWL	National Institute for Working Life, Solna, Sweden

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1. Introduction

1.1 Gasoline and additives

Gasoline is a complex mixture of many constituents in varying proportions and about 150-200 different compounds have been identified (168, 170). In gasoline vapor, about 95% (by volume) of the components are various alkanes (e.g., n-butane, isopentane, n-pentane and isobutane) and typically less than 2% are aromatics (toluene, benzene and xylenes) (16, 66, 168, 170). Further, several compounds are added to the gasoline e.g., antiknock, detergent, antirust, antioxidant and anti-icing additives (77, 168). With the restrictions on use of lead as an antiknock additive other means of octane enhancement are used today. These include (in order of increasing cost) (113):

• the metallic additive, methylcyclopentadienyl manganese tricarbonyl
• changes in the refining process - increase of aromatics or alkenes
• addition of oxygen containing compounds (called oxygenates).

Use of the manganese compound is not approved in Europe (113), but in the USA and Canada it is a source of manganese contamination (47, 177). Increasing the amounts of aromatic compounds in gasoline has been the most common method, but also the most controversial, due to the known carcinogenic effect of e.g., benzene (75, 113). Oxygenates as gasoline additives will be discussed in this thesis.

Oxygenates enhance the octane number in gasoline and improve combustion, thus reducing emissions (69, 160). Today, especially methyl *tertiary*-butyl ether (MTBE) is used and the world-wide production has grown by 20% per year over the last decade, particularly in North America and in Europe (1, 53). The total production capacity was $21 \cdot 10^9$ kg MTBE in 1994 (53). Other aliphatic ethers, such as ethyl *tertiary*-butyl ether (ETBE) and *tertiary*-amyl methyl ether (TAME) or alcohols may be used as oxygenates. MTBE is usually added to unleaded gasoline in quantities between 2 and 5% (by volume), to raise the octane level, or up to 15% (by volume) to improve combustion efficiency (53, 69). The ethers are preferred to alcohols as gasoline additives for instance because ethers do not increase the volatility of the gasoline or cause problems with corrosion (27, 37). However, these ethers themselves are highly volatile and the risk for human exposure is mainly through inhalation. Occupational exposure to oxygenates occurs mostly during handling of oxygenates alone or in combination with gasoline. The general public is primarily exposed to oxygenates during gasoline filling, but may be exposed to low levels of MTBE through the drinking water in areas where the groundwater has been contaminated with MTBE from leaking underground storage tanks (primarily reported from California, USA) (28, 69). After the introduction of oxygenated fuels and the addition of MTBE to gasoline,

complaints about acute health symptoms, e.g., headache, irritation and nausea, have been reported in the USA (69, 160).

1.2 Toxicokinetics

Toxicological basic research, directed toward risk assessment relates to the relationship between exposure, tissue dose, initial tissue interactions and toxic responses. Two areas that are of key importance to make it possible to understand and interpret toxicological information are:

- *toxicokinetics*, specifically defined as the *uptake, distribution, metabolism (biotransformation)* and *excretion* of a chemical in the body and
- *toxicodynamics*, the mechanism of action of a compound or its metabolites and their potency at the site of action.

Kinetics (from Greek for motion or movement) in this field is defined as the mathematical description of the time-course of chemicals in living organisms. One of the rationales for a toxicokinetic study is that the response in a target tissue should be related to the concentration profile of the parent compound or metabolites in that particular tissue. In animals it is possible to sample different tissues to measure the concentration of a compound. This is often not possible in humans. Instead computer models have been developed and used to estimate tissue doses and toxicokinetics in situations when measured data cannot be obtained e.g., to extrapolate from exposed animals to humans. Physiologically based toxicokinetic (PBTK) models are especially useful, since PBTK models are based on actual organ volumes and blood flows in individual tissues. A PBTK model consist of several compartments where organs are grouped together according to blood flow and fat content. Mathematical differential equations are applied on data from actual physiological tissue blood flow and organ volumes and tissue/blood partition coefficients to describe the fate of the chemical in the body (7, 84).

1.3 Biological Monitoring

Sometimes it may be more relevant to measure the absorbed dose (internal exposure or body burden) than to analyze the exposure dose (e.g. external air level). Biological exposure monitoring should be considered complementary to air monitoring and should be conducted when it offers an advantage over air monitoring alone. Further, biological monitoring should be used to e.g., validate air monitoring, to test the efficiency of personal protective equipment, to determine absorption via the skin and the gastrointestinal system, to detect non-occupational exposure or when physically demanding work is performed. The individual exposure is usually determined from levels in blood, urine or expired air and a biological exposure marker (biomarker) adjusts for dermal absorption and individual factors that may influence the uptake of a chemical (e.g. non-

occupational exposure, metabolic ability, diet, physical activity, body build, age, alcohol intake and smoking) (106, 144).

2. The Present Thesis

2.1 Background

An exposure chamber (20 m^3) with controlled climate is at our disposal in the laboratory and it is therefore possible to perform well-defined controlled experiments in which humans are exposed to a substance through the air they breath. During these experimental exposure studies, the toxicokinetics (uptake, distribution, metabolism and excretion) are studied quantitatively, and in addition, potential acute effects are measured during and after the exposure. This present thesis applies primarily to human toxicokinetics, but also to acute effects of the two fuel additives MTBE and ETBE.

At the time the MTBE study were initiated there was no data on the toxicokinetics in humans despite an increasing industrial use of MTBE. Further, complaints about acute health effects (headache, nausea, nasal irritation and eye irritation) have been associated with MTBE, since they appear to have emerged after the addition of MTBE to gasoline (69, 120).

The ETBE research project was initiated since it was considered essential that the uptake, disposition and acute effects of ETBE in humans should be explored before this oxygenate was introduced on a broad scale as an additive to gasoline. One reason for the interest in ETBE in Sweden is the potential to increase the market for renewable fuels, as ETBE is produced from bioethanol.

A further goal in performing these studies was to address the issue of biological exposure monitoring. The unmetabolized ethers or some metabolites may be useful biomarkers for exposure to gasoline vapor as these ethers, added in controlled amounts to gasoline, are highly volatile. Biomarkers already exist for the less volatile compounds predominantly present in liquid gasoline, e.g., for benzene there are several biomarkers including e.g. *trans, trans*-muconic acid (128) and for trimethylbenzenes dimethylhippuric acids is proposed (89). However, no suitable biomarker for the vapor phase of gasoline has yet been characterized.

2.2 Aims

The main purposes of this thesis were to study the uptake, distribution, metabolism and excretion of MTBE and ETBE in humans, to address the issue of biological monitoring and, in addition, to characterize acute effects during and after the exposure to these ethers.

More specifically, the aims of these studies were to:

- Study the inhalation toxicokinetics, i.e. the respiratory uptake, distribution, metabolism and excretion of MTBE, ETBE and of their common metabolite, *tertiary*-butyl alcohol (TBA), respectively (Studies I and II).
- Map additional metabolites of MTBE in urine (Study III).
- Determine partition coefficients of MTBE, ETBE, TAME and TBA (Study IV).
- Develop a physiologically based toxicokinetic (PBTK) model for inhalation exposure to ETBE (Study V) using the partition coefficients (Study IV) and experimental kinetic data (Study II).
- Use the PBTK model to address the issue of biological exposure monitoring (Study V).
- Screen acute effects of MTBE and ETBE by symptoms questionnaire and by objective measures of eye and nasal irritation (Studies VI and VII) and lung function (Study VII).

3. Review of the Literature on Methyl and Ethyl *tertiary*-Butyl Ether

Several reviews discussing the toxicity and acute effects of MTBE are available (42, 53, 69, 104, 160). No major work related to health effects of ETBE has been published. The following chapter will give a brief summary of the physical properties, toxicity and biological effects of MTBE and ETBE.

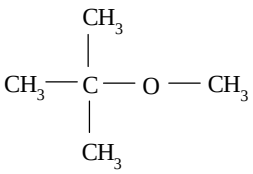
3.1 Chemical Structure, Nomenclature and Physical Properties

MTBE and ETBE are aliphatic branched ethers with the molecular formulas $C_5H_{12}O$ and $C_6H_{14}O$, respectively (Figure 1). These ethers are colorless and inflammable liquids at room temperature. Further, the ethers have a distinct smell with a low odor threshold (69, 135). The taste threshold is also low for the ethers and mixed in water the taste threshold is 47 ppb for ETBE and 134 ppb for MTBE (69). Chemical and physical properties of MTBE and ETBE are summarized in Table 1.

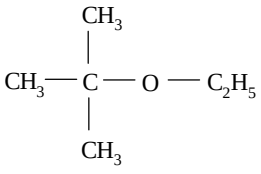
Highly branched ethers have much less peroxide formation after UV-light exposure compared with unbranched ethers. Hence, ETBE has a somewhat higher formation of peroxides compared to MTBE (37, 54, 115).

Table 1. Chemical and physical properties of MTBE and ETBE (30, 34).

	MTBE	ETBE
CAS number	1634-04-4	637-92-3
Synonyms	Methyl *tertiary*-butyl ether, MTBE 2-metoxy-2-methylpropane, methyl *tert*-butyl oxide, *tert*-butyl methyl ether, methyl-1,1-dimethylethyl ether, 1,1-dimethylethyl metyl ether	Ethyl *tertiary*-butyl ether, ETBE 2-etoxy-2-methylpropane, ethyl *tert*-butyl oxide, *tert*-butyl ethyl ether, ethyl 1,1-dimethylethyl ether, 1,1-dimethylethyl etyl ether
Molecular weight (g/mol)	88.15	102.18
Density (g/cm^3)	0.7404 (20°C)	0.7364 (25°C)
Boiling point (°C)	55.2	73
Vapor pressure (kPa)	32.7 (25°C)	17.3 (25°C)
Water solubility (g/100gwater)	4.8	1.2
Odor detection threshold (ppb)	53	13
Conversion factor (20°C, 101.3 kPa)	1 ppm = 3.60 mg/m^3 1 mg/m^3= 0.278 ppm	1 ppm = 4.24 mg/m^3 1 mg/m^3 = 0.236 ppm



Methyl *tertiary*-butyl ether (MTBE)



Ethyl *tertiary*-butyl ether (ETBE)

Figure 1. Structural formulas of MTBE and ETBE.

3.2 Occurrence

The first large scale production of MTBE started in Italy around 1975, followed by production units in Germany and the USA (27, 69). Today, MTBE is manufactured world-wide from methanol (mainly from natural gas) and isobutene (from petroleum refinery sources or from dehydration of *tertiary*-butyl alcohol) to a very large extent. The production of MTBE was $21 \cdot 10^9$ kg in 1994 and is increasing (53). In Sweden, $37 \cdot 10^6$ kg of MTBE was produced during 1996, and an additional $33 \cdot 10^6$ kg was imported (137).

MTBE is the most used oxygenate in motor fuel, however TAME has been in use in Finland since 1995 (145). In Sweden, with an interest in the potential for renewable fuel sources, the replacement of MTBE with ETBE has been suggested. ETBE can be manufactured from ethanol (obtained from agricultural and forestry products) and isobutene.

Since 1981, MTBE has been used therapeutically in humans to dissolve gallstones (3, 70, 83, 99). Further, MTBE has been used as a eluent in liquid and thin-layer chromatography (103, 124) and as solvent in the determination of resin and fatty acids in pulp mill effluents (173).

3.2.1 Oxygenates in Gasoline

Oxygenates are released to the atmosphere during the manufacture and distribution of oxygenated fuels, in the vehicle-refueling process and from evaporative and, to a lesser extent, exhaust emissions from motor vehicles.

In the USA, the Clean Air Act Amendments of 1970 initiated a commitment by the US Government to reduce exposure of the general population to air pollutants that may cause adverse health effects. A further step was taken later on toward reducing motor vehicle emissions, and fuel reformulations with oxygenates started to be used. Oxygenates increase the octane number and improve the combustion efficiency and reduce emissions (69, 160). The reduction of carbon monoxide (CO) is a main argument for the use of MTBE in the USA. MTBE has been put to more widespread use after the legislation of the Clean Air Act, especially in the winter when levels of automotive CO emissions are higher. Typically, the maximum allowed oxygen content in gasoline is 2.7% by weight,

corresponding to 15% MTBE or 17% ETBE by volume (69), but in Sweden is the maximum allowed oxygen content in gasoline 2% oxygen by weight (27). In Sweden, the use of oxygenates was mainly introduced when lead was outlawed from gasoline starting in 1986 when an extra tax was added on gasoline containing lead (27). Other reasons for the use of oxygenates in Sweden have been to decrease the oil dependence and decrease those emissions that might cause adverse health and environmental effects. Further, if ETBE is going to be used a decrease of the emission of fossil carbon dioxide is initiated.

Examples of environmental and health observations related to oxygenates in motor fuels are given bellow:

- The total level of toxic air pollutants decreases with the addition of oxygenates to gasoline, but levels of specific toxic substances in air may increase. Oxygenates in motor fuel decrease the emission of CO, aromatic compounds, e.g. benzene, and, in addition, 1,3-butadiene and ozone (69, 150). However, an increase in the emissions of aldehydes (formaldehyde or acetaldehyde from MTBE and ETBE respectively) is seen after the addition of oxygenates in the gasoline (69, 110, 150).
- Oxygenates cause a substantial reduction in the odor detection threshold of various gasoline blends (69, 150).
- The main atmospheric fate of the ethers is probably a reaction between hydroxyl-radicals and the ether (161). A number of degradation products have been identified: the primary products are tertiary-butyl formate, formaldehyde (from MTBE), acetaldehyde (from ETBE), methyl acetate and acetone (91, 157). In the soil, biodegradation of the oxygenates by microorganisms has been shown (118, 151).
- Complaints about acute health effects (headache, nausea, nasal irritation and eye irritation) have been associated with the exposure to gasoline containing MTBE (further described in section 3.5) (69, 120).
- Neoplasia in animals (liver tumors in female mice, kidney and testicular tumors in male rats) have been reported after high and chronic exposures to MTBE (further described in section 3.4) (15, 21).

3.2.2 Occupational and Non-Occupational Exposure

Oxygenates are highly volatile and exposure is therefore mainly via the air. People exposed occupationally to oxygenates include clinical laboratory technicians, production testers, workers involved in transport of oxygenates, gasoline station attendants and professional drivers (69, 170). Commuters, drivers who pump gasoline and people who live or work near service stations and plants, and, in addition, gallstone patients and health care workers may also be exposed to oxygenates (3, 70, 83, 99).

Table 2. Occupational exposure and exposure of the general public to MTBE (69). Peak exposure (range) and median values are given.

	Neat MTBE		MTBE in gasoline	
	Peak (ppm)	Median values (ppm)	Peak (ppm)	Median values (ppm)
Manufacturing	0.01-250	0.03 [d]	-	-
Blending	0.01-97	2.2 [d]	0-100	0.04 [d]
Transport	0.03-1000	0.2 [d]	0.001-510	0.1 [d]
Distribution	-	-	0-14	0.1 [d]
Service station attendants	-	-	0.3-140 [b]	0.3-5.8 [b]
				0.2-0.6 [d]
Mechanics	-	-	0.3-32	0.1 [d]
Refueling	-	-	0-38 [a]	0.4-5.8 [a]
In automobile while refueling	-	-	0.006-0.17	0.02-0.04 [d]
Automobile cabin for commuters	-	-	0.002-0.02	0.005 [c]
Community air	-	-	0-0.004	0.00013 [e]

[a] exposure during 1-2 min.
[b] exposure during less than 30 min.
[c] median during 1 h.
[d] median during 6-9 h.
[e] median during 24 h.

The exposure to gasoline and oxygenates at gas stations and during loading of gasoline are generally reduced when a vapor recovery system is used (16, 65, 67, 149). A review of MTBE exposure studies performed in the USA is presented in a document from Health Effect Institute (69) and summarized in Table 2.

3.3 Toxicokinetics

No toxicokinetic studies in humans were available when this thesis work was initiated. In the following chapter, work from the thesis is not cited.

3.3.1 Uptake

The predominant uptake of oxygenates is via inhalation. Absorption through the skin and from the gastrointestinal tract does occur, mainly when potable water is contaminated with MTBE (138). In a Finnish study, the absorption after inhalation is reported to be approximately 40% of the MTBE dose after a 4 h exposure at rest (130).

