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Methyl *tert*-Butyl Ether
(MTBE)
Health Risk Characterisation

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SUMMARY

Use

Methyl *tert*-butyl ether (MTBE) is a colourless flammable liquid with a distinctive ethereal odour. It is used almost exclusively as a gasoline additive. It is added to unleaded gasoline in quantities between 2 and 5% (w/w), to raise the octane level, or up to 15% (w/w) to improve combustion efficiency as a measure to reduce air pollution from automotive exhaust emissions.

Minor applications include its use in clinical practice as a solvent for the dissolution of gallstones.

Exposures

Although the data on occupational exposure available to the Task Force were limited, they provided an indication of the magnitude of the potential exposure for workers. Occupational exposure may occur during MTBE production and loading, when handling gasoline containing MTBE or when working as a service station attendant or garage worker. The relevant route of such exposure is inhalation. Whereas MTBE production is associated with relatively low exposures (< 10 mg/m^3), loading operations may result in higher exposures (mean values of 20 mg/m^3, peak values of about 200 mg/m^3). Mean short-term exposure measurements for loading and delivery of gasoline containing 10-15% MTBE were between 13 and 91 mg/m^3 with a maximum of 226 mg/m^3. Since most fuels in Europe currently contain only 2 to 5% MTBE as an octane enhancer, these findings were considered by the Task Force to be a 'worst case' situation. For service station attendants and garage workers in the US, the mean exposures were < 3.5 mg/m^3 and 7.56 mg/m^3, respectively. The most likely source of consumer exposure to MTBE arises from gasoline evaporation during car refueling and its duration is very short. A study of consumer exposure in Finland measured concentrations of 6.0-7.5 mg/m^3 at service stations delivering gasoline containing 11% of MTBE.

Toxicity

Biotransformation of MTBE leads to the formation of *tert*-butanol (TBA) and formaldehyde, which in turn are further metabolised. TBA excretion proceeds relatively slowly (half-life of 8 h in humans). For formaldehyde the detoxification rate is much faster than its rate of formation from MTBE and therefore this route of metabolism is judged not to contribute to the toxic effects of MTBE discussed in this report. Toxicokinetic data do not indicate reasons for concern with regard to bioaccumulation of MTBE or any of its metabolites.

Skin and respiratory irritation are regarded as effects of prime concern following acute exposure. MTBE posesses a low order of acute toxicity in experimental animals exposed via oral, dermal and inhalation routes. LD_{50} values exceed 2,000 mg/kg for oral and dermal exposure, and the inhalation LC_{50} value is 85,000 mg/m^3 for 4 hours. Sub-lethal acute exposure evokes local irritation at the site of contact and transient clinical signs characteristic of central nervous system (CNS) depression. Skin contact with MTBE causes reversible moderate to severe irritation in rabbits whereas MTBE was found to be only slightly irritant to the rabbit eye. MTBE vapour at concentrations above 300 mg/m^3 evokes slight and transient irritation to the respiratory system of laboratory animals. For sensory and respiratory irritation an RD_{50} value (50% reduction of breathing rate) of 16,600 mg/m^3 was determined for MTBE in the mouse. The Task Force recommended that MTBE be labelled as irritant (Xi) with the corresponding R-phrase 38 (irritating to skin).

There have been no cases reported of sensitisation to MTBE in humans exposed by skin contact to the neat material or to gasoline containing MTBE. Studies in animals also failed to demonstrate skin sensitising potential on the part of MTBE, adding weight to the conclusion that MTBE is not a skin sensitiser.

MTBE caused anaesthesia in experimental animals when inhaled at concentrations of 28,800 mg/m^3. Reversible CNS effects were detected in a rat study at 14,400 mg/m^3 (LOAEL) using a functional observation battery and 6 hours of exposure. The NOAEL in this study was 2,880 mg/m^3. Observations suggesting transient CNS depression were consistently made also in animal studies using repeated inhalation and oral exposure. However, all effects were reversed when exposure ended and repeated exposure did not lead to NOAELs that were lower than for single exposure.

Principle effects observed following repeat oral and inhalation exposure of rats and mice to MTBE are local irritation, transient anaesthetic effects (as observed with many other low molecular weight ethers), chronic nephropathy and hepatocellular hypertrophy. The NOAEL for sub-chronic oral exposure is 300 mg/kg and for chronic inhalation exposure 1,440 mg/m^3. The latter value corresponds to daily retained doses (for calculation see page 29) of 102 and 113 mg/kg for male and female rats, respectively and 182 and 184 mg/kg for male and female mice, respectively.