In rats, the absorption through the gastrointestinal tract is fast and complete, whereas the dermal absorption is low (116, 121).

3.3.2 Metabolism

MTBE is metabolized to *tertiary*-butyl alcohol (TBA) and formaldehyde, and ETBE is metabolized to TBA and acetaldehyde, by oxidative demethylation

(Figure 2). MTBE and TBA have been detected in human blood, exhaled air, urine and breast milk after experimental, clinical and environmental MTBE exposures (29, 33, 67, 99, 119, 120, 130, 166).

In vitro experiments using rat liver microsomes show that MTBE is metabolized to TBA and formaldehyde (26) and in addition, TBA is biotransformed to formaldehyde (36). Further, it has been reported that MTBE and ETBE are metabolized to TBA in rats (23, 26, 116, 147). Four additional metabolites have been detected in rats exposed to carbon-14 labeled MTBE and two metabolites were identified as α-hydroxyisobutyric acid (HBA) and 2-methyl-1,2-propanediol (MPD) (116). In a recent study, rats were exposed to 2000 ppm MTBE or 2000 ppm ETBE during 6 h and three major metabolites, HBA, MPD and an unidentified conjugate of TBA were found in addition to low levels of TBA, acetone and another TBA-conjugate (17). All these metabolites have also been detected in rats exposed to labeled TBA (13, 17).

MTBE activates UDP-glucuronosyltransferase and P450 isoenzymes 2B1, 2A6 and 2E1 in liver microsomes from both man and rats (26, 74, 147, 158). The metabolic activity, measured as the formation of TBA from MTBE in microsomes from different organs of rats, was found to be markedly higher in the nasal mucosa compared with the liver (73).

3.3.3 Distribution

Linear kinetics have been seen in blood of rats exposed for 2 weeks to MTBE at 50 ppm to 300 ppm in the air (147). In humans, linear kinetics have been reported after 4 h exposure to up to 75 ppm MTBE (130).

The MTBE level in blood increases rapidly during exposure and decreases soon after the exposure ends (29, 33, 130, 135). In contrast, the concentration in blood of the first metabolite, TBA, increases slowly and a plateau is reached after the exposure. The levels start to decrease slowly approximately 2-4 h after the exposure (29, 130, 135). The same distribution pattern has been seen in patients exposed to MTBE through the gallbladder (99). MTBE was found to be exhaled, distributed to fatty tissues and excreted in urine together with TBA. In addition, MTBE and TBA have been detected in breast milk at levels only slightly lower than the concentration in blood.

3.3.4 Excretion

Of the MTBE taken up by the lung in humans, about 58% was eliminated unchanged to the air, 1.4% in urine and less than 1.2% as TBA in the urine (130).

The excretion of carbon-14 labeled MTBE in rats was fast and independent of sex and delivery route (116). After intravenous administration (40 mg MTBE/kg bw), 60% was eliminated through the lungs (90% of this fraction within 3 h post exposure) and 35% in the urine (70% within 24 h and 90% within 48 h post exposure). Only minor amounts were recovered in feces (2%) and tissues (0.4%).

At higher MTBE doses (8000 ppm and 400 mg/kg bw), the fraction recovered decreased in urine and increased in exhaled air. In another study (176), mice that were given MTBE intraperitoneally (50-500 mg/kg bw) eliminated 23-69% of the dose as MTBE through the lungs, and most of this fraction (90%) within 3 h.

In a study reported in a conference abstract, rats and mice were exposed to carbon-14 labeled ETBE at a low level (500 ppm, 6 h exposure) after which most of the radioactivity was eliminated in the urine (23). However, at a higher exposure level (5000 ppm, 6 h), rats eliminated most of the ETBE in exhaled air, whereas mice eliminated equal parts in urine and exhaled air. Furthermore, rats repeatedly exposed to ETBE have higher urinary levels of TBA compared with rats exposed only once.

These MTBE and ETBE studies implies that saturable metabolism, enzyme induction or inhibition occurs at high exposure levels.

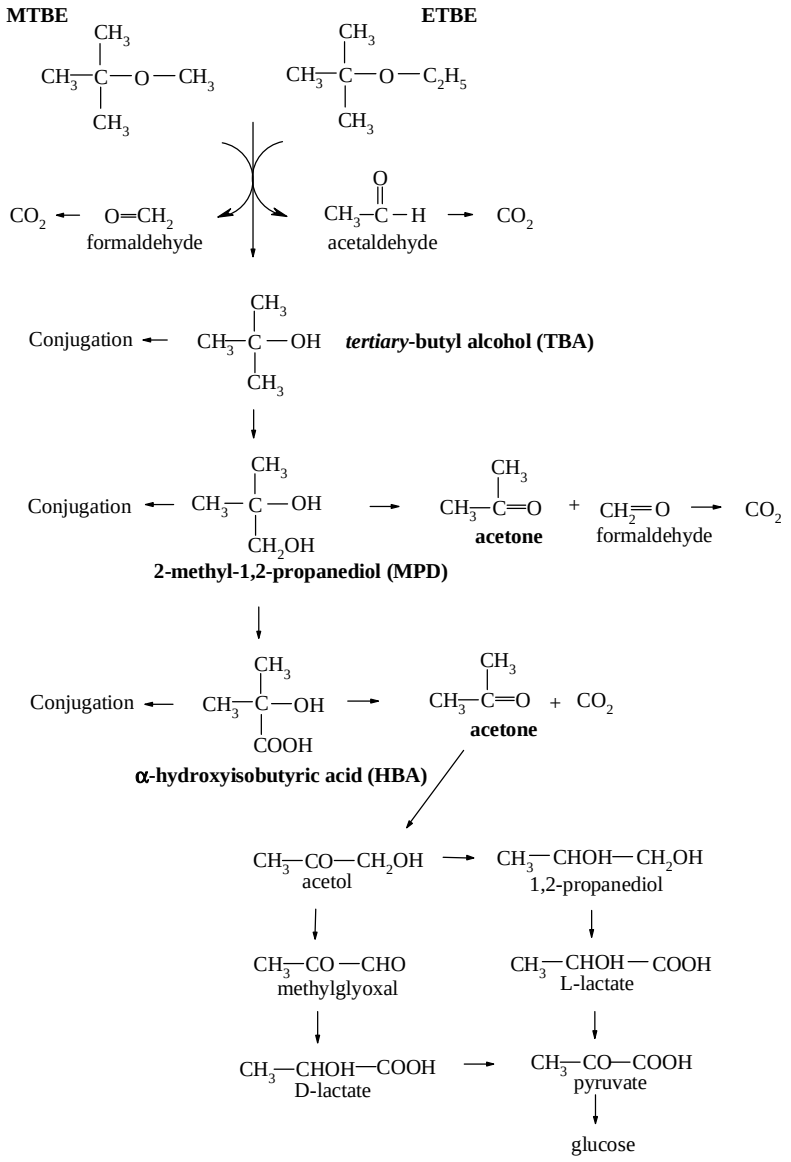


Figure 2. Potential metabolism of MTBE and ETBE (69, 98). The parent ethers and metabolites that are mainly dealt with in the present thesis are marked with bold.

3.4 Toxicity in Animals and *In Vitro* Tests

The LD_{50}-value for MTBE in mice is 4 g/kg (103) and for ETBE in rats >5 g/kg (123). The LC_{50}-value in mice after 15-min inhalation exposure is 39000 ppm for MTBE and 29000 ppm for ETBE (109). This implies low acute oral toxicity of MTBE and ETBE.

Acute and reversible *neurotoxic effects* (e.g., eyelid twitching, hypoactivity, lack of startle reflex, labored respiration and ataxia) have been seen after short term (45, 164), subchronic (50, 102, 112, 167) and chronic (21) exposure to high levels of MTBE and ETBE.

The concentration of α_{2u}-globulin increased in male rats after MTBE and ETBE inhalation exposure and, in addition, after exposure to TBA in drinking water (100, 136, 171). The toxic syndrome, referred to as *α_{2u}-globulin nephropathy*, is well known in male rats. The low molecular weight protein, α_{2u}-globulin, is synthesized in the liver under androgen control. Characteristic symptoms include a progressive increase of the size and number of protein droplets in the kidney, which can progress to mild tubular degeneration, necrosis and scattered foci of tubular regeneration (24, 148). These changes have also been reported after exposure to unleaded gasoline (107) and isoparaffinic fractions of unleaded gasoline, e.g. 2,2,4-trimethylpentane (148).

In mice, a reversible decrease in the *breathing frequency* is seen during the first 5-10 min of a 1 h exposure to 83-2800 ppm MTBE (155). At a higher exposure level, 8300 ppm MTBE, the breathing frequency is decreasing during the entire exposure, which according to the authors indicate both sensory and pulmonary irritation. However, cells recovered in lung lavage did not show any morphological or biochemical changes.

Since MTBE is used in medical treatment, *tissue injury* has been studied in rats after injection of MTBE into the hepatic parenchyma and after intravenous and intraperitoneal administration (2). MTBE was found to induce local tissue damage and could cause severe lung injury when infused into a large vein.

In unpublished studies (11, 121, 122), *dermal tests* of MTBE and ETBE caused reversible erythema and edema in rabbits. However, the authors did not consider MTBE to be a primary skin irritant whereas ETBE seemed to give a slightly higher degree of irritation. Further, *ocular tests* were performed and eye redness was found in rabbits after application of 0.1 ml MTBE or ETBE. The irritation was reversible and the eyes were normal 3 days after MTBE and one to two weeks after the ETBE exposure. No sensibilization was seen in guinea pigs after ten intradermal injections of 0.5 ml MTBE (0.1%) (11).

Mutagenicity tests for MTBE (108, 111, 141) and ETBE (178) have been predominantly negative. Neither MTBE nor ETBE showed *genotoxic* effects in bone marrow micronucleus tests (38, 90, 111, 164), in chromosome aberration or in forward mutation assays (38, 90, 108, 163, 165) with or without the addition of metabolic enzymes. In the evaluation of the toxicity of MTBE and ETBE using a

structure relational models, no genotoxicity or carcinogenicity could be predicted (142, 179).

Chronic inhalation exposure of MTBE resulted in an increased incidence in kidney tumors in male rats (3000 ppm) and in liver tumors in female mice (8000 ppm) (21). In another study, chronic exposure (up to 1000 mg MTBE/kg bw) increased the incidence of Leydig cell tumors in male rats and the incidence of leukemia and lymphoma in female rats (15). This latter study has been criticized for the incomplete descriptions of methods and results: Leydig cell tumors were common also in the control material (i.e. the increase might be random) and only combined data on leukemia and lymphoma were reported (results were not presented for each respective neoplasm) (114).

MTBE tested negative for teratogenic and reproductive effects in rabbits and rats up to 8000 ppm and in mice up to 1000 ppm (18, 19, 20, 41).

No studies showing cancinogenic effects or effects on reproduction after ETBE exposure in animals have been published.

MTBE and ETBE are metabolized to at least two intermediates each that are suspected carcinogens in rodents. TBA causes kidney tumors in male rats and thyroid gland adenomas in mice exposed to high concentrations in drinking water (39). Formaldehyde is genotoxic in a wide variety of *in vitro* assays and carcinomas have been reported in the nose of rats at high exposure levels (78). Acetaldehyde is genotoxic in *in vitro* assays and may cause cancer in the upper airways of rodents (76). No data on carcinogenic potential is available for other metabolites, e.g., HBA and MPD.

3.5 Health Effects in Humans

In Fairbanks, Alaska, acute health effects have been reported during the use of oxygenated gasoline (MTBE) (120). Different symptoms, e.g., headache, nausea, nasal irritation and eye irritation were common: among 18 workers exposed to an average of 0.1 ppm MTBE (air measurements) between 5 and 13 reported one or more of a variety of symptoms. These workers had an average concentration of 0.02 µM MTBE in blood. At a follow-up three months later (no MTBE in the gasoline), only up to 2 among 28 gasoline-exposed subjects reported symptoms. At that time, 0.04 ppm MTBE was detected in air (8 h average) and MTBE levels in blood averaged 0.003 µM from the studied individuals. In another study, subjects with high levels of MTBE in the blood (>0.03 µM MTBE) after a workshift, reported one or more symptoms more frequently than subjects with lower levels of MTBE in the blood (166). Other epidemiological studies have failed to show any correlation between rating of acute health effects and MTBE exposure levels (8, 57, 61, 64, 119). The above mentioned study in Fairbanks has been criticized because several factors may have influenced the ratings, e.g. the change in gasoline odor, lower odor threshold, winter climate, outbreaks of complaints due to increase in gasoline price, and media reports (53, 69, 160).

Headache, dizziness, nausea, and dyspnea were reported in a family of five individuals who were exposed to water contaminated by gasoline. The levels of MTBE found in the well water analysis were 1.3 to 1.6 mg/l and the family used the water for everything except drinking. In a double-blind test, all members of this family indicated MTBE as the most objectionable odor of several other compounds existing in gasoline (9).

The majority of human MTBE studies relate to the percutaneous transhepatic catheterization of the gallbladder and direct dissolution of gallstones with MTBE (70, 83, 99, 134, 156). In this medical procedure MTBE is continuously supplied in amounts that fill the gallbladder (1-15 cm^3) for up to 6 h/day for 1 to 3 days. Mild acute side effects (nausea, vomiting, and abdominal discomfort) have been reported. Overflow of MTBE during instillation has been associated with sedation (reversible coma in one patient), unpleasant odor of MTBE in the breath, low blood pressure, acute renal failure and mild duodenitis.

No acute effects (rated symptoms or objectively measured nasal or eye irritation) were observed in healthy volunteers experimentally exposed to up to 1.7 ppm MTBE for 1 h at rest (n=80) (33, 135). In a Finnish experimental study, thirteen males were exposed to 0, 25 and 75 ppm MTBE for 4 h (140). In this study, measurements of subjective symptoms and mood as well as reaction time and posturography (body sway) were performed during the exposure (1 and 3 h) and at 1 h post exposure. At 75 ppm MTBE, mild symptoms such as “heaviness in the head” and mild mucous membrane irritation were reported after 3 h of exposure, but the symptoms had disappeared 1 h post exposure. In total 6 out of 13 subjects reported MTBE-related symptoms.

No reports on the health effects of ETBE have been published.

3.6 Occupational Exposure Limits and Classifications

The present Swedish occupational exposure limit (OEL) for MTBE is 50 ppm or 180 mg/m^3 during an 8 h work day (10, 104). The short-time limit (15 min) is 75 ppm or 270 mg/m^3. No OEL value for ETBE is available in Sweden (December 1998).

OEL values for MTBE have recently been established in several countries (listed in Table 3), whereas it is still under evaluation in e.g. Germany (48). At present, the lowest 8 h time-weighted average (TWA) value has been adopted in the United Kingdom (68). In the International Labour Office summary list for the year 1991 (81), Czechoslovakia and the Soviet Union were included. No OELs for ETBE has been found in the literature.

The categorization of MTBE and some metabolites by the National Board of Occupational Safety and Health in Sweden (10), the International Agency for Research on Cancer (IARC) (76, 78) and the American Conference of Governmental Industrial Hygienists (ACGIH) (5) is summarized in Table 4. ACGIH has classified MTBE as a confirmed animal carcinogen with unknown

Table 3. The time-weighted average value during an 8 h work day and the short-time exposure value (15 min time-weighted average exposure) for MTBE.

Country	Time-weighted average value (ppm)	Short-term exposure value (ppm)
Sweden (10, 104)	50	75
Finland (159)	50	-
the Netherlands (117)	50	100
the USA (5)	40	-
United Kingdom (68)	25	-
Czechoslovakia (81)	28	56
Soviet Union (81)	-	28

relevance to humans (A3) based on animal studies and inadequate (or no) data from epidemiological studies. Gasoline vapors have been classified by IARC as "possibly carcinogen to humans", based mainly on the established carcinogenicity of some constituents such as benzene and 1,3-butadiene (77). The following are known for humans of the three metabolites, TBA, formaldehyde and acetaldehyde:

- The carcinogenicity of TBA in humans has not been evaluated. TBA has an OEL in Sweden, but is not classified as a carcinogen in the OEL list (10) or by the IARC. TBA is categorized by ACGIH (5) as a non classifiable human carcinogen (A4), based on inadequate data.
- Formaldehyde occurs as a natural product in most living systems and in the environment, e.g., from cigarette smoke. For the highly reactive formaldehyde an increase in relative risk for nasopharyngeal cancer was associated with occupational exposure (78). The IARC evaluation of formaldehyde is based on "limited evidence in humans for carcinogenicity and sufficient evidence of carcinogenicity in experimental animals".
- The IARC evaluation of acetaldehyde is "inadequate evidence for carcinogenicity to humans and sufficient evidence for carcinogenicity in animals".

Table 4. Summary of classification of MTBE and some metabolites.

	Sweden	IARC	ACGIH
MTBE	-	-	A3 [c]
TBA	-	-	A4 [c]
Formaldehyde	C and D [a]	2A [b]	A2 [c]
Acetaldehyde	C [a]	2B [b]	A3 [c]

[a] classified as a human carcinogen (Group C) and sensibility agent (Group D) (10)

[b] classified as a probably carcinogenic agent to humans (Group 2A) and possibly carcinogenic agent to humans (Group 2B) (76, 78)

[c] classified as a suspected human carcinogen (A2), animal carcinogen (A3) and not classifiable as a human carcinogen (A4) (5).

4. Methods

An overview of the methods used is given in this section, however, all details are further described in the original papers.

The human exposures were performed according to the Helsinki Declaration, after approval from the regional ethical committee at the Karolinska Institute, Solna, Sweden and after informed consent (verbal and written) from the subjects.

4.1 Whole-Body (Studies I and II) and Face-Mask (Study III) Exposures

Healthy male volunteers were exposed to MTBE (n=10, Study I) or ETBE (n=8, Study II) in a 20 m^3 exposure chamber (Figure 3). Two subjects were exposed at a time and different subjects participated in the MTBE and ETBE studies. In study III, four out of the ten subjects that participated in the MTBE study were exposed to ^{13}C-labeled MTBE ([1,2-$^{13}C_2$]-MTBE). These volunteers were exposed via a face-mask and one subject at a time (Figure 4).

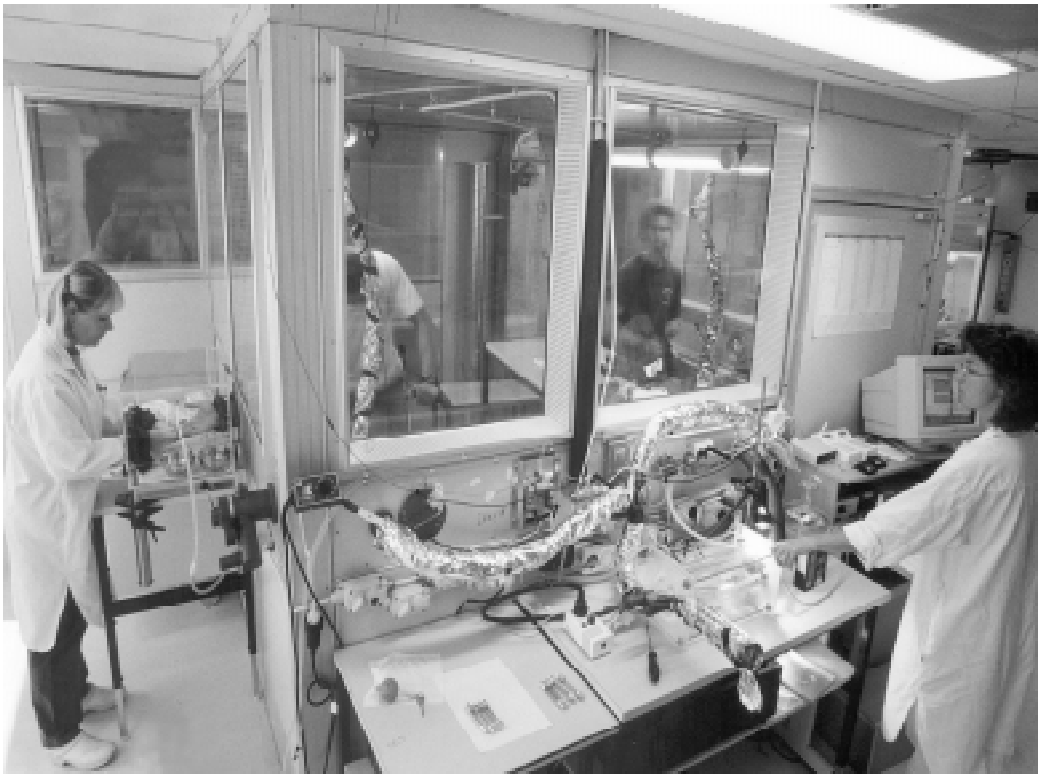


Figure 3. Inhalation exposure of MTBE or ETBE vapor in a 20 m^3 chamber with controlled climate. The two volunteers performed light physical exercise on bicycle ergometers during the entire exposure and capillary blood samples were taken outside the chamber.

The *chamber exposures* (Studies I and II) were conducted during 2 h at the nominal levels of 5, 25 and 50 ppm MTBE or ETBE and in addition, 0 ppm (clean air) in the ETBE study. A pump transferred the liquid MTBE solution to a preheated glass tube, where MTBE was vaporized and the completely vaporized solvent followed the influent air stream into the exposure chamber. The concentrations of MTBE or ETBE in the chamber air were monitored at 5-min intervals by a Fourier transform infrared spectrophotometer (MTBE) and by a gas chromatograph (GC) equipped with a gas sample injection loop (ETBE). The chamber climate was carefully controlled with an average temperature of 19-20°C, a relative humidity of 40-43% and 16-19 air changes per hour. To prevent leakage of solvent, the air pressure in the chamber was kept about 5 Pa lower than in the surrounding laboratory.

In the *face-mask inhalation study* (Study III), volunteers were exposed for 2 h from polyester-laminated aluminum foil bags containing 180-200 liter air and 50 ppm ^{13}C-labeled MTBE vapor (analyzed by GC). During the entire exposure, the subject wore a breathing mask fitted with separated inlet and outlet valves. The inlet valve of the breathing mask was connected to the exposure bag, which was attached on the outside of the chamber (Figure 4). In this study, the volunteers performed the exercise inside the chamber during the exposure because: i) the chamber had controlled climate, ii) for practical reasons, as there was insufficient space in the surrounding laboratory and iii) since all equipment for the exhaled air analysis was fixed on the chamber wall.

Figure 4. Exposure to ^{13}C-labeled MTBE vapor (via a face-mask) from exposure bags. The volunteer wore a breathing mask during the entire 2 h exposure and performed light exercise on a bicycle.

The highest exposure level (50 ppm MTBE and ETBE) was chosen considering the current Swedish OEL value for MTBE (10) in both studies, since no restrictions have yet been assigned to ETBE. There was at least two weeks between successive exposures (Study I and II) and all subjects were unaware of the actual exposure sequence. During all exposures, the subjects performed light physical exercise (50W) on a computer-controlled bicycle ergometer and heart rate, pedal frequency, workload and speed were recorded every 20 seconds. Further, the individual pulmonary ventilation and respiratory frequency were recorded as one-minute averages during the entire [1,2-$^{13}C_2$]-MTBE exposure and during each 5-6 min exhalation period of MTBE, ETBE and post exposure of all exposures.

4.2 Toxicokinetics of MTBE and ETBE (Studies I, II and III)

4.2.1 Sampling and chemical analysis of exhaled air, blood and urine

Identical time points for collecting blood, urine and exhaled air were used in all exposure studies (Studies I, II and III).

Exhaled air was collected through a mouthpiece (Figure 5), fitted to two valves with separate in and outlets (Studies I and II). Each exhalation period lasted for 5-6 min and the exhaled air was analyzed during the last 2 min. MTBE in exhaled air was analyzed by a Fourier transform infrared spectrophotometer (Study I) and ETBE (Study II) and MTBE (Study III) by a GC equipped with a gas sample injection loop.

After the exposure exhaled air was collected on adsorbent tubes. In study I, the adsorbent was eluted with carbon disulfide and MTBE was analyzed with GC. In studies II and III, the adsorbent tubes were desorbed in an automatic thermal desorption unit and ETBE, MTBE and TBA were analyzed with a GC.

Figure 5. Sampling of exhaled air every 30 min (5 min each time) during the 2 h whole-body exposure to MTBE and ETBE. Physical exercise was performed on a bicycle ergometer during exposure and pulmonary ventilation and breathing frequency were measured with a spirometer.

Figure 6. Capillary blood sampled on the outside of the chamber and inside a glove box, which was flushed with clean air.

Capillary blood was sampled from the fingertips of the volunteers (Figure 6) before, during and up to 46 h after the MTBE and ETBE exposures and up to 20 h after the [1,2-$^{13}C_2$]-MTBE exposure. In total, 23-24 blood samples were collected and MTBE and TBA (Study I), ETBE, TBA and acetone (Study II) and MTBE, TBA and acetone (Study III) were analyzed by head-space GC.

All *urine* was collected until 24 h post exposure (Studies I, II and III) and in addition, a spot urine sample was collected at 46 h post exposure (Studies I and II). After recording the volume, the urine samples were analyzed in the same way as the blood samples

4.2.1 Calculations

Individual kinetic calculations were performed on data from all exposures. Each subjects concentrations of MTBE and ETBE in blood versus sampling time were fitted to a linear kinetic four compartment mamillary model with zero-order input and first-order elimination (metabolism and excretion) in the central compartment. Thus, other ways of elimination, e.g., renal clearance were considered negligible, as well as skin uptake. Additional input data were net uptake, time of exposure and the amount exhaled post exposure. The model was written as an Excel spreadsheet macro (Johanson, unpublished). In fitting the model to the exposure data, optimization was carried out by finding the most likely values of rate constants by minimizing the unweighted residual sum of squares using the Solver add-in macro in Microsoft Excel.

In the calculations the absorbed dose was regarded as the sum of the net respiratory uptake and the amount exhaled during exposure. Previously in studies of this kind, the absorbed dose (net respiratory uptake) has been estimated as the

difference between the concentration of the chemical in inhaled and exhaled air, multiplied by the pulmonary ventilation. However, by inclusion of the exhalation during exposure, dose related parameters (such as clearance) become independent of the length of exposure and a more accurate estimation of body burden was achieved. Thus, absorbed dose in the present studies was referred to in two ways: the respiratory uptake and the net respiratory uptake (previous approach).

Decay curves of MTBE and ETBE in urine (starting at 2-4 h) were fitted to biexponential functions by nonlinear regression analysis using Solver in Microsoft Excel. Data from the elimination phase of TBA in blood and urine, starting approximately at 6 h, were fitted to a monoexponential function again by nonlinear regression analysis.

The concentrations of MTBE and ETBE in blood at steady state was calculated as the respiratory uptake rate divided by the total clearance. The area under the concentration-time curve (AUC) of ETBE in blood was obtained from the four-compartment model, while the AUC of TBA and acetone was calculated by the trapezoidal rule. The renal clearance of TBA was calculated as the total amount of TBA excreted in urine divided by the AUC of TBA in blood.

The toxicokinetics after the face-mask exposure (Study III) was analyzed (as described above) and compared with the previous whole-body exposure (Study I).

4.3 Characterization of Metabolites in Urine by ^{13}C NMR Spectroscopy (Study III)

The natural abundance of the stable isotope ^{13}C is 1.1%, and thus, 1.1% of carbon atoms in compounds excreted in normal urine give usually single resonance in NMR-spectra (one ^{13}C-labeled). Thus, adjacent ^{13}C atoms give multiple signals in NMR-spectra as a result of carbon-carbon coupling and the natural probability of that is 0.01%. The subjects in this study were exposed to MTBE that was 99% ^{13}C-enriched at *two adjacent carbons* (i.e. *doublet signals* in the NMR spectra) at the center carbon (C*, called b) and at one of the three CH_3-groups (*CH_3, called a or c). Since only one of the three methyl groups was labeled three chemically identical metabolites were formed, but with two different labeled structures (marked with or without prime in Figure 14).

^{1}H-Decoupled carbon-13 nuclear magnetic resonance (NMR) spectra were obtained for urine samples collected prior to the [1,2-$^{13}C_2$]-MTBE exposure and at 2, 4, 7, 11, and 20 h post exposure (Study III). The urine was treated in two ways: diluted in D_2O and concentrated after protein precipitation. The latter was done to concentrate the sample and reduce the signal to noise ratio in the NMR spectra.

4.4 Determination of Partition Coefficients (Study IV)

The kinetics of a volatile substance depends largely on the partitioning between blood and air and blood and other tissues. By definition, the partition coefficient (λ) of a compound is the quotient of the concentration (C) in two different phases at equilibrium (146):

$$\lambda_{liquid/air} = C_{liquid} / C_{air}$$

Liquid/air partition coefficients were determined *in vitro* by a closed vial equilibration method previously described by Sato *et al.* (146) and used to determine partition coefficients for several other solvents (55, 85, 87). In brief, aliquots of olive oil, physiological saline or fresh human blood were added to sample vials. Further, known amounts of MTBE, ETBE, TAME and TBA were added both to sample vials and to reference vials. The reference vials were empty except for inert glass pearls, which corresponded to the same volume as the liquid in the sample vials (due to the pressure-injection method of the GC). The capped vials were allowed to equilibrate at 37°C for at least 20 minutes, before head-space GC analysis. The liquid/air partition coefficients were calculated from GC peak areas, air (head space), and liquid volumes of the sample vials and the reference vials.

Blood samples, obtained from five males and five females, were used to calculated the inter individual variation (correlation of variance, CV) of the $\lambda_{blood/air}$.

Liquid/air partition coefficients (Study IV) and tissue composition (12, 58, 131) were used to calculate the tissue/blood partition coefficients needed in the PBTK modeling (Study V).

$$\lambda_{tissue/air}=(\%\ \text{water content in tissue}\cdot\lambda_{water/air})+(\%\ \text{fat content in tissue}\cdot\lambda_{fat/air})$$

4.5 Physiologically Based Toxicokinetic Modeling (Study V)

A PBTK model (Figure 7) was developed for inhalation exposure to ETBE using the previously determined partition coefficients (study IV) and the experimental data obtained in study II. To describe the kinetics of ETBE the following compartments were used: lungs (including arterial blood), liver, fat, rapidly perfused tissues (including kidney, brain and other tissues), resting muscles and working muscles (to account for the leg exercise during bicycling, throughout the exposure). The skin was included in the muscle compartments. The same set of compartments and, in addition, a urinary excretion compartment were used for the metabolite TBA. First order metabolism was assumed in the model, since linear kinetics have been shown experimentally up to 50 ppm ETBE (Study II). Organ volumes and blood flows were calculated from individual body weight and height according to published equations (52) and the tissue/blood partition coefficients were calculated as described in section 4.4.

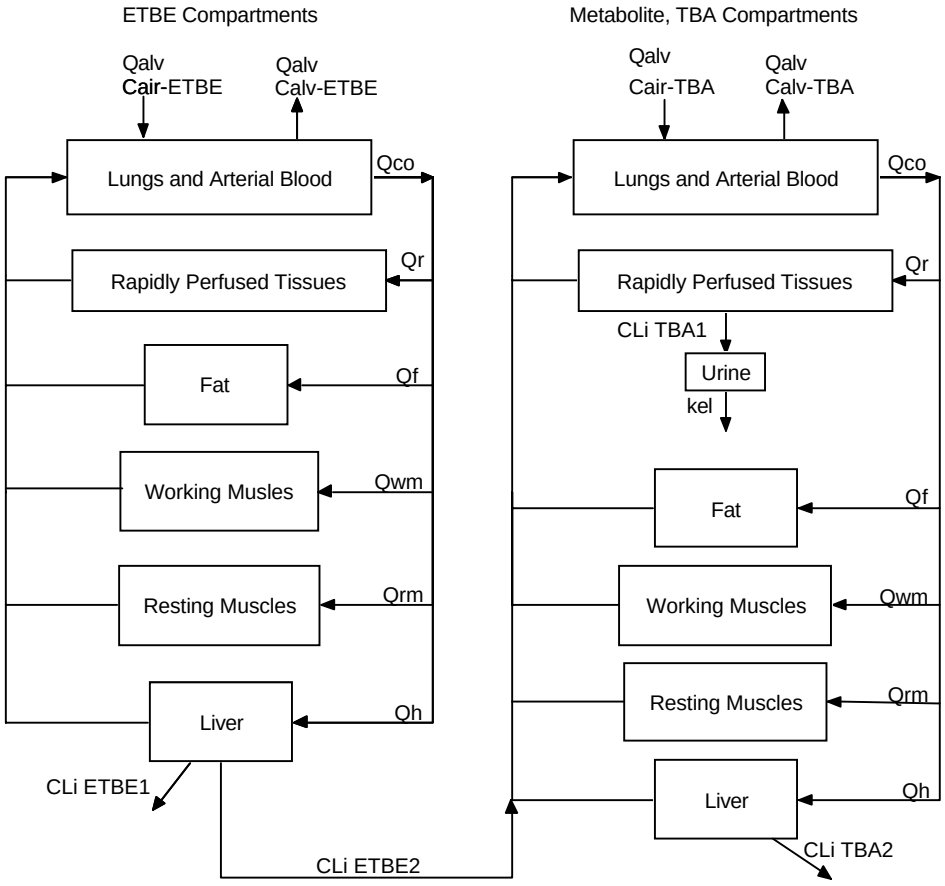


Figure 7. Physiologically based toxicokinetic model used in the simulations of ETBE and the metabolite TBA.