MTBE has been tested extensively *in vitro* and *in vivo* for its genotoxic potential. The weight of evidence shows MTBE is not genotoxic . This conclusion is supported by the information for TBA, which is not genotoxic in several *in vitro* and *in vivo* tests, and formaldehyde, which though genotoxic in a number of tests, is rapidly detoxified by the body thereby removing the potential to damage the cell.

Tumours in rodents result from exposure to MTBE at doses exceeding the Maximum Tolerated Dose (MTD). An inhalation study in rats demonstrated a tumourigenic response in the male kidney at 10,800

and 28,800 mg/m^3 (corresponding to daily retained doses of 384 and 1023 mg/kg, respectively), but a non-genotoxic mechanism unique to the male rat is probably involved. An apparent increase in the incidence of Leydig cell tumours in male rats treated via inhalation was not considered to be relevant to humans. An inhalation study with mice showed an increase in the incidence of liver adenomas in female animals at 28,800 mg/m^3 (corresponding to a daily retained dose of 1824 mg/kg). A non-genotoxic mechanism is likely to be involved.

Further mechanistic studies are currently under way to clarify the mechanisms for the induction of these tumours.

Effects reported in an oral gavage study include an increase in rat Leydig cell tumour incidence and elevated combined lymphoma/leukaemia incidence in female rats. The Task Force considered the rat Leydig cell tumour findings as not predictive of hazard to humans. Furthermore, the importance of the combined lymphoma/leukaemia incidence from this oral gavage study was unclear due to deficiencies in the study report.

Overall, the Task Force concluded that the doses necessary to evoke neoplastic effects are equal to or greater than the doses that induce non-neoplastic effects in female mouse liver and male rat kidney. Therefore, protection against non-neoplastic effects should also protect from any theoretical carcinogenic effect. The Task Force concluded that MTBE is not carcinogenic according to the criteria in EU Directive on Dangerous Substances 67/548/EEC (EEC, 1993B).

Effects of MTBE vapour on reproduction and development have been evaluated in well-conducted inhalation studies with rats, mice and rabbits. Foetal toxicity and developmental toxicity were observed only at concentrations clearly toxic to the mother. MTBE was not embryotoxic or teratogenic at exposure levels not causing maternal toxicity and did not adversely affect reproduction.

Human Experience

A large body of data is available from human experience with MTBE, including case reports of clinical use of MTBE for gallstone dissolution, studies reporting subjective complaints by garage workers and service station attendants, large population studies with sophisticated study design and controlled short-term exposure of volunteers. Whereas the early studies suggested a relationship between MTBE exposure concentration and health complaints, this has not been confirmed in subsequent studies. This absence of an association is in line with short-term experimental studies that showed no specific effects at concentrations (< 3.6-180 mg/m^3) similar to or greater than those observed in the population studies. Human experimental data do not indicate irritation of the respiratory tract at concentrations of 180 mg/m^3 for two hours. Exposure to 270 mg/m^3 for three hours caused mild mucous membrane irritation in some volunteers. Objective symptoms on the CNS have not been observed in volunteer

studies up to 270 mg/m^3. Subjective symptoms at this concentration were reported by volunteers (mainly feeling of heaviness in the head). At 180 mg/m^3 no symptoms were reported.

Risk Characterisation

Table 1 on page 5 summarises the conclusions with regard to MTBE-related health effects. Irritation observed after short-term exposure in humans as well as liver and kidney toxicity observed after long-term exposure in experimental animals are considered to be the critical effects for the health risk characterisation of MTBE. Mild respiratory irritation occurred at a concentration of 270 mg/m^3 for three hours in human volunteers, whereas 180 mg/m^3 for two hours did not evoke such effects. The lowest NOAEL for liver and kidney effects after chronic inhalation exposure was 102 mg/kg/day (retained dose in male rats). The basis for the risk characterisation is a comparison of these three different doses/concentrations with occupational and consumer exposure data.

The available data on short-term peak exposure levels (about 200 mg/m^3) did not indicate concerns with regard to respiratory irritation. Comparison of the NOAEL for long-term liver and kidney effects revealed margins of safety between 180 to 300 fold for workers involved in MTBE production, about 70 fold for workers handling gasolines containing MTBE, and between 250 to 800 fold for service station attendants and garage workers. A 17,000 fold margin of safety was calculated for consumer exposure during car refuelling.