A sensitivity analysis, according to Pierce *et al.* (132), was performed to determine the influence of parameter values on blood, urine and exhaled air levels.

Further, biomarker levels at the end of the workshift and the following morning prior to the next workshift were predicted. The model was used to study how various factors such as long-term exposure, fluctuations in exposure levels and workload influenced the levels of biomarkers in blood, urine or exhaled air.

4.6 Acute Effects after MTBE and ETBE Exposures (Study VI and VII)

Acute effects were measured, objectively and subjectively, in parallel with the toxicokinetics during the same exposures. The battery of test for acute effects in this study was extensive, laborious and, for some analyses, expensive. Therefore, all subjects were first exposed to 50 ppm, whereas the following exposures to 5 or 25 ppm levels were random. End-points that did not result in any positive findings at the highest exposure levels were excluded from the test battery during exposure to the lower levels (see Table 5).

Table 5. Acute effect tests performed under various exposure conditions (- indicates not studied).

	MTBE			ETBE			
Exposure levels (ppm)	5	25	50	0	5	25	50
Subjective ratings	x	x	x	x	x	x	x
Ocular							
Blinking frequency	-	-	x	-	x	x	x
Eye redness	-	-	x	-	-	-	x
Tearfilm stability	-	-	x	-	-	-	x
Conjunctival epithelial damage	-	-	x	-	x	x	x
Nasal							
Blockage index	x	x	x	-	-	-	-
Acoustic rhinometry	x	x	-	x	x	x	x
Nasal lavage							
Eosinophilic cationic protein	-	-	x	x	x	x	x
Myeloperoxidase	-	-	x	x	x	x	x
Lysozyme	-	-	x	x	x	x	x
Albumin	-	-	x	x	x	x	x
IL-8	-	-	-	-	-	-	x
Cell count	-	-	x	-	-	-	x
Pulmonary							
PEF	x	x	x	-	x	x	x
Spirometry	-	-	-	x	x	x	x
Transfer factor, diffusing capacity	-	-	-	x	x	x	x

The subjects were informed about the experimental design, but were unaware of the exposure level sequence. The 5 ppm level was chosen so the subjects could smell the solvent in all exposures and thus not know the actual exposure level. The original plan was that the level of 5 ppm would be the control condition, and it was so in the MTBE study. However, some acute effects were observed at 5 ppm in the ETBE study and it was necessary to include a zero level (clean air) exposure. The subjects were not informed that the last exposure was to be to clean air.

4.6.1 Ratings of Symptoms

The subjects were asked to complete a questionnaire with 10 items related to symptoms of irritation and effects on the central nervous system (discomfort in the eyes, nose, throat and airways, difficulty in breathing, smell of solvent, headache, fatigue, nausea, dizziness, intoxication). Answers were given by marking with a pen along a 100 mm visual analog scale graded from “not at all” to “almost unbearable” (Figure 8). During exposure, the ratings were performed while exercising on the bicycle.

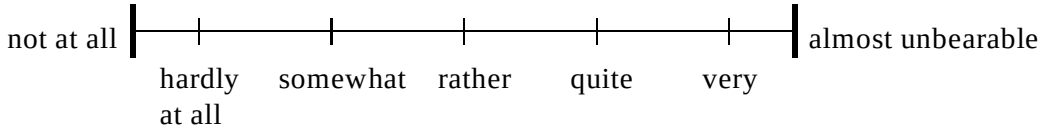


Figure 8. A visual analog scale (0-100 mm) used for ratings of various symptoms in a questionnaire. All verbal grading and questions were written in Swedish.

4.6.2 Ocular Measurements

Blinking Frequency
Blinking frequency was determined from video tape recordings where the number of blinks was counted during 3 minutes at each occasion.

Eye Redness
The difference in eye redness was scored on a four-grade scale (94) by comparing photographs (diapositives). The scorings were made in a blinded fashion, i.e. without the observer knowing when the photo was taken.

Tearfilm Stability
Tearfilm stability was assessed in two ways. First, the self-reported break-up time was monitored, i.e. the time the subject was able to keep his eyes open without blinking (174). Second, the tearfilm stability was measured by observing the tearfilm in a slitlamp microscope and recording the break-up time after instillation of Fluorescein into the lower conjunctival sac (126). The tearfilm measurements were performed in both eyes. The measurements at the end of the exposure were performed at rest inside the chamber.

Conjunctival Epithelial Damage
Conjunctival epithelial damage was visualized by instilling a drop of Lissamine Green into the lower conjunctival sac in the left eye. The eye was inspected in a slitlamp microscope and the degree of corneal and conjunctival damage was scored semi-quantitatively (126). To minimize interference the Lissamine Green staining was performed after the other ocular measurements.

4.6.3 Nasal Measurements

Acoustic Rhinometry
The degree of swelling of the nasal mucosa was estimated by acoustic rhinometry (63, 71). This method describes the geometry of the nasal cavity by analyzing the reflections of an acoustic signal. The nasal volume, the minimal nasal cross-sectional area and the minimal nasal diameter were determined as an average of three measurements of each nostril.

Blockage Index
A peak expiratory flow (PEF) meter (measuring capacity 0 to 800 l/min) was connected to a face mask to determine the nasal PEF. The subject exhaled maximally into the flow meter through the nose with his mouth closed (mean of three measurements). The blockage index, a measure of the nasal airway resistance (153), was calculated as:

Blockage index = (pulmonary PEF - nasal PEF) / pulmonary PEF

(pulmonary PEF see section 4.6.4).

Nasal Lavage
Nasal lavage was performed by rinsing each side of the nasal cavity with 5 ml of sterile physiological saline (62). Leukocytes and epithelial cells were counted in the nasal lavages and in addition, inflammatory markers (eosinophilic cationic protein, myeloperoxidase, lysozyme, albumin and interleukin 8) were analyzed. The levels of inflammatory markers were calculated and compared in two ways: as the concentration and as the total amount in the recovered lavage fluid.

4.6.4 Pulmonary Measurements

Peak Expiratory Flow
The pulmonary PEF rate (average of three measurements) was performed by blowing in a flow meter with a measuring range of 0 to 800 l/min (6).

Spirometry
Spirometry was carried out with a calibrated wedge spirometer (6). The subject wore a nose clip and was asked to inhale as much as possible and thereafter exhale completely in a mouthpiece. Three slow vital capacity (VC) maneuvers were carried out followed by three forced vital capacity (FVC) exhalations. One second forced expiratory volume (FEV_1) was measured as the highest value of three attempts and VC was measured as the maximum exhaled air from three slow and three forced exhalations.

Transfer Factor
The transfer factor (TLco), which reflects the gas exchange characteristics of the lung parenchyma, was measured with the single breath holding method (diffusing capacity) using the Morgan Transfer test equipment (43). In brief, the subjects inhaled a test gas consisting of 0.3% carbon monoxide (CO), 14% helium and 85.7% oxygen. The transfer factor (average of two measurements, TLco within 5%) was calculated from the rate of disappearance of CO from the alveolar gas during a 10-sec breath holding period. The transfer of CO into the pulmonary capillary is limited solely by diffusion and the concentrations is measured by an infrared analyzer. Helium is added to the inspired gas to give a measurement of lung volume by dilution.

4.7 Statistical Analysis

Unless otherwise stated, the results are presented as mean values with 95 % confidence intervals. Student’s paired t-test was used to compare results from different exposure occasions. Analysis of variance, repeated measures model, was used to compare the subjective ratings and the acute effect measurements. The level of significance was set to 0.05.

5. Results and Discussion

5.1 Toxicokinetics of MTBE and ETBE (Studies I, II and III)

Results of uptake, excretion and clearance after exposure to 50 ppm MTBE, ETBE and [1,2-$^{13}C_2$]-MTBE are presented in Table 6. Small differences in the kinetics of the two oxygenates were found. MTBE had higher uptake and lower respiratory excretion than ETBE. This was in agreement with the determined *in vitro* partition coefficients (see section 5.3).

The concentration of parent ether in blood increased during the entire exposure with a tendency to level off but without reaching any plateau (see Figure 9). Similar concentration curves have been seen in other MTBE studies (33, 130, 135). A somewhat lower ETBE level in blood compared with MTBE was seen and this was illustrated by a 15-30% lower blood AUC for ETBE, and is explained by the lower uptake of ETBE (Table 6).

During these 2 h exposures no steady state (i.e. when the rate of uptake matches the rate of elimination) was reached: neither for MTBE nor for ETBE. Nonetheless, steady state concentrations could be calculated from the respiratory uptake rate and the total blood clearance. For MTBE in blood the following steady state concentrations were estimated: 2.3, 8.8 and 19 µM after the 5, 25 and 50 ppm MTBE exposure. After the 2 h exposure the following concentrations were reached in blood: 1.3, 6.0 and 14 µM MTBE. The calculated steady state levels in blood for ETBE were 1.5, 5.9 and 13 µM and the corresponding measured levels after the 2 h exposure were 1.1, 5.4 and 10 µM ETBE, at 5, 25 and 50 ppm ETBE exposure, respectively.

Table 6. Results after whole-body exposure to 50 ppm MTBE (n=10) and 50 ppm ETBE (n=8) and face-mask exposure to 50 ppm [1,2-$^{13}C_2$]-MTBE (n=4) during 2 h and at a workload of 50 W (mean values ± 95% confidence interval).

	MTBE	ETBE	[1,2-$^{13}C_2$]-MTBE
Parent ether			
Respiratory uptake (%)	49 ± 10	34 ± 3	47 ± 7
Respiratory excretion (%)	32 ± 8	46 ± 5	29 ± 8
Urinary excretion (%)	0.09 ± 0.03	0.06 ± 0.02	0.1 ± 0.04
Total clearance (l/h/kg)	0.8 ± 0.2	0.8 ± 0.2	0.8 ±0.04
Exhalatory clearance (l/h/kg)	0.2 ± 0.08	0.4 ± 0.08	0.2 ± 0.07
Metabolic clearance (l/h/kg)	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.05
Mean residence time (h)	6.1 ± 1.7	11 ± 7	4.0 ± 2.2
Metabolite, TBA			
Respiratory excretion (%)	nd[a]	3.8 ± 0.9	2.0 ± 0.4
Urinary excretion (%)	0.6 ± 0.1	0.9 ± 0.5	0.6 ± 0.2
Renal clearance (ml/h/kg)	0.6 ± 0.1	1.0 ± 0.5	0.9 ± 0.4

[a] not determined

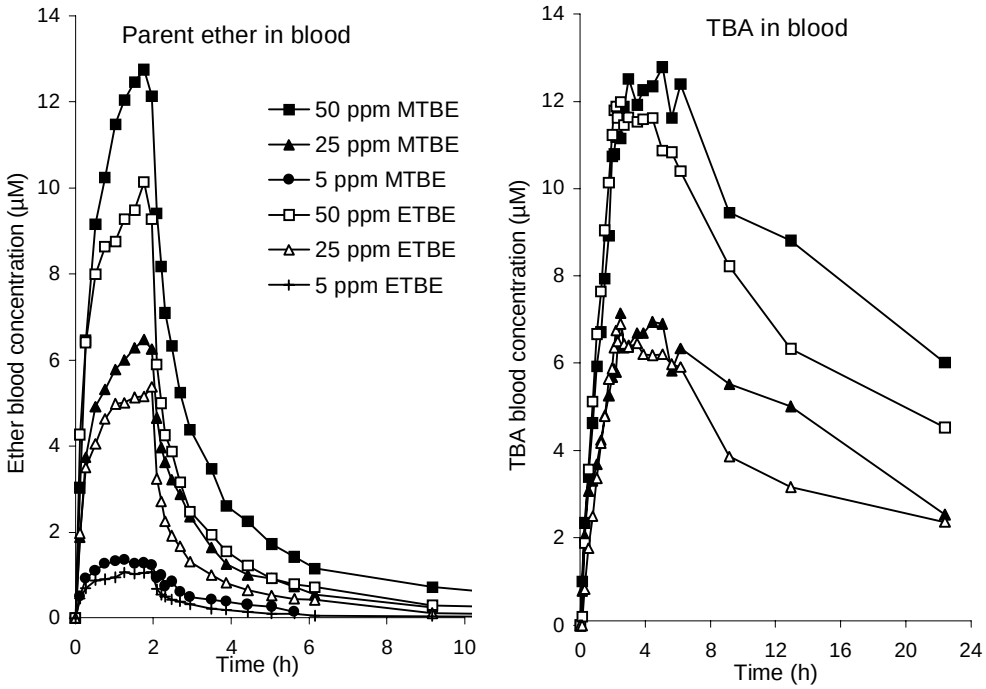


Figure 9. A comparison of average concentration of MTBE and ETBE in blood after MTBE (n=10) and ETBE (n=8) exposure.

The process of elimination of MTBE and ETBE from blood was separated into four phases. The two fastest elimination phases had average half-times of 1 and 10 min and 2 and 18 min for MTBE and ETBE, respectively. The mean values for the intermediate and the slowest phase were 1.5 and 19 h for MTBE and 1.7 h and 28 h for ETBE. The half-times as well as the mean residence time (Table 6) indicate a somewhat slower elimination of ETBE compared with MTBE.

The half-times of parent ether in urine were separated in two elimination phases, with approximately 20 min and 3 h for MTBE and 8 min and 9 h for ETBE. In agreement with the blood half-times, elimination of ETBE was slower than elimination of MTBE.

In contrast to the parent ethers profile, TBA in blood increased steadily during the exposure and remained high for several hours after TBA was eliminated slowly from the body, as illustrated in Figure 9. About 4 h after the end of exposure, TBA started to decline and the half-times of TBA in blood and in urine were 8-12 h and 7.5-9 h, respectively.

Low urinary recovery of parent ether (<0.1%) and TBA (<1%) was seen after all exposures (Table 6). This implies further metabolism or other excretion routes. Further metabolism of MTBE has indeed been shown in humans, in Study III (section 5.2) and in animals (17, 116). The excretion of minor amounts of unchanged ETBE and MTBE in the urine was not a surprise, since these ethers are only slightly water soluble. In fact, even less water-soluble chemicals e.g., styrene and trimethylbenzenes, are recovered in small amounts in human urine (88, 127). Urinary excretion of small amounts of unmetabolized MTBE has been

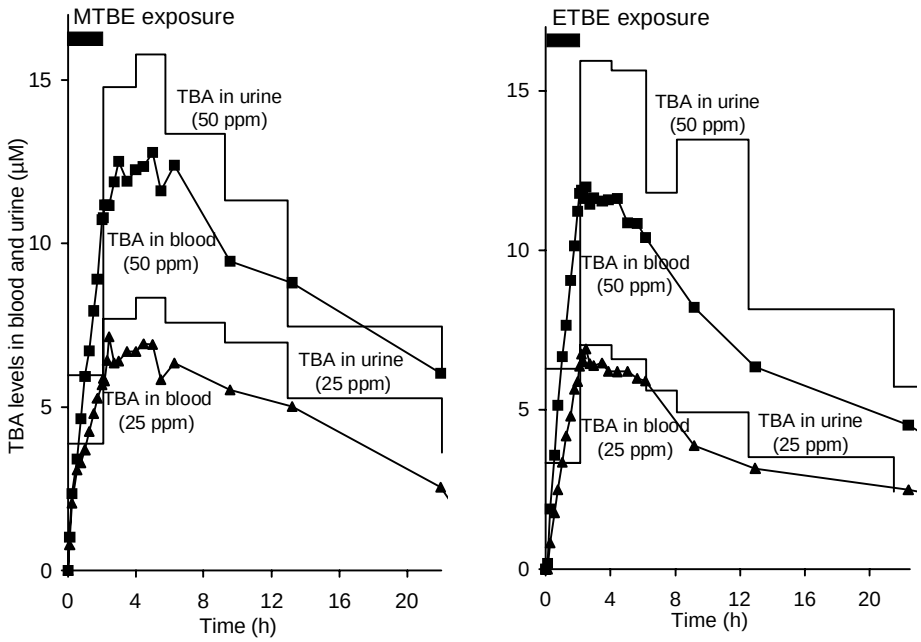


Figure 10. Comparison of average TBA levels in blood and urine after ether exposures.

demonstrated in two other experimental studies on volunteers (29, 130). The renal clearance for TBA was low, less than 1 ml/kg/h, which implies extensive blood protein binding or tubular reabsorption of TBA and makes a logical framework for the possibility of further metabolism. The concentration of TBA in urine was in general higher than the levels of TBA in blood (Figure 10). This could be explained by the water/blood partition coefficient of TBA which was determined to be 1.3 (study IV, Table 8), i.e. the level in urine should in average be 30% higher than in blood. The water/blood partition coefficient is approximately equal to the urine/blood partition coefficient, and this has been shown for MTBE (79).

The main excretion route for the unchanged ethers was via exhalation (Table 6), whereas a small amount TBA was also exhaled and detected in the ETBE study. Approximately the same figures have been seen in other MTBE studies (29, 130, 135). In total, less than 50% recovery (parent ethers and TBA) was found in exhaled air and in urine within 24 h. However, taking into account the quantification of the other metabolites (HBA and MPD) in the 20 h post exposure urine sample, this indicates that a substantial amount is further metabolized and excreted in the urine (section 5.2).

The area under the concentration-time curve (AUC) of parent ether and TBA in blood was proportional against exposure levels. This illustrates linear kinetics up to 50 ppm MTBE and ETBE.

The level of acetone (analyzed by GC) in blood (Figure 11) and in urine increased during exposure and up to approximately two hours after the MTBE and ETBE exposures (Studies II and III). In the ETBE-study, the average concentration of acetone in blood (and AUC) was highest at the 50 ppm exposure, but otherwise poorly related to the exposure levels. Acetone was detected in urine as well. The highest cumulative urinary excretion was seen at the 50 ppm

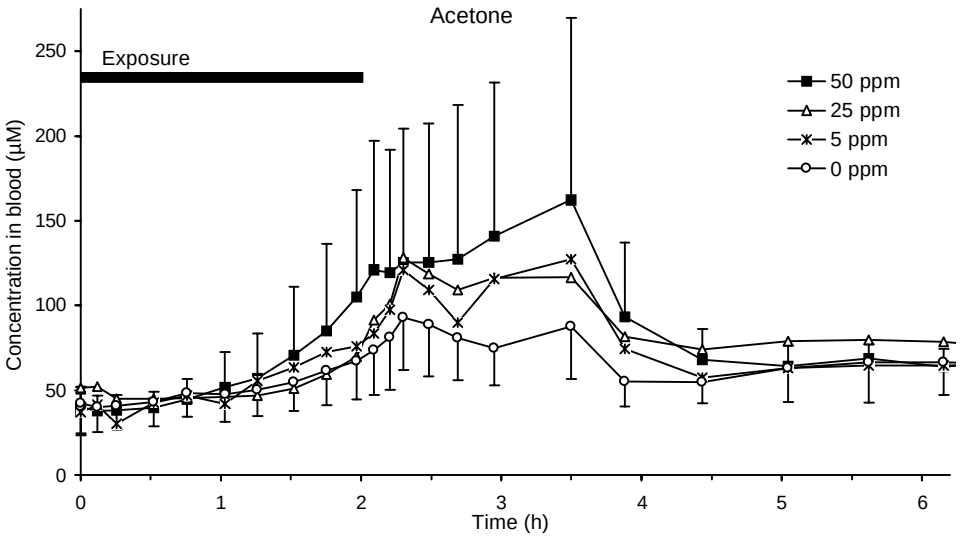


Figure 11. The concentration of acetone in blood after exposure to 0, 5, 25 and 50 ppm ETBE. Mean levels and 95% confidence interval are given for eight subjects.

exposure level and the lowest at the control exposure; however, apart from this there were no dose correlation of acetone. A wide inter individual variation in acetone levels in blood and in urine was seen before, during and after the exposure. This could probably be explained by the endogenous production of acetone, since the acetone levels may change naturally through the day due to e.g., physical exercise and food intake (86).

5.1.1 Whole-Body versus Face-Mask MTBE Exposure (Studies I and III)

The concentration curves for MTBE and TBA in blood after the face-mask exposure (Study III) were similar to those obtained in the whole-body exposure (Study I) (Figure 12). However, a somewhat higher dose was absorbed in the face-mask study. The slight differences between these studies could be related to e.g., the slight difference in analytical methods used in the two studies, continuos versus intermittent breathing via valves respective measurement of pulmonary ventilation, differences in exhalation based on breathing mask versus mouthpiece and nose-clip, weight gain of about 2 kg for the participants over the past 3 years and the breathing pattern. A statistically significant higher breathing frequency was seen in the whole-body exposure compared with the face-mask exposure study (Table 7). The subjects in the whole-body MTBE study were not used to mouthpiece breathing and started to hyperventilate during the four exhalation periods (5 minutes each) (Study I). During hyperventilation the alveolar ventilation decreases and the dead space increases, relatively speaking (181). In practice, this means that less MTBE was absorbed and the calculated absorbed dose in the whole-body exposure (Study I) was then probably underestimated since the estimations were based on the mouthpiece exhalations during exposure. However, it could be assumed that the volunteers had normal breathing frequency

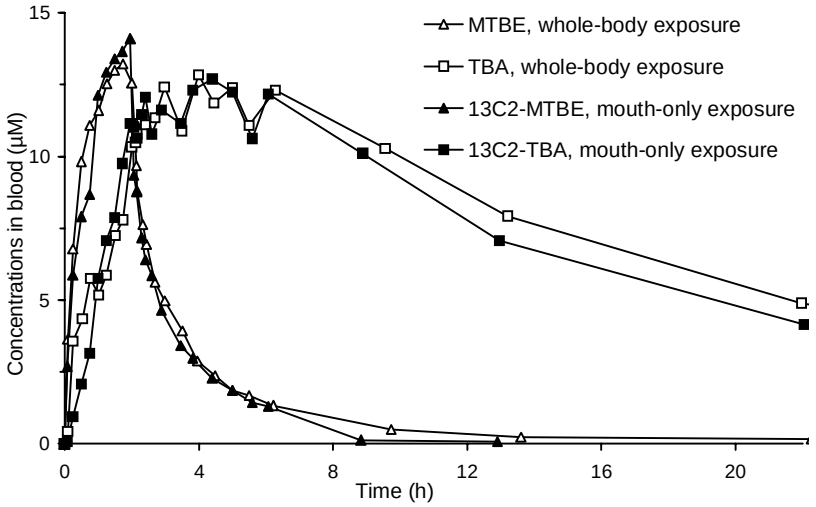


Figure 12. Comparison between the whole-body MTBE exposure (Study I) and face-mask [1,2-$^{13}C_2$]-MTBE exposure (Study III). Mean values of MTBE and TBA in blood from four subjects exposed to 50 ppm MTBE are given.

during the rest of the exposure, since the concentration curves of MTBE in blood match so well in the two different exposure studies (Figure 12).

The breathing frequency measured during the four exhalation periods (5 minutes each) in the ETBE study did not differ from the [1,2-$^{13}C_2$]-MTBE breathing frequency measured during the entire exposure, even though different subjects participated in the two studies.

In conclusion, the absence of major differences in the toxicokinetics implies that dermal uptake does not contribute significantly to the total uptake of MTBE during the whole-body exposure.

Table 7. Pulmonary ventilation and breathing frequency during and after whole-body exposure to 50 ppm MTBE and 50 ppm ETBE and face-mask exposure to 50 ppm [1,2-$^{13}C_2$]-MTBE are given (mean values ± 95% confidence interval). During the 2 h exposure the subjects performed physical exercise (50 W) on ergometer bicycles.

	MTBE (n=10)	ETBE (n=8)	[1,2-$^{13}C_2$]-MTBE (n=4)	MTBE [a] (n=4)
During exposure				
Pulmonary ventilation (l/min)	23 ± 3.3 [b]	24 ± 0.9 [b]	23 ± 2.4 [c]	25 ± 2.1 [b]
Breathing frequency (breaths/min)	22 ± 2.0 [b]	18 ± 1.2 [b]	18 ± 3.5 [c]	24 ± 3.5 [b]
After exposure				
Pulmonary ventilation (l/min)	12 ± 1.5	11 ± 0.7	12 ± 2.4	10 ± 1.3
Breathing frequency (breaths/min)	17 ± 2.2	15 ± 1.2	15 ± 2.4	19 ± 5.0

[a] Results from the same four subjects in the unlabeled MTBE exposure (Study I) as in the [1,2-$^{13}C_2$]-MTBE exposure (Study III).

[b] Average of approximately 20 min of exhalation via mouthpiece during whole-body exposure.

[c] Average of all exhalations during the entire 2-h face-mask exposure.

5.2 Urinary Metabolites after [1,2-$^{13}C_2$]-MTBE Exposure (Study III)

5.2.1 Characterization

In this study, signals from the ^{13}C-labeled portion of [1,2-$^{13}C_2$]-MTBE and derived metabolites should arise in doublet patterns (two adjacent ^{13}C-nuclei) enabling the distinction from endogenous compounds (singlet patterns). Chemical shifts for the ^{13}C-labeled portion of the urinary metabolites were consistent with the shifts obtained for spiked standards of HBA and MPD, see Figures 13 and 14. NMR signals were not detected for labeled MTBE, TBA, or possible MTBE-derived glucuronide conjugates, probably due to insufficient sensitivity.

In a previous study, rats were exposed for 6 h to 2000 ppm [2-^{13}C]-MTBE and a human consumed 400 mg of [2-^{13}C]-TBA (17). TBA-conjugates (glucuronide and sulfate) and an unknown metabolite in urine were attributed to [2-^{13}C]-MTBE. In the present study, a number of signals were observed in those spectral regions both in the control urine and in urine from MTBE exposed humans. These signals could not be defined as doublets. However, singlets in these regions could arise from [1,2-$^{13}C_2$]-MTBE metabolites that may possess only one labeled carbon, such as acetone and metabolites formed from acetone, e.g., 1,2-propanediol and lactate (Figure 2) or from endogenous compounds that are excreted at different concentrations throughout the day. Conjugates of e.g., TBA and MPD, on the other hand, should give rise to doublets in the present study, since they would contain two labeled carbons from [1,2-$^{13}C_2$]-MTBE. In the previous study (17), the administered MTBE or TBA was labeled only at the carbon 2-position, prohibiting the use of carbon-carbon coupling patterns to confirm that the assigned singlets were derived from labeled MTBE or TBA. In another previous study, conjugates from [1,2,3-$^{13}C_3$]-TAME have been assigned in urine obtained from rats and mice administered (152). In the present study, it was possible that MTBE-derived glucuronide and sulfate conjugates did exist in the urine but below the detection limit of the NMR method. In addition, given that the ratio of HBA:MPD increases with time after termination of exposure, it was also possible that conjugates would form to a greater extent after 20 h post exposure, which was the time point when the last urine sample was taken.

5.2.2 Quantification

Quantification of MPD and HBA was performed by NMR in two urine samples (collected 20 h post exposure from two volunteers) that had sufficient amounts of metabolites to quantify. Previously only one metabolite, TBA, has been identified in human urine and with a low recovery (<1%) (Study I). In study III, the two urine samples contained 1% MPD (0.12 mM) and 11% HBA (0.96 mM) (urine diluted in D_2O) or 1% MPD (0.06 mM) and 3% HBA (0.17 mM) (concentrated urine after protein precipitation). The percentage values are expressed as percentage of MTBE uptake. This indicates that HBA and MPD occur at markedly higher levels in the urine (detected by NMR) than MTBE and TBA

(detected by GC). Hitherto, this is the first characterization of MTBE metabolites, other than TBA, in humans.

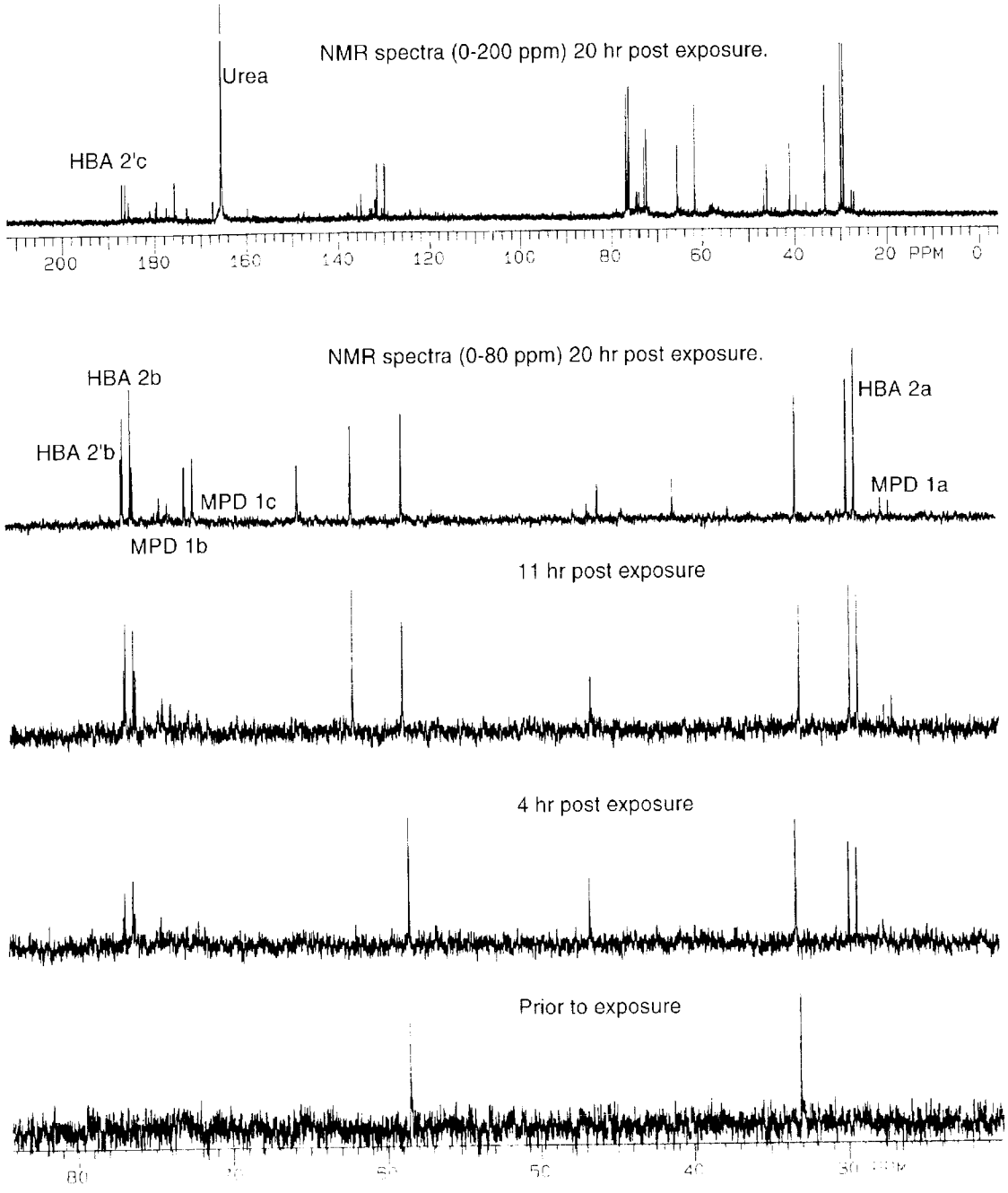


Figure 13. ^{1}H-decoupled ^{13}C NMR spectra of urine from one male volunteer following a 2 h exposure to 50 ppm [1,2-$^{13}C_2$]-MTBE. The top spectrum was obtained from concentrated urine, after protein precipitation, collected 20 h post exposure. The four spectra below were obtained from urine diluted in D_2O and the urine was collected prior to exposure and 4 h, 11 h and 20 h post exposure.