Compliance with an occupational exposure limit of 90 mg/m^3 or 25 ppm MTBE (8-h TWA) is considered by the Task Force to protect workers from any potential health hazards. This concentration corresponds to a daily retained MTBE dose of about 5.1 mg/kg for a 70-kg adult (on the basis of a ventilation volume of 10 m^3/8-h shift and a relative respiratory uptake of 40%) and provides a margin of safety of 20 when compared with the lowest NOAEL determined in chronic animal inhalation experiments. Respiratory irritation is regarded as the critical effect for higher short-term exposures. In humans, no effects were observed at a concentration of 180 mg/m^3 for 2 hours, while at 270 mg/m^3 for three hours only weak irritating effects on the mucous membranes were reported in some volunteers. Therefore, a limit of three times the TWA (270 mg/m^3 or 75 ppm) is considered to be an appropriate short-term, peak exposure limit (15-min STEL).

Conclusion

The risk characterisation for MTBE does not indicate concern for human health with regard to current occupational and consumer exposures.

Table 1: Principal effects of MTBE and NOAELs

End point	Species	Route	Exposure Time	Principal Effects	NOAEL	Remarks	Reference
acute effects	human	inhalation	2 hours (during light physical exercise)	mucous membrane irritation	180 mg/m^3	subjective symptoms (like slight irritation and heaviness in the head) were reported at 270 mg/m^3 (3 h exposure)	Johanson *et al*, 1995; Riihimäki *et al*, 1996
subchronic toxicity	rat	inhalation	90 days	liver and kidney toxicity (males)	2,880 mg/m^3	equivalent to 228 mg/kg bw/day (males)	Dodd and Kintigh, 1989
chronic toxicity and neoplastic effects	rat	inhalation	105 weeks	liver and kidney toxicity, kidney tumours (males)	1,440 mg/m^3	equivalent to 102 mg/kg bw/day (males)	Chun *et al*, 1992
	mouse	inhalation	18 months	liver tumours (females)	10,800 mg/m^3	equivalent to 669 mg/kg bw/day (females)	Burleigh-Flayer *et al*, 1992
neurotoxicity	rat	inhalation	6 hours	functional CNS effects	2,880 mg/m^3	LOAEL was 14,400 mg/m^3; effects were reversible	Gill, 1989
effects on fertility	rat	inhalation	two generations	no treatment-related effects	> 28,800 mg/m^3	for parental toxicity a NOAEL of 1,440 mg/m^3 was determined	Myhr *et al*, 1991
developmental toxicity	mouse	inhalation	gestation days 6 to 15	no direct effect on the fetus	3,600 mg/m^3	higher concentrations caused maternal toxicity and secondary developmental toxicity	Tyl and Neeper-Bradley, 1989

1. INTRODUCTION

Methyl *tert*-butyl ether (MTBE) is a colourless flammable liquid with an ethereal distinctive odour. MTBE is used as gasoline additive, with minor applications as a solvent. It is added to unleaded gasoline in quantities ranging between 2 and 5% (w/w) to raise the octane rate, although some countries (notably Canada, Finland and the USA - the latter with its "oxygenated fuel program") have implemented higher blending levels of up to 15% MTBE (w/w) to improve combustion efficiency in order to reduce car emissions.

The widespread introduction of MTBE and the public awareness of health-related issues with regard to gasoline have caused interest in the toxicological and epidemiological database on MTBE. A programme of toxicity studies was conducted in the 1980s. After the start of the oxygenated fuel programme in the USA in 1992, however, reports on health complaints in Fairbanks, Alaska, initiated a scientific and public debate in the USA on the risk/benefit of MTBE use in gasoline. This was further stimulated by a report that suggested an increased tumour incidence in rats receiving high oral doses of MTBE. In view of this discussion, ECETOC established a Task Force to review the toxicity of MTBE. In the meantime, MTBE was selected for inclusion in the third priority list of the EC existing substances program. In anticipation of the EU risk assessment, the Task Force prepared a review of the toxicological and epidemiological database, an estimation of the occupational and consumer exposures, and a risk characterisation for occupational and consumer exposures. The structure of this report on the health risk assessment of MTBE follows the general principles outlined in the EU Technical Guidance Documents (EEC, 1994).

2. IDENTITY AND CHEMICAL PROPERTIES

Common name:	Methyl *tert*-butyl ether, MTBE, *tert*-butyl methyl ether
IUPAC name:	2-Methoxy-2-Methyl Propane
CAS registry No.:	1634-04-4
EINECS No.:	216-653-1
EU-Labelling	R11 - Highly flammable + R38 - Irritant
Chemical Group:	Dialkyl ethers
Formula:	$H_3C\text{-}O\text{-}C(CH_3)_3$
Molecular mass:	88.15
Purity of the technical product:	97.0 to 99.9%
Conversion Factor (20°C, 1.018 hPa):	1 mg/m^3 = 0.277 ppm
	1 ppm = 3.6 mg/m^3

Table 2-1: Physical and Chemical Properties[a]