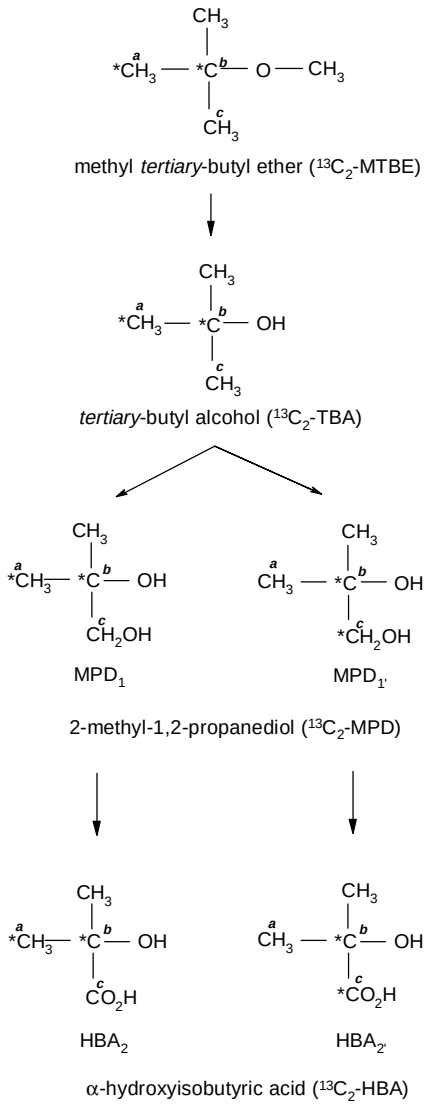


Figure 14. Metabolites of [1,2-$^{13}C_2$]-MTBE shown in study III (*; ^{13}C-labeled carbons).

5.3 Partition Coefficients (Study IV)

The partition coefficients illustrate that MTBE, ETBE and TAME have higher affinity to adipose tissue than to blood, as do many other solvents. Further, the partition coefficients implies that ETBE would have the lowest respiratory uptake of these ethers and that TAME might have longer half-times since it accumulates for a longer time in adipose tissue. The low oil/water and oil/blood values for TBA indicate that TBA was preferentially distributed in the body water. Average values for measured liquid/air partition coefficients and calculated tissue/blood partition coefficients are given in Table 8. The present partition coefficients agree rather well with partition coefficients for MTBE obtained from blood and tissues in rats (25, 133), from oil/nitrogen (32), and from a recent study (79) of human

Table 8. Measured liquid/air partition coefficients (Study IV) of oxygenates and a metabolite in blood, water[a] and oil (average values of 25 samples are given) and in addition, calculated tissue/blood partition coefficients are given.

	Measured			Calculated[b]				
Partition coefficients	blood / air	water [a] / air	oil / air	water [a] / blood	fat / blood	liver / blood	muscle / blood	rpt [c] / blood
MTBE	18	15	120	0.86	5.3	1.1	1.1	1.4
ETBE	12	8.4	190	0.72	12	1.7	1.7	2.3
TAME	18	12	340	0.66	14	1.8	1.9	2.6
TBA	460	600	170	1.3	0.57	1.0	1.0	1.0

[a] Strictly physiological saline

[b] Calculated from tissue composition (percentage water and oil content) (12, 58, 131) and measured liquid/air partition coefficients *in vitro* (Study IV).

[c] Partition coefficient of the rapidly perfused tissues/blood (rpt/blood) calculated from tissue composition (percentage water and oil content) of the brain (58) and measured liquid/air partition coefficients *in vitro* (Study IV).

blood/air, urine/air, saline/air and oil/air partition coefficients (λ: 20, 16, 15, and 140, respectively).

Further, inter individual variation (CV) of the $\lambda_{blood/air}$ (10 subjects) was calculated and estimated to 14% for MTBE, 20% for ETBE, 20% for TAME and 30% for TBA. These coefficients of variation are in agreement with previously reported CV of other compounds (7.3, 13, 16 and 16% respectively for acetone, 1,1,1-trichloroethane, toluene and styrene) determined from 73 subjects (20 analyses per individual) (49). In the present study as well as in the previously mentioned study, sex was not seen to be a significant grouping variable for the individual blood/air partition coefficients.

5.4 Physiologically Based Toxicokinetic Modeling (Study V)

The model could adequately describe the experimental data (Figure 15). The sensitivity of the model towards different parameters was studied by sensitivity analysis. The parameters that influenced the levels of ETBE in blood and exhaled air were mainly alveolar ventilation, the blood/air partition coefficient of ETBE, and parameters associated with fat, such as body weight. The sensitivity analysis indicated that parameters having the largest influence on TBA levels in blood and exhaled air were associated with the liver, the blood/air and liver/blood partition coefficients for ETBE and TBA. Further, the sensitivity coefficients illustrate that parameters have a complex time-dependent behavior.

Predicted levels in venous blood and in fat tissue after two weeks of exposure at 50 ppm ETBE (8 h a day for 5 consecutive days) are shown in Figure 16. Only slightly increased biomarker levels (in blood, exhaled air and urine) were seen at the end of the workshift and the following morning (day 2 to day 5). ETBE, and to a lesser degree TBA, accumulated slightly in fat tissue after weeks of exposure

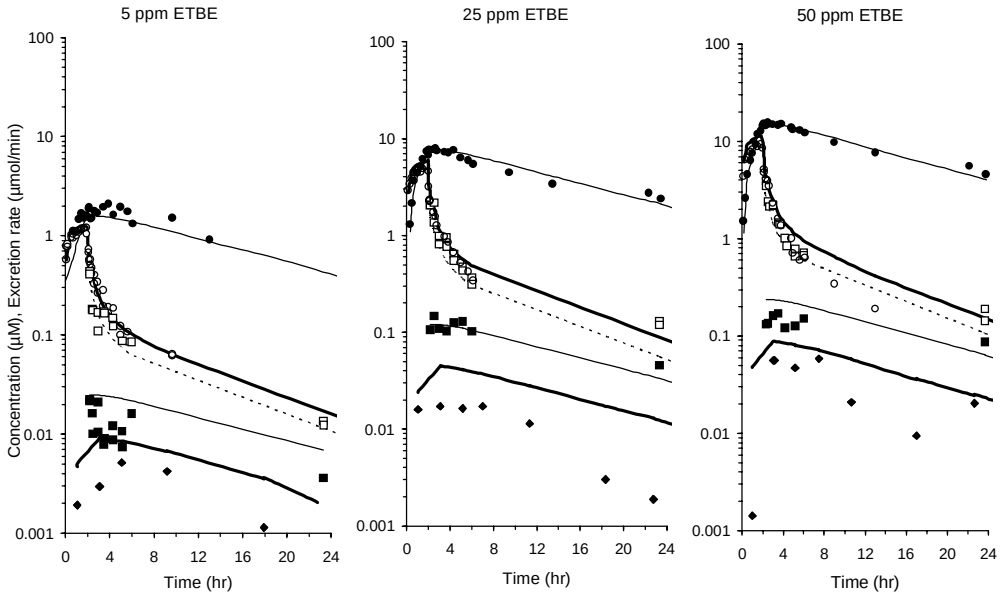


Figure 15. Experimental (dots) (Study II) and predicted values (lines) for one individual after exposure to 5, 25 and 50 ppm ETBE for 2 h at 50 W. Symbols; ○ - ETBE in blood (µM), □ - ETBE in exhaled air (µmol/min), ● - TBA in blood (µM), ■ - TBA in exhaled air (µmol/min), and ◆ - urinary excretion rate of TBA (µmol/min).

at 50 ppm ETBE. According to the model, workload (0 to 100 W) increases all biomarker levels by approximately 2-fold at the end of the workshift, and by 3-fold the next morning. This illustrates that biological exposure monitoring is very important, because the internal exposure might be strongly underestimated by air monitoring if physically demanding work tasks are performed. The effect of workload depends on the blood/air partition coefficients, alveolar ventilation, cardiac output and distribution in a complex manner.

Table 9. Results from 500 Monte Carlo simulations of random fluctuating exposure to 50 ppm ETBE at 50W. *Scenario 1* illustrates continuous ETBE emission with rapid air exchange and *scenario 2* intermittent emission with slow air exchange. The coefficients of variation (CV) in biomarker levels are given for ETBE and its metabolite, TBA, at the end of an 8 h shift and the next morning prior to next shift (16 h post exposure).

	ETBE		TBA			
CV (%)	Venous blood	Exhaled air	Venous blood	Exhaled air	Urine[a]	Urine[b]
Scenario 1						
End of workshift	4.4	17	0.8	0.7	1.9	0.7
Next morning	1.1	1.1	1.0	1.0	1.6	1.0
Scenario 2						
End of workshift	44	84	6.8	7.3	7.4	7.2
Next morning	13	13	11	11	12	11

[a] variability in urinary excretion rate levels

[b] variability in urinary concentration levels

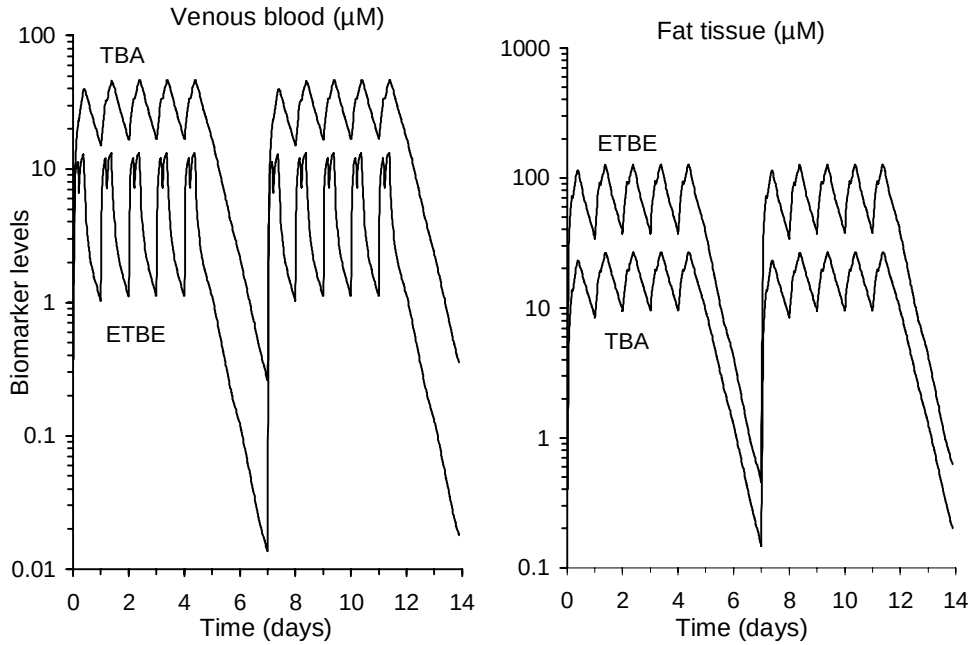


Figure 16. Predicted levels of ETBE and TBA in blood, exhaled air, urine and fat tissue after two weeks (8 h/day, 8 AM to noon and 1 PM to 5 PM, 5 consecutive days) of exposure to 50 ppm ETBE and at a workload of 50W. The workload was set to zero at night (11 PM to 7 AM), whereas light physical activity (25 W) was assumed for the remaining time.

Fluctuations in an 8 h exposure were estimated with 500 Monte Carlo simulations. These simulations shows that ETBE in blood and exhaled air at the end of the workshift was highly sensitive to exposure fluctuations, whereas ETBE next morning and TBA in general was less sensitive to fluctuations (Table 9).

Predicted inter individual variability (eight subjects) in biomarker levels is higher the next morning than at the end of the workshift, and higher for TBA than for ETBE (Table 10). Further, a rather high variability of biomarker levels was seen on Monday morning after one working week of exposure (37-48% for ETBE and 49-58% for TBA).

Table 10. The variability (coefficient of variation, CV) between eight individuals simulated levels of ETBE and TBA in blood, exhaled air and urine after exposure to 50 ppm ETBE (50 W) 8 h daily for five consecutive days (end of workshift = average CV Monday to Friday; next morning = average CV Tuesday to Saturday).

	ETBE		TBA			
CV (%)	Venous blood	Exhaled air	Venous blood	Exhaled air	Urine[a]	Urine[b]
End of shift	7.1	4.2	9.6	8.5	44	9.5
Next morning	7.6	9.1	20	20	39	21

[a] variability in urinary excretion rate levels

[b] variability in urinary concentration levels

5.5 Acute Effects after MTBE and ETBE Exposures (Studies VI and VII)

The *ratings* of solvent smell increased dramatically in the beginning of the exposure and then gradually decreased during exposure (MTBE and ETBE). Further, the solvent smell ratings were related to exposure levels, illustrating the potential of the questionnaire to pick up feelings of discomfort. No significant ratings (except solvent smell) were seen regarding the other questions in the MTBE study. However, in the ETBE study, significantly elevated ratings of discomfort in throat and airways were seen at 50 ppm ETBE compared to the clean air level. Further, the average ratings for the other questions corresponded verbally to something between “not at all” and “hardly at all”, i.e. in the range of 0-10 mm on the visual analog scale. The questionnaire was elaborated for solvent exposure and has been used in several similar inhalation studies at our laboratory (56, 82, 88). The scale was originally developed and validated for measuring annoyance from noise (95).

All *eye* measurements were negative after the MTBE exposure and in the majority of the ocular tests in the ETBE study. But an increase in blinking frequency, from on average 10 blinks per min before exposure to 14 blinks per min during exposure was seen in the ETBE study. Still, this effect did not correlate with exposure levels.

Nasal swelling, expressed as a 6-15% decrease in the nasal volume measured by acoustic rhinometry, was observed at all exposure levels in the ETBE study. A nasal swelling effect was also seen in the MTBE study. However, neither in the ETBE nor in the MTBE study did this effect correlate to exposure levels and no significant increases in cell count or in biochemical inflammatory markers were seen in the nasal lavage fluid.

Since the nasal swelling and the blinking frequency did not correlate with the exposure level, these effects may be related to other factors than ETBE. Plausible explanations might be e.g. bicycle exercise, possible daily variation or slightly different climate in the chamber.

Pulmonary effects were measured in the ETBE study but not in the MTBE study. Impaired lung function, expressed as reductions in vital capacity and forced vital capacity (significant), FEV_1 (not significant) and TLco (of borderline significance), was noted at the two highest exposure levels (25 and 50 ppm) compared with the lower ones (0 and 5 ppm). These impairments seemed to fall within the normal inter and intra individual variability and presumably have no clinical relevance as such. However, it cannot be excluded that other subjects, maybe more sensitive ones, may react more strongly or that a longer exposure duration might give a more pronounced effect.

The acute effects in volunteers after controlled experimental exposure to MTBE and ETBE are summarized in Table 11.

Table 11. Comparison of acute effects in man after controlled experimental exposure (2 h, 50 W) to MTBE (n=10) and ETBE (n=8).

Effect measure	MTBE	ETBE
Effect versus exposure level and time		
Rating of smell	↑	↑
Rating of discomfort in throat and airways	↔	↑
Vital capacity	nd	↓
Forced vital capacity	nd	↓
Effect versus time		
FEV_1 (forced expiratory volume in 1 sec)	nd	↓
TLco (transfer factor of diffusing capacity)	nd	↓
Blinking frequency	↔	↑
Nasal swelling	↑	↑
Albumin in nasal lavage	↓	↓
Myeloperoxidase in nasal lavage	↓	↔
Interleukin 8 in nasal lavage	nd	↓

↑ : Increased significantly ($p<0.05$, repeated measures ANOVA)

↓ : Decreased significantly ($p<0.05$, repeated measures ANOVA)

↔ : No significant effect ($p>0.05$, repeated measures ANOVA)

nd: Not determined

6. General Discussion

6.1 Measures of Exposure

Paracelsus pointed out half a millennium ago: *"All substances are poisons, there is none which is not a poison. The right dose differentiates a poison from a remedy"* (4). In toxicology, it is essential to establish the dose-response (the proportion of a population showing a specified change) and dose-effect (the magnitude of change in a parameter).