Parameter	Value
Boiling point	55.2 °C
Freezing point	-109 °C
Flash point	- 30 °C
Autoignition temperature	425 °C
Flammability limits in air	1.5 - 8.5%
Vapour pressure	245 mm Hg at 25 °C
	361.7 to 413.8 mm Hg at 38 °C
Relative Density	0.7405 at 20 °C
Refractive index	1.3690 at 20 °C
Colour	Colourless
Odour	Strong ethereal odour
Odour threshold	0.18 mg/m^3 (0.24 µl/l; 0.05 ppm) (Prah *et al*, 1994)
Solubility	less than 10% in water; miscible with ethanol and diethyl ether.
Partition Coeff. log P_{ow}	1.06
The technical product is stable.	
Conditions to avoid include:	open flame and other ignition sources, heat, sparks
Substances to avoid:	oxidising agents, strong acids, 2-Fluorel^R and Viton^R.

[a] From Material Safety Data Sheet for MTBE provided by ARCO Chemical Co.

3. PRODUCTION AND USE

The raw materials for the manufacture of MTBE are isobutylene and methanol. Isobutylene is obtained from either petroleum refinery sources (e.g. steam cracker operation, fluid catalytic cracker operation, butane dehydrogenation) or from dehydration of *tert*-butanol. Production is in closed systems.

Commercial production of MTBE started in Europe in 1976 and in the US in 1979. World-wide capacity has grown at a rate of 20% per year over the past decade, particularly in North America and Europe. Total world-wide production capacity in 1994 was 20.6 million tonnes and is expected to grow to 25 million tonnes. Of this amount 23% originated from steam cracker, 31.5% from fluid catalytic cracker and 32.5% from dehydrogenation operations. The remaining 13% was produced through dehydration processes. Most of the recent growth is based on butane dehydrogenation and fluid catalytic cracker units. European production of MTBE is approximately 3.5 million tonnes per annum.

MTBE has been added to gasoline blends since the second half of the 1970's, initially at low levels (2-5% w/w) to boost the octane rating of unleaded premium or high performance grades. More recently MTBE has been added at higher levels (11-15% w/w) to promote more efficient combustion of the gasoline. Blends meeting the latter specifications are widely used in North America and parts of Europe to improve air quality (oxygenated gasolines).

High purity MTBE (>99.9%) is being used as a process reaction solvent in the pharmaceutical industry, and as a gallstone dissolver in clinical practice.

4. TOXICOKINETICS

4.1 INTRODUCTION AND OVERVIEW

The information presented in this chapter has been arranged according to the processes that influence the fate of chemicals in the body, i.e. absorption, distribution, metabolism and excretion. Major studies addressing these aspects are listed in table 4-1 (studies indicated as A to J) and experimental details are described either in tables 4-2 to 4-4 or are given in the text.

MTBE has been studied extensively with regard to its toxicokinetic properties. MTBE is absorbed by all routes of exposure with quantitative differences with regard to the extent of absorption. Absorbed material is distributed uniformly in all tissues according to the relevant tissue/blood partition coefficients which for most tissues is around one. The fat/blood partition coefficient is about 10 and consequently the fat concentrations are about 10-fold higher than blood concentrations at steady state. Overall, due to rapid removal via exhalation and metabolism, there is no tendency for MTBE to accumulate. Metabolism proceeds via two principal metabolites, i.e. *tert*-butanol (TBA) and formaldehyde (see figure 4-1), both of which are further transformed and also show no tendency to accumulate. The products resulting from TBA metabolism are mainly eliminated via urine, whereas formaldehyde and its breakdown products enter the normal physiological pathways. This general description of the MTBE toxicokinetics appears to be valid for all investigated species, including man.

4.2 ABSORPTION

Information on absorption following oral, dermal and inhalation exposures is available from several studies. In study A-2 peak plasma concentrations of MTBE were reached within 1 hour after oral (gavage) administration, indicating rapid uptake from the gastrointestinal tract. The area under the plasma concentration-time curve (AUC), a measure of the total absorption of MTBE, after oral administration was greater than the AUC after intravenous administration (A1) of an approximately equivalent dose. Since the theoretical bioavailability for intravenous administration is 100% , this result was attributed by the authors to a higher proportion of MTBE being exhaled after intravenous administration. Following dermal application (A3 - closed chamber, 6-hour contact), the peak plasma concentrations were 20-fold lower than observed after oral administration and were reached about two hours after the start of the experiment. This demonstrates a slower uptake of MTBE through the skin compared with the uptake from the gastrointestinal tract. The bioavailability of MTBE after dermal administration was 20% and 39% (40 and 400 mg/kg, both routes respectively) of the oral bioavailability.