A dose, or the amount of xenobiotics entering the body, can be expressed as exposure dose (in the case the concentration measured in air), absorbed dose (internal dose or body burden), tissue dose or target tissue dose (the dose in tissue relevant for the adverse effect). Air concentration is measured by air monitoring, and body burden by biological monitoring. Tissue doses in humans are usually predicted, e.g. using a PBTK model.

6.1.1 Biological monitoring

The definition of biological exposure monitoring in the occupational health field is "the measurement and assessment of workplace agents or their metabolites either in tissues, secreta, excreta, expired air or any combination of these to evaluate exposure and health risk compared with an appropriate reference" (172). The chemical, or a metabolite, is usually assayed from blood, urine and exhaled air. Saliva, fat, milk, hair, nails or tissue biopsies are more rarely used.

Urine samples are the most commonly used body fluid in biological exposure monitoring mainly because of the practical and non-invasive sampling technique. When a metabolite is monitored, urine levels are much less influenced by peak exposures than the concentration of the unchanged chemical in blood or exhaled air. The time of urine collection and the urine output are the most influential factors for the urine concentration. Urine output changes with e.g., water intake, surrounding temperature, humidity and physical activity. However, the urinary output is not influencing that much if the urinary excretion rate of a chemical is reported, instead of the concentration. In order to exclude over-diluted or over-concentrated samples it is sometimes advisable to relate the urine concentration to the endogenous urinary creatinine levels or urine density (22, 72). In controlled experimental exposures, as in the present thesis, all urine produced during one day is usually collected. Therefore, the urinary excretion rate can be calculated and the correction for dilution is not necessary.

Blood samples are primarily used when the exposure is too low for a detectable amount of chemical or metabolite in urine analysis. The determination of unchanged chemical in blood usually has higher specificity compared to that of a metabolite which may originate from several other substances. The concentration

in blood or alveolar air frequently has the same significance, i.e. it reflects the most recent exposure (sampling during or at the end of the workshift) or the integrated exposure (sampling next morning).

In the present studies, capillary blood samples were analyzed. Capillary blood mainly represents arterial blood. A direct comparison to venous blood levels is not possible, since the pulmonary uptake or wash-out of volatile chemicals creates a difference between arterial and venous blood. Further, sampling venous blood from body parts that have been in direct contact with a chemical may result in inaccurate concentrations because of dermal absorption. In the present studies the subjects washed their hand in water before we took a capillary blood sample.

For levels in *exhaled air*, the most important factor is the affinity to blood - the blood/air partition coefficient. A chemical less soluble in blood will show higher concentration in exhaled air (lower uptake). In general, chemicals that are less soluble in blood and/or are eliminated mainly unchanged in the lung, are the most suitable for use as biomarkers in exhaled air. These criteria are met by the parent ethers in this study. The time of sampling exhaled air is very critical. Exhaled air can be sampled as mixed-exhaled air or as end-exhaled air. The former represents the gas mixture displaced from the dead space and from alveoli. Mixed-exhaled air was collected in the present exposure studies.

When a monitoring method is based on the determination of a chemical or its metabolite in biological media, it is essential to know the *toxicokinetics* (further discussed in Section 6.2), i.e. how the substance is absorbed (lung, gastrointestinal tract and skin), distributed to different compartments in the body, biotransformed and finally excreted. The rate of uptake depends on the affinity of a chemical for the tissues and on the tissue perfusion rate. For many chemicals, the concentration in alveolar air and in blood increases rapidly during exposure, after which the chemical is eliminated very quickly, as illustrated in Figures 9 and 12 for the unmetabolized ethers. It is also important to know if the chemical will accumulate in the body. For example, levels of TBA in blood and of the parent ethers in e.g. adipose tissue, build up slowly and are eliminated at a lower rate. If the exposure is intermittent, measurements of the concentrations in blood and exhaled air are of little value during exposure, whereas at the end of exposure, smooth elimination curves are observed. The different kinetic aspects of the unchanged compound and the metabolite must be considered when choosing the *time of sampling*. If the biological half-time is not too short (> 2 h) (172), the sampling after the work shift or next morning may provide a reliable picture of the exposure during the preceding shift. When the half-time is so short that the timing cannot be controlled in field conditions, biological monitoring is unsuitable (59). This is usually the case when monitoring solvents in exhaled air collected during the first hour post exposure (exemplified by the present ethers). Further, some substances may have several half-times corresponding to the elimination from different organs or tissues, as illustrated with the unmetabolized ethers. Other chemicals or metabolites have a relatively slow monoexponential decay and, hence, the time of sampling is then less crucial, as illustrated for TBA in blood and urine. Further,

the concentration of the substance is of importance, since higher levels often mean less analytical variability and make the detection limit of the method less important. Furthermore, the volatility is important, since volatile substances are sensitive to sampling procedures. If the concentration the next morning has not returned to the pre-shift level, a progressive accumulation during a week of exposure is to be expected and the longer the half-time, the larger the contribution of previous exposure (59).

6.1.2 Complexities

Several *inter individual variables* affect the uptake and metabolism of a chemical, e.g. (4, 72, 106, 144, 169):

- pulmonary ventilation: respiratory rate, fitness and exercise,
- body composition: obese - lean or pregnant,
- work practices: route of exposure, fluctuations of exposure intensity, coexposure to other chemicals, temperature and humidity during exposure,
- partitioning between air/blood and blood/fat,
- addiction / aversion to the solvent,
- inter individual variation in metabolic clearance rates: genetic variability in biotransformation including genetic polymorphism,
- age, sex and ethnicity,
- confounding factors due to individual life style e.g., drugs, disease, alcohol, smoking, diet, after work activities and exposure, personal hygiene, working and eating habits.

A better understanding of individual factors is needed to improve risk assessments and biological monitoring.

In the experimental exposure studies in the present thesis, some affecting factors have been standardized. For example, the volunteers were all healthy Caucasian males (20-50 years) and they had to refrain from alcohol and drugs before and during the exposures. Further, the dose, route of exposure, workload and the climate were standardized and the pulmonary ventilation was measured. Diet, genetic variability or variation in partition coefficients are other factors that may vary between subjects and this was not accounted for in the present controlled exposures. Not all of these factors can be controlled in fields studies and, e.g., an increased physical activity will most probably result in an increased body burden as illustrated earlier in the PBTK predictions (section 5.4). The effect of exercise is more pronounced for highly blood-soluble compounds (58). Further, if an exposure period is short, the results may not be representative for exposure during longer periods due to saturable metabolism, cofactor depletion, enzyme induction or inhibition.

6.1.3 Ethers and their Metabolites as Biomarkers

There may be both advantages and disadvantages to use the unmetabolized ethers and TBA, respectively, as biological exposure markers for gasoline vapor. For example, TBA may be a more suitable biomarker compared to the ethers for the following practical and toxicokinetic reasons:

- TBA is cleared more slowly from the body compared to the parent ether, i.e. timing is not crucial for TBA. In blood, TBA has a plateau several hours post exposure and thereafter one linear single elimination phase was seen in blood and urine (MTBE and ETBE have several elimination phases) (Studies I and II).
- TBA is found to be less sensitive to fluctuations in the exposure air levels compared to the parent ether, especially at the end of the workshift (Study V).
- TBA has a higher recovery in the urine compared to unmetabolized ethers (Studies I and II), i.e. less analytical limitations.
- TBA is less volatile, i.e. less sensitive to sample losses, than the parent ethers.

For reasons discussed previously (section 6.1.1) sampling of urine is preferable to sampling of blood or exhaled air. In the PBTK predictions using ETBE data from 8 subjects (Table 10) a higher variability is seen for TBA in urine compared with TBA in blood or exhaled air (Study V). However, this is seen only for the urinary excretion rate and not the urinary concentration of TBA (Figure 17 and Table 10). Therefore the urinary concentration of TBA is most likely preferred to excretion rate in biological monitoring. For other small molecules e.g., acetone and methanol ACGIH has recommended to measure urinary concentration (5). In the present studies, data on the unmetabolized ether and TBA have been analyzed and used in the PBTK predictions. Two other urinary metabolites have recently been characterized and excreted in higher amounts than TBA (Study III). These two metabolites, HBA and MPD, may be even more suitable as biomarkers for the oxygenates and gasoline vapor.

A potential drawback against the use of TBA (or metabolites of TBA) as a biomarker of ether and gasoline is that TBA itself might also be present in the environment (154). First, TBA may be present in commercial oxygenate liquid as was the case in the ETBE study (0.4% TBA). Second, TBA may be added as an

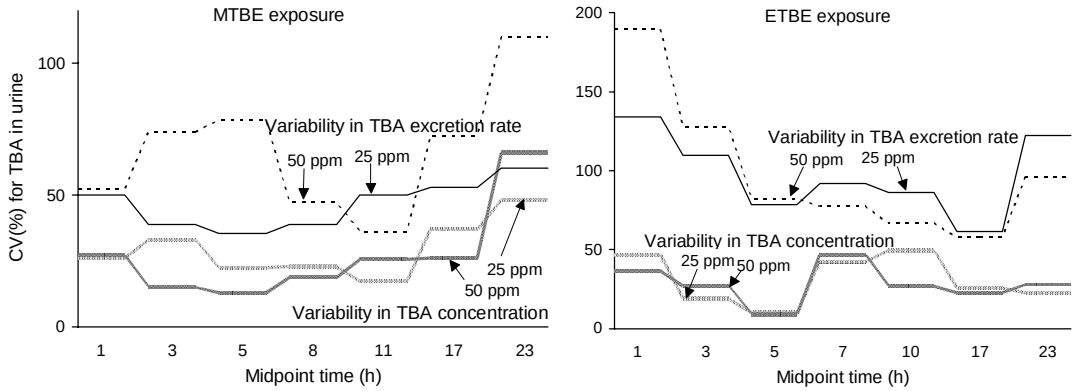


Figure 17. Average coefficient of variation (CV) for TBA in urine is given for urinary concentration (µM) and excretion rate (µmol/h) after 50 ppm and 25 ppm MTBE (n=10) and ETBE (n=8) exposures.

oxygenate to gasoline. A third possibility is that TBA is used for other purposes, e.g., as an industrial solvent (dehydrating agent, denaturant in ethanol and other alcohols, and in extraction processes) (143, 154).

If there is reason to suspect exposure to TBA then the unmetabolized ethers may be considered as biomarkers. Venous blood samples are preferable to urine and exhaled air samples. This is because the low urinary recovery, risk of losses due to the volatile ether, and that e.g. physical activity increases the excretion of the ethers through the exhaled air. The timing is crucial for the unchanged ethers due to the fast decay and the multiple elimination phases. The PBTK simulations of ETBE (Study V) illustrates that it is more appropriate i.e. lower variability (Table 9), to measure the unmetabolized ether the following morning prior to the next shift compared to at the end of the workshift. The former alternative is more insensitive to timing, but may suffer from e.g., analytical limitations.

6.2 Toxicokinetics

Three main factors determine the uptake, distribution and elimination of inhaled vapors and gases:

- the rate by which the vapor is transferred from the environment to the tissue and vice versa (mainly affected by cardiac output, blood/air partition coefficients, alveolar ventilation),
- the capacity of the body to retain the inhaled substance and
- excretion and metabolism.

The *uptake* (absorption) in occupational settings is usually via inhalation or through the skin and large surface areas are exposed in both cases.

In the two present MTBE studies, the same subjects were exposed to MTBE vapor both whole-body (Study I) and via face-mask (Study III). Theoretically, both respiratory and dermal absorption might take place in Study I. However, after the face-mask study we could conclude that due to the absence of major differences in the toxicokinetics, it is plausible that dermal uptake does not contribute significantly to the total uptake during whole-body exposure to MTBE vapor.

The *solubility* of compounds plays an essential role in the uptake, distribution and elimination. The *in vitro* determined blood/air partition coefficients reflect both the solubility and the binding to the red blood cell (hemoglobin) and plasma proteins (97). The blood/air partition coefficients for the oxygenates and TBA were determined in a wide concentration range and no saturable binding was seen.

Xenobiotics are *distributed* via the blood circulation from the site of absorption to various tissues. The uptake rate of a compound may be limited either by diffusion or blood flow (perfusion). If the diffusion of a chemical across membranes is slow, the rate of entry into the tissues is limited. Conversely, if diffusion across membranes is rapid, the rate of entry is limited by the rate of delivery, i.e. perfusion rate limitation. Highly water soluble compounds (such as

phase II conjugates) are generally diffusion rate limited, whereas blood flow limitation applies more often to lipid-soluble compounds in slowly perfused systems such as adipose tissues. Small molecules like the ethers are assumed to diffuse rapidly across the membranes and blood flow may be the rate limiting step. Hence, the PBTK model was perfusion-limited (Study V).

The main *elimination* processes by which the circulating levels of a compound are irreversibly reduced are biotransformation (metabolism) and excretion. Generally, biotransformation leads to generation of hydrophilic substances that are readily excreted. The elimination is usually in urine, but excretion may also be e.g. via the lungs or feces. Metabolism usually results in a detoxification of the compound, but the reverse may also happen and more reactive intermediates may be formed.

For the ethers dealt with here it is not known whether it is the unmetabolized ethers or metabolites, such as formaldehyde, acetaldehyde, TBA or other metabolites, that make the oxygenates “toxic” in animals at high levels. For substances lacking functional groups, like MTBE or ETBE, an initial oxidation step (phase I) is required before conjugation (phase II) to more water soluble products can occur. MTBE activates P450 isoenzymes (phase I metabolism) and the UDP-glucuronosyltransferase (phase II - conjugates) in liver microsomes from both man and rats (26, 74, 147, 158), and it has been shown that extrahepatic metabolism occurs in microsomes obtained from rats (73). In the present studies low levels of TBA were found in the exhaled air after the ETBE and [1,2-$^{13}C_2$]-MTBE exposures. Pekari *et al.* (130) have also reported negligible amounts of TBA in the exhaled air after MTBE exposure.

Metabolic clearance of MTBE and ETBE was moderate and presumably limited by both perfusion and enzymatic capacity. No steady state was achieved after these 2-h exposures. This could be explained by the relatively high blood/air partition coefficient and the low metabolic rate. The latter in combination with the large blood flow through the lungs explains the relative importance of the exhalation as elimination route for the ethers. For MTBE (Study I), however, the levels in exhaled air may be somewhat overestimated and consequently the adsorbed dose underestimated due to hyperventilation during exposure (section 5.1.1).

6.3 Modeling of Kinetic Data

PBTK models have an essential advantage compared to traditional computer models, since the aim of PBTK models is to describe events in the entire body. However, like all models, PBTK models are simplifications of reality that help us to predict the elapse of a chemical in tissues in the body.

In the present PBTK model (Study V), the partition coefficients used are group averages and not individual estimates, since it has been shown that most subjects can be represented by group averages (49). Body build (described by body height

and body weight) influenced the tissue volumes and the blood flow used in the PBTK model (52) and, in addition, the blood flow was influenced by physical exercise. Individual exposure duration, workload, body weight and body height were used as individual input values in the model. Two muscle compartments, one resting and one working, were used in the model in order to account for physical exercise (bicycling, increasing blood flow in the legs) during exposure (Study II) and indeed the model had an overall good fit to the experimentally collected data. Further, workload was set to 25 W post exposure instead of the commonly used 0 W. The former is probably a more correct estimate of physical exercise during the leisure time.

Linear kinetics was seen up to 50 ppm MTBE (Study I) (75 ppm MTBE in another study (130)), up to 50 ppm ETBE (Study II) in humans and in addition, up to 300 ppm MTBE in animals (147). Deviations from linearity have been seen at high doses in animals, e.g. at 8000 ppm or 400 mg/kg MTBE (116) and at 5000 ppm ETBE (23). Linear kinetics was assumed in the present PBTK model, since no saturation has been seen up to 50 ppm ETBE in humans. This exposure level is very high compared to observed occupational and non-occupational levels of MTBE (see Table 2) (64, 69). This makes the assumption of first order kinetics plausible, and the model should be adequate for most exposure situations.

Further, in this PBTK model, *metabolism* was postulated to exist only in the liver. This is probably a simplification, since TBA has been measured *in vitro* in extrahepatic microsomes obtained from rats (73). In the PBTK model, TBA was excreted both from the lungs and via the urine. The uptake of TBA was due to the contamination of the ETBE liquid (0.4% TBA) in the experimental study and also to the fact that the metabolite TBA was probably excreted through the lungs too. The latter we found plausible since the pulmonary excretion of TBA was higher than the TBA exposure levels (Study II).