Figure 1: Major Metabolic Pathways for MTBE and MTBE Metabolites

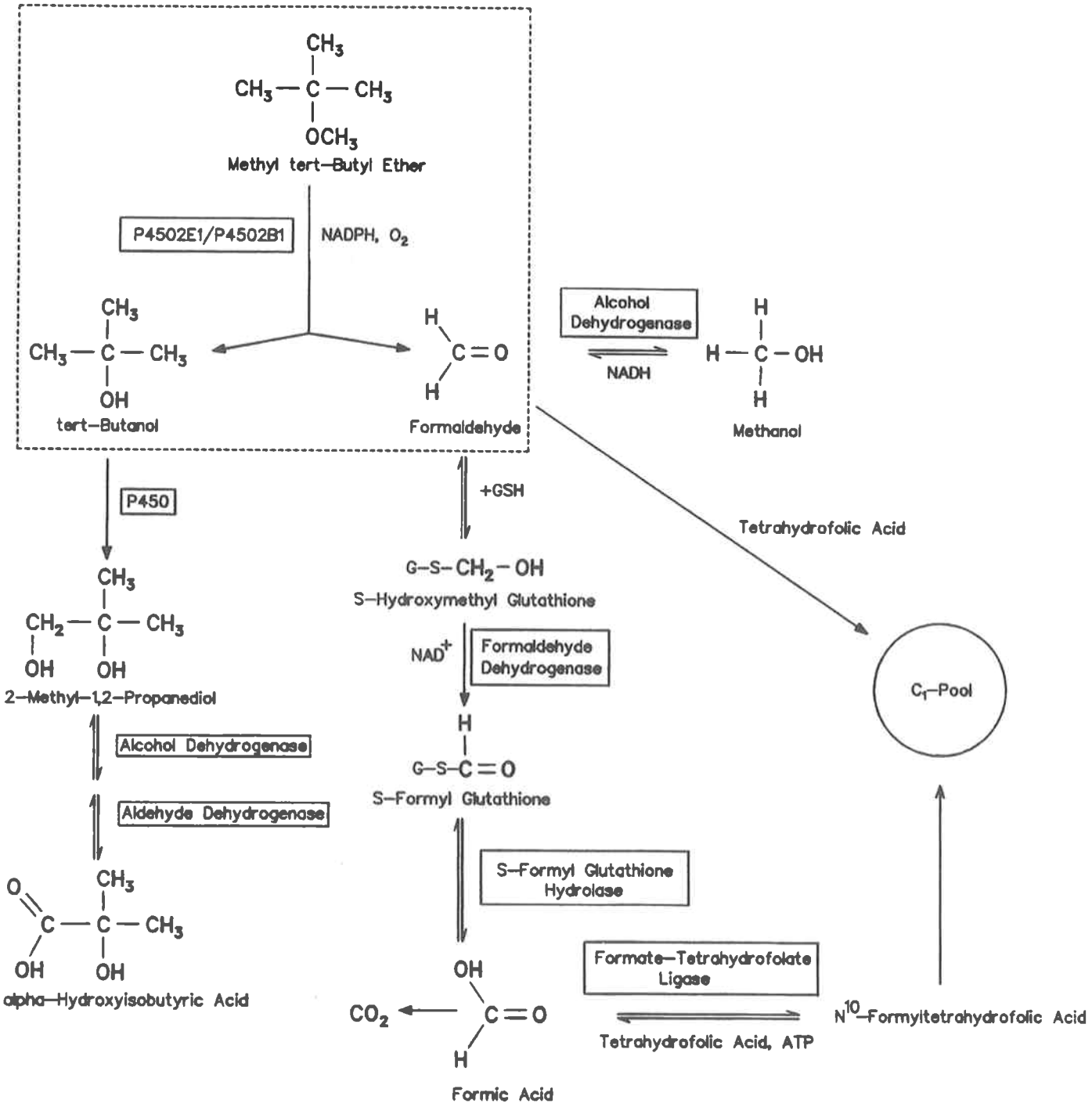

Table 4-1: Major Studies Addressing Several Aspects of MTBE Toxicokinetics

Study	Species/ Strain/Sex/ No/group	Route	Dose/ Concentration	Material	Reference	Confidence in results
A-1	rat/Fischer 344/ M+F/ 40/group	intravenous (bolus)	40 mg/kg	unlabelled MTBE	Bio-Research Laboratories, 1990a	high
A-2	rat/Fischer 344/ M+F/ 40/group	oral (gavage)	40, 400 mg/kg			high
A-3	rat/Fischer 344/ M+F/ 60/group	dermal (application chamber)	40, 400 mg/kg			high
B-1	rat/Fischer 344/ M+F/ 52/group	single inhalation (nose only, 6h)	1,440 mg/m^3 28,800 mg/m^3	unlabelled MTBE	Bio-Research Laboratories, 1990b	high
B-2	rat/Fischer 344/ M+F/ 40/group	15-day inhalation (nose only, 6h/d)	1,440 mg/m^3			high
C-1	rat/Fischer 344/ M+F/ 6/group	intravenous (bolus)	40 mg/kg	^{14}C-MTBE (label on central butyl carbon)	Bio-Research Laboratories, 1990c, 1991	high
C-2	rat/Fischer 344/ M+F/ 6/group	oral (gavage)	40, 400 mg/kg			high
C-3	rat/Fischer 344/ M+F/ 6/group	dermal (application chamber)	40, 400 mg/kg			high
D-1	rat/Fischer 344/ M+F/ 6/group	single inhalation (nose only, 6h)	1,440 mg/m^3 28,800 mg/m^3	^{14}C-label as in C-1	Bio-Research Laboratories, 1990d	high
D-2	rat/Fischer 344/ M+F/ 6/group	15-day inhalation (nose only, 6h/d)	1,440 mg/m^3	no label first 14 d, further as D-1		high
E	rat/Sprague-Dawley M+F/ 3/group (11 time points)	intraperitoneally	232 mg/kg	^{14}C-label on methyl and central butyl carbon	Zacharias and Eschbach, 1984	high
F	rat/Charles River/ M+F/ 2/gr	intraperitoneally	7.3 mg/kg 14.6 mg/kg	^{14}C-label position not indicated	Kennedy and Keplinger 1972	low
G	monkey/Rhesus / F/2/group	intraperitoneally	58.4 mg/kg	as F	as F	low
H	rat/Wistar/ M/ 5/group	15-week inhalation(whole body, 6 h/d, 5 d/w)	180 mg/m^3 360 mg/m^3 1,080 mg/m^3	unlabelled MTBE	Savolainen *et al*, 1985	high
J	rat/Wistar/ M/ 5 (8 time points)	oral (gavage)	0.379 mg/kg	unlabelled MTBE	Li *et al*, 1991	low

In study J, the peak plasma concentration of 5.9 µg/ml was reached after 0.9 h, also demonstrating rapid uptake of MTBE from the gastrointestinal tract.

During the single inhalation exposures in study B plasma levels of MTBE increased rapidly between 10 min to 2 h after the start of exposure and then more gradually up to a maximum concentration (Cmax), at approximately 4 to 6 h after the start of exposure. This indicates a rapid pulmonary absorption of MTBE.

In the mass balance study C, the percentages of the radioactive doses recovered after oral (gavage) and after the intravenous dose were about the same, demonstrating virtually complete absorption from the gastrointestinal tract. In addition, the time course for exhalation of radioactive material and for the appearance of radioactivity in the urine indicated that most of the material was absorbed from the gastrointestinal tract within 3 hours after dosing the animals. Following dermal application of 40 mg/kg, about 60% of the radioactive material was still present in the application chambers, after administration of 400 mg/kg about 35% remained in the application chambers. This demonstrated a limited dermal absorption in comparison with the oral dose. The time course for exhalation of radioactive material and for the appearance of radioactivity in the urine after dermal application showed the uptake via the skin to be much slower in comparison with the uptake after oral administration.

Data from the inhalation experiments in study D do not allow any conclusions regarding the extent of absorption of MTBE after inhalation. However, a recent publication by Borghoff *et al* (1995) described a physiologically based pharmacokinetic (PBPK) model for MTBE in the rat and reported a blood/air partition coefficient of 11.5. Johanson *et al* (1995) determined a partition coefficient blood/air of 17.7 for human blood. These values indicate efficient uptake from inhaled air, as well as excretion via exhaled air. The net uptake at steady state is dependent on the MTBE concentration and decreases with increasing MTBE concentrations. A detailed discussion is provided in chapter 4.7.

In study E radioactivity in whole blood peaked 5 min after intraperitoneal administration, decreased sharply until one hour post-treatment and then decreased more gradually during the rest of the observation period. Forty eight hours after dosing, only small amounts of radioactivity could still be detected. The half life of MTBE, calculated on the basis of the ^{14}C-labelled material in whole blood, was 60 min for male rats and 49 min for female rats. In study F using two rats of each sex, 67 to 72% of the radioactive material was excreted within 24 hours of dosing by intraperitoneal injection, suggesting rapid absorption also via this route of administration.

It is not possible to derive clear data on the uptake kinetics for monkeys after intraperitoneal injection from the results of study G. Of the radioactive material 72 to 75% was excreted via exhalation, urine and faeces within 24 hours, indicating that primates and rats show similar kinetics via this route of administration.