6.4 Acute Effects

With the battery of tests for acute effects in Studies VI and VII we tried to examine acute effects, since complaints (e.g. headache, irritation and nausea) appear to have emerged after the addition of MTBE to gasoline (described in section 3.5) (69, 120, 166).

6.4.1 Oxygenates

The chosen acute effect test battery has been used in other MTBE exposure studies (33, 135) and in previous chamber exposures at our department (44, 56, 82). Although some of the end points in the acute effect battery have not been thoroughly validated, the tests have nonetheless been used and effects have been seen in several field and experimental studies (60, 93, 96, 125, 129, 162, 180).

Statistically significant increases in *ratings* of smell were seen during and after the exposures, and in addition the ratings correlated well to exposure levels. In the

MTBE study (Study VI) no question, except smell, showed any significant elevated ratings, whereas significantly elevated ratings of discomfort in throat and airways were seen after the 50 ppm ETBE exposure compared to the control exposure. Most of the remaining questions in the ETBE study were in average rated highest at the 50 ppm ETBE exposure, but no such trend was seen for MTBE. The ratings were in generally low, nevertheless, single subjects might have rated up to "somewhat" (30% of the scale) at some occasion during the 50 ppm exposure. Further, after the two highest ETBE exposures, some of the participants reported a bad taste in their mouth and the taste lasted for more than one day post exposure. This was not mentioned or noticed after the MTBE exposures, but it is possible that it have been oversighted in the previous study. Nevertheless, this finding may partly be explained by the more annoying smell of ETBE, the lower odor detection threshold for ETBE (69, 160), and of the smell of possible exhaled metabolites or contaminants.

Nasal swelling, e.g., is a commonly reported symptom during exposure to airborne substances. Nasal swelling has been previously seen in woodworking teachers exposed mainly to wood dust (180) and in healthy volunteers exposed to volatile organic compounds (125). Further, the biochemical markers analyzed in nasal lavage are indicators of early nasal inflammation. Increasing levels of albumin and eosinophilic cationic protein have previously been seen after formaldehyde, nitrogen dioxide and ozone exposure (96, 129, 162). In a recent study, a correlation between nasal swelling (measured by acoustic rhinometry) and biomarkers in nasal lavage (ECP and lysozyme) has been seen in personnel working in schools with a low air exchange rate (175). In the present studies (Studies VI and VII), nasal swelling was indeed seen; however, it seems unlikely that the effect is related to the ether exposure since there was no dose-effect correlation. Other exposure-related factors, such as bicycling, or climate change or daily variation may have influenced the observed effect.

Ocular measurements have previously given positive findings, e.g., eye redness is found among tobacco workers (93) and reduced break-up time and increased Lissamine green staining is seen in office workers due to indoor climate problems (60). In the present studies, no ocular effects were seen after the MTBE or ETBE exposures apart from an increased blinking frequency (without a dose-effect relationship) that was seen after the ETBE exposures.

Impaired *lung* function was noted at the two highest ETBE exposure levels (25 and 50 ppm) compared to the control exposure, suggesting an ETBE-related effect in these healthy volunteers. Several different pulmonary measures point in the same direction and a small but significant impairment is shown in Study VII. Still, it may be the case that in sensitive individuals, a prolonged or repeated exposure to ETBE could cause a more pronounced effect. Unfortunately, pulmonary function was not assessed in the MTBE study; therefore comparisons between the two ethers cannot be made in this respect.

6.4.2 Gasoline

The acute health effects reported after exposure to gasoline containing MTBE, are in most cases similar to the effects of exposure to other constituents of gasoline and emissions from motor vehicles (35, 139). Irritation in the eyes and respiratory tract gives the first signs of toxic effects of gasoline, whereas higher concentrations affect the central nervous system and e.g., headache, nausea, dizziness and ataxia have been reported (168). Associations between gasoline exposure and renal and liver cancer (seen in some studies and not in others (80)), acute myeloid leukemia (101), changes in CNS (31), skin alterations and modifications of the mucous membranes (105) have been reported in some epidemiological studies (35).

Several decades ago, volunteers were experimentally exposed to gasoline vapor (51). In an 8 h controlled exposure study, thirteen men were exposed to up to 270 ppm and eight women to up to 160 ppm gasoline vapor. The most distinctive symptoms were irritation of the eyes and the throat. Further, after exposure for up to five minutes at higher concentrations (up to 11000 ppm) the subjects felt intoxicated (dizziness, incoordination and drunkenness), i.e. CNS symptoms. The authors nonetheless concluded that commercial gasoline - as formulated in 1943 - was an “innocuous substance and that it is difficult to tell whether the gasoline smells at 500 ppm or 2000 ppm”. In another experimental study, dated 1960, volunteers were exposed in a chamber to up to 1000 ppm gasoline vapor for 30 min (46). Eye irritation was the only significant effect seen both by ratings and by the change in the pattern of conjunctival blood vessels as determined from photographs.

6.4.3 Possible Sources of Error

“As scientists it is our duty to maximize the amount of relevant information gathered from volunteer studies” (40). In the present studies, to achieve this aim, the acute effects were measured during the same exposure session as the toxicokinetic variables. This limits the number of exposure occasions and indeed the number of subjects, as only two subjects at a time can be exposed if samples of blood and exhaled air are to be taken. Therefore, the number of experimental subjects was by necessity few and this unfortunately reduces the power of the acute effect study. Further, the subjects were a homogenous group of healthy white men. There was a possibility of further selection bias in the volunteer recruitment as sensitive individuals may avoid participating because of previous negative experiences with volatile chemicals or odors in general. Since we seek volunteers by advertising, the magnitude of this possible self-selection bias is unknown. Hence, oxygenate-related effects may have escaped unnoticed in the present study because of the small group, exclusion of sensitive individuals in the sample population and the relatively short exposure duration. On the other hand, the exposure conditions used (up to 50 ppm) are at least one order of magnitude higher than expected environmental exposure levels for the general public.

To compare the acute effects of two chemicals such as ETBE and MTBE it would obviously be best to expose the same volunteers in a randomized blinded trial. However, as two years passed between these two studies and because the experiments are rather extensive and time-consuming for the volunteers, we judged it difficult to successfully recruit all 10 subjects for a second exposure series. Instead we chose to recruit new volunteers for the ETBE study, excluding those that had already participated in the MTBE study.

Further confounding factors in acute effect studies in general are e.g., air pollution and pollen. In the present studies, at least pollen was not a matter of concern, since these studies were performed during the autumn and winter seasons (September through March). However, other forms of air pollution could confound these kinds of studies.

Physical exercise may confound the battery of tests for acute effects, especially the pulmonary function test (92). However in the ETBE study it is clear that the exposure and not the physical activity gave the minor lung function impairments, since these effects were not seen during the control exposure.

In occupational settings people are often exposed to a mixture of substances and gasoline is indeed a complex mixture. This may entail variations in terms of both toxicokinetics and acute effects. Any single chemical could have independent, additive, synergistic, antagonistic or potentiating effects when combined with any other (172). Chemical interactions between the oxygenates and other components of gasoline have hitherto not been considered in these or other exposure studies (33, 130, 135, 140), since volunteers were exposed to the oxygenates alone. The acute health complaints seen after environmental exposure to gasoline containing MTBE may perhaps be explained by a combination effect between MTBE and other compounds in the gasoline, or possibly with some other environmental contaminant. It is also a possibility that MTBE in gasoline may have acute effects on particularly sensitive individuals under some special circumstances. However, there is a high degree of confidence in the conclusion that MTBE-containing fuels are not a cause of acute toxicity in the general population (14).

7. Conclusions

Experimental toxicokinetics:

- A moderate respiratory uptake and a high respiratory excretion was seen for the ethers compared to many other solvents. MTBE showed a higher uptake, lower respiratory excretion and a faster terminal elimination in blood compared with ETBE.
- Two additional metabolites, α-hydroxyisobutyric acid (HBA) and 2-methyl-1,2-propanediol (MPD), were characterized in urine by ^{13}C-NMR after exposure to [1,2-$^{13}C_2$]-MTBE.
- Acetone level in blood (detected by GC) was higher after ETBE exposures compared to control exposure, and acetone is probably partly formed from ETBE.
- Urinary excretion of MTBE, ETBE and TBA was low, below 1% of the ether uptake, however, markedly higher amounts of HBA and MPD were found in the urine.
- Linear kinetics was seen after exposure up to 50 ppm MTBE and ETBE.

PBTK modeling:

- Physical activity has a profound influence on the internal exposure.
- ETBE, as well as lesser amounts of TBA accumulate in a small extent in tissues during a week of repeated exposure.
- The levels of TBA in blood, urine and exhaled air were less influenced by fluctuations in the exposure levels than were the amount of ETBE in blood and exhaled air (especially at the end of the workshift).
- Inter individual variability (eight subjects) in biomarker levels is higher the next morning than at the end of the workshift, and higher for TBA than for ETBE.
- The concentration of TBA in urine is a suitable biological exposure marker for ether and gasoline vapor. However, other metabolites that are excreted in higher amounts in the urine (e.g., HBA), may be even better candidates as biomarkers.

Acute effect test battery:

- Elevated ratings of discomfort in the throat and airways were recorded at 50 ppm ETBE.
- Minor impairment in pulmonary function was seen after ETBE exposure at 25 and 50 ppm (lung function tests were not performed in the MTBE study).
- Nasal swelling (MTBE and ETBE) and an increased blinking frequency (ETBE) was seen. Still, these effects did not correlate with exposure levels and may not be related to the oxygenate exposure.

8. Summary

Nihlén, A. Ethers as Gasoline Additives: Toxicokinetics and Acute Effects in Humans. *Arbete och Hälsa* 1998; 28.

Ethers or other oxygen-containing compounds are used as replacements for lead in gasoline and to ensure complete combustion. Methyl *tertiary*-butyl ether (MTBE) is already in use world-wide and ethyl *tertiary*-butyl ether (ETBE) may be used increasingly in the future. The aims of the present thesis were to study the uptake and disposition (toxicokinetics) of MTBE and ETBE in humans, to address the issue of biological monitoring and to measure acute effects (assessed by questionnaire, lung function, nasal and ocular measurements).

Healthy male volunteers were experimentally exposed during 2 h to vaporized MTBE (5, 25 and 50 ppm, n=10) or ETBE (0, 5, 25 and 50 ppm, n=8) in a chamber (whole-body) and ^{13}C-labeled MTBE (50 ppm, n=4) via a face-mask.

The toxicokinetics of MTBE and ETBE in humans were quite similar, judging from breath, blood and urine profiles. However, the respiratory uptake of MTBE was higher (45% MTBE and 33% ETBE) and respiratory exhalation of unmetabolized MTBE was lower (38% MTBE and 47% ETBE) compared to ETBE. Linear kinetics was seen for the ethers and TBA up to 50 ppm. Low urinary excretion of unmetabolized ether (<0.1%) and of a metabolite, *tertiary*-butyl alcohol (TBA) (<1%), was seen. After ^{13}C-labeled MTBE exposure two urinary metabolites, 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid, were characterized by ^{13}C-NMR. These metabolites were excreted in markedly higher amounts than TBA.

A physiologically based toxicokinetic model was developed for ETBE using the determined partition coefficients and data from the chamber exposure. The model indicates that workload has a profound influence on internal dose. TBA excretion seemed to be less sensitive to fluctuations in exposure levels compared to the ether, especially in samples taken at the end of a workshift. Only a small accumulation of ether and TBA in the body was seen throughout a week of repeated exposure.

MTBE vapor had very small or no effects up to 50 ppm. After ETBE exposure, elevated ratings of irritation in the throat and airways (50 ppm), and, in addition, minor pulmonary function impairments were seen (25 and 50 ppm ETBE, lung measures not performed for MTBE). This effect as such is not of clinical concern in healthy individuals. However, it cannot be excluded that other subjects may show more severe reactions.

In conclusion, the toxicokinetic results as well as considering practical issues (i.e. sampling and analysis), implies that the concentration of TBA in urine is a good biological exposure marker for ether and gasoline vapor. However, further studies of other metabolites that are excreted in higher amounts in the urine (e.g., α-hydroxyisobutyric acid), may identify even more suitable candidates as

biomarkers. The knowledge about the toxicokinetics and acute effects of MTBE and ETBE in humans presented in this thesis is relevant in e.g., risk assessments.

Key words: acute effects, biological monitoring, ethyl *tertiary*-butyl ether, ETBE, man, methyl *tertiary*-butyl ether, MTBE, oxygenate, petrol, physiologically based toxicokinetic model, PBTK, PBPK, *tertiary*-butyl alcohol, TBA, toxicokinetics.

9. Sammanfattning (Summary in Swedish)

Nihlén, A. Ethers as Gasoline Additives: Toxicokinetics and Acute Effects in Humans. *Arbete och Hälsa* 1998; 28.

Etrar eller andra ämnen som innehåller syre används i bensin för att ersätta bly och förbättra förbränningen. Metyl *tertiär*-butyl eter (MTBE) används globalt, men kan komma att ersättas av etyl *tertiär*-butyl eter (ETBE). Syftet med studien var att studera MTBE och ETBEs upptag och omsättning (toxikokinetik) hos människa, relatera till biologiska exponeringsmarkörer och mäta akuta effekter (frågeformulär, lungfunktion, näs- och ögonmätningar).

Friska frivilliga män exponerades i 2 timmar under lätt arbete dels för förångad MTBE (5, 25 och 50 ppm) eller ETBE (0, 5, 25 och 50 ppm) i en kammare och dels för ^{13}C-märkt MTBE (50 ppm) via en ansiktsmask.

Blod-, utandnings- och urinprofiler av MTBE och ETBE hos människa visade på likartad toxikokinetik för båda ämnena. Dock var upptaget av MTBE högre (45% MTBE och 33% ETBE) och utandningen av ometaboliserad MTBE lägre (38% MTBE och 47% ETBE) än för ETBE. Etrar och TBA följde linjär kinetik upp till 50 ppm. Urinutsöndringen av eter (<0.1%) och av en metabolit, *tertiär*-butyl alkohol (TBA) (<1%), var låg. Efter ^{13}C-märkt MTBE exponering identifierades två urinmetaboliter, 2-metyl-1,2-propandiol och α-hydroxi isosmörsyra, med ^{13}C-NMR. Dessa metaboliter utsöndrades i högre grad i urin jämfört med TBA.

En fysiologiskt baserad toxikokinetisk modell utvecklades för ETBE med hjälp av fördelningskvoter och data från de tre ETBE exponeringarna. Modellen indikerade att arbetsbelastningen har en betydande inverkan på den interna kroppsdosen. TBA verkade vara mindre känslig för fluktuationer i exponeringsnivån jämfört med etern, speciellt vid slutet av ett arbetspass. Endast en liten ackumulering av eter och TBA i kroppen observerades efter en veckas upprepad exponering.

Minimala akuta effekter observerades efter exponering upp till 50 ppm MTBE. Efter ETBE exponering observerades förhöjda skattningar av obehag i svalg, hals eller luftvägar (50 ppm) samt en något försämrad lungfunktion (25 och 50 ppm ETBE, lungfunktionsmätningar utfördes ej efter MTBE exponering). Den lätta försämringen har ingen klinisk relevans som sådan, men det kan inte uteslutas att andra individer kan reagera betydligt kraftigare än dessa få och friska män.

Sammanfattningsvis, de toxikokinetiska resultaten samt hänsyn till praktisk användning (vid t.ex. provtagning och analys) visar att halten av TBA i urin är en lämplig biologisk exponeringsmarkör för bensinånga. Men studier av andra urinmetaboliter, som utsöndras i högre halter än TBA (t.ex. α-hydroxi isosmörsyra), kan möjligen identifieras vara ännu bättre biomarkörer. Informationen om MTBE och ETBEs toxikokinetik och akuta effekter hos

människa, som har presenterats i denna avhandling, är relevant vid t.ex. riskbedömningar.

Nyckelord: akuta effekter, bensin, biologisk monitorering, etyl *tertiär*-butyl eter, ETBE, fysiologiskt baserad toxikokinetisk modell, metyl *tertiär*-butyl eter, MTBE, människa, oxygenat, PBTK, PBPK, *tertiär*-butyl alkohol, TBA toxikokinetik.

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Annsofi Nihlén, December 1998.

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