Table 4-2:Toxicokinetics of MTBE and TBA in Male and Female F-344 Rats After Administration of MTBE by the Intravenous, Oral, Dermal and Inhalation Routes (Bio-Research Laboratories 1990a, 1990b, see table 4-1) (group mean values are shown)

Study	Route	Target Dose/ Concentration	Received Dose [mg/kg]	MTBE-Cmax [µg/ml]	MTBE-tmax [h]	MTBE-AUC [µg•h/ml]	MTBE-t1/2 [h]	TBA-Cmax [µg/ml]	TBA-tmax [h]	TBA-AUC [µg•h/ml]	TBA-t1/2 [h]	CL [ml/h]
						Male Animals						
A-1	*iv*	40 mg/kg	36.7	na	na	10.7/na	0.45	7.2	2	26.7/na	0.9	413
A-2	oral	40 mg/kg	33.5	17.2	0.25	17.0/na	0.52	10.0	0.75-2	39.0/na	1.0	392
A-2	oral	400 mg/kg	420	124.0	0.15-0.75	230/na	0.79	50.3	2-4	304/na	1.6	358
A-3	dermal	40 mg/kg	33.5	0.3	2-6.5	7.9/2.6	2.30	0.4	4-7	26.3/6.7	2.1	389
A-3	dermal	400 mg/kg	373	5.4	4	46.9/14.6	1.80	13.3	4	93.9/34.0	1.9	364
B-1	inhal.[a]	1,440 mg/m^3	242[c]	14.9	4 - 6	84.3/10.5	0.52	39.7	6.5	404/116	3.3	531
B-1	inhal.[a]	28,800 mg/m^3	4,709[c]	556.0	6	2,960/406	0.57	536	6.5	6,010/1,790	3.4	298
B-2	inhal.[b]	1,440 mg/m^3	220[c]	9.0	6	na/6.7	0.51	37.1	6 - 6.5	na/127	1.8	na
						Female Animals						
A-1	*iv*	40 mg/kg	33.5	na	na	7.9/na	0.46	6.4	1-2	32.2/na	1.3	466
A-2	oral	40 mg/kg	42.7	11.2	0.25	12.5/na	0.62	8.9	2	36.7/na	1.0	481
A-2	oral	400 mg/kg	388	115.0	0.25-0.5	193/na	0.93	48.8	4	289/na	1.9	287
A-3	dermal	40 mg/kg	39.4	0.1	4	1.1/nd	0.90	0.5	4-6	3.8/1.2	2.2	458
A-3	dermal	400 mg/kg	441	7.8	2	34.4/6.9	1.40	16.3	6	101/37.1	1.9	273
B-1	inhal.[a]	1,440 mg/m^3	329[c]	15.1	4	77.9/11.8	0.63	39.4	6	374/94.2	3.0	573
B-1	inhal.[a]	28,800 mg/m^3	6,991[c]	563.0	4	2,870/369	0.53	245	6	2550/574	2.8	315
B-2	inhal.[b]	1,440 mg/m^3	318[c]	9.1	6	na/6.3	0.48	44.2	6	na/125	1.5	na

iv Bolus intravenous injection of MTBE dissolved in isotonic saline into the caudal vein (40 rats/group)
oral Intragastric gavage administration of 10 ml MTBE solution in isotonic saline per kg body weight (40 rats/group)
dermal Dermal application of 10 ml MTBE solution in isotonic saline via an occluded dermal chamber for 6 hours (60 rats/group)
inhal.[a] Single 6-hour inhalation exposure
inhal.[b] Repeated 6-hour inhalation exposure for 14 days; on day 15 the indicated values were determined after the end of the last exposure
[c] Calculated from target concentrations using a respiratory minute volume of 0.169 l and an uptake of 0.5 with no subsequent elimination over the course of the 6-hour exposure
Cmax Maximum plasma concentration reached during sampling schedule
tmax Time to reach Cmax after MTBE administration
t1/2 Apparent half life calculated from one or two compartment models
AUC Area under the plasma concentration-time curve; the first value gives AUC(0->4 hours), the second number AUC(6->24 hours)
na Not available
CL total plasma clearance of MTBE.

Table 4-3: Total percent of radioactive MTBE dose recovered from Fischer 344 rats during the 0 - 48 h interval after administration (Bio-Research Laboratories 1990c, 1990d, 1991; see table 4-1)(group mean values are shown)

Study	Route	Target Dose/ Concentration	Received Dose [mg/kg]	Radioactive Dose [µCi/kg]	Percent of Dose Received: Urine	Faeces	Expired Air	Tissues/ Carcasses	Chamber/ Appl. Sites	Total
Male Animals										
C-1	*iv*	40 mg/kg	31.54	37.66	27.9	0.89	46.8	2.14	na	77.7
C-2	oral	40 mg/kg	33.38	43.57	36.2		45.8	2.00	na	85.2
C-2	oral	400 mg/kg	349.65	43.68	16.0	0.28	65.2	0.31	na	81.9
C-3	dermal	40 mg/kg	34.64	44.30	6.49	0.14	6.05	0.67	57.10	70.5
C-3	dermal	400 mg/kg	350.95	43.80	12.4	0.19	19.6	0.28	38.29	70.8
D-1	inhal.[a]	1,440 mg/m^3	215.0[b]	na	64.7	0.76	21.2	13.40	na	100.0[c]
D-1	inhal.[a]	28,800 mg/m^3	4220.0[b]	na	41.6	0.75	53.6	4.07	na	100.0[c]
D-2	inhal.[d]	400 mg/m^3	245.0[b]	na	71.6	0.62	16.9	10.90	na	100.0[c]
Female Animals										
C-1	iv	40 mg/kg	30.96	3.6.98	222.4	0.81	54.60	2.97	na	80.9
C-2	oral	40 mg/kg	34.22	44.21	29.0	0.87	54.40	1.94	na	86.3
C-2	oral	400 mg/kg	341.74	42.82	10.8	0.26	68.70	0.53	na	80.2
C-3	dermal	40 mg/kg	35.79	45.85	6.12	0.13	9.67	1.02	59.91	76.8
C-3	dermal	400 mg/kg	358.44	44.60	10.7	0.11	23.20	0.67	33.71	69.4
D-1	inhal.[a]	1,440 mg/m^3	293.0[b]	na	65.4	0.74	21.90	11.90	na	100.0[c]
D-1	inhal.[a]	28,800 mg/m^3	5840.0[b]	na	35.0	1.06	59.00	5.02	na	100.0[c]
D-2	inhal.[d]	400 mg/m^3	344.0[b]	na	67.4	0.77	21.40	10.40	na	100.0[c]

after intravenous, dermal, and oral dosing all rats were returned to metabolism cages immediately.

iv Bolus intravenous injection of MTBE dissolved in isotonic saline into the caudal vein (6 rats/group)

oral Intragastrical gavage administration of 10 ml MTBE solution in isotonic saline per kg body weight (6 rats/group)

dermal Dermal application of 10 ml MTBE solution in isotonic saline via an occluded dermal chamber on the shaved dorsal flanks for 6 hours (6 rats/group);

inhal.[a] Rats were exposed nose-only for 6 hours to ^{14}C-MTBE and were returned to metabolism cages immediately after the end of exposure (6 rats/group)

inhal[d] Rats were exposed for 14 days, 6 h/d to unlabelled MTBE and on day 15 to for 6 hours and then placed in metabolism cages (6 rats/group)

[b] Calculated assuming a respiratory minute volume of 0.169 l and 0.5 uptake in the lungs with no subsequent elimination over the course of the 6-h-exposure

[c] The exhalation of radioactive material during the exposure was not measured; a true mass balance therefore could not be determined after the ihalation exposures. Consequently the recovery of radioactivity was calculated as percent of the total radioactivity recovered from each animal. comparison of the numbers derived from inhalation exposures with another route of administration is only possible to a limited extent.

na Not available

Three experimental studies with human volunteers exposed by inhalation are described in section 4.6. The results of these studies also indicate a rapid uptake of MTBE in man.

Evaluation

Absorption of MTBE has been investigated in a number of well-designed studies conducted in rats showing that MTBE is absorbed via all routes of exposure. Absorption from the gastrointestinal tract is complete and rapid. The dermal absorption rate, following occluded exposure, is about 1/3 of the oral rate as determined by bioavailability. It may be assumed that non-occluded skin contact results in lower bioavailability. Absorption from the respiratory tract is rapid resulting in a steady state MTBE concentration in rat blood within about 3 hours after the exposure starts (supporting information is also available from mice and monkeys). The limited data from studies with monkeys and human volunteers indicate that the uptake kinetics in these species are similar to those in rats. The relative respiratory uptake for experimental animals and humans is further discussed in section 4.7.

4.3 DISTRIBUTION

MTBE concentrations were determined in blood, brain and fatty tissues in rats, following repeated inhalation exposure for 6 hours daily, 5 days per week for 15 weeks (study H, see also table 4-4). They were linearly related to exposure concentration at all time points. The individual tissue concentrations measured after 2, 6, 10 and 15 weeks of exposure were similar, indicating that the distribution equilibrium was already reached during the first 2 weeks and that no further bioaccumulation occurred during the study. The results further indicate that MTBE did not accumulate in rapidly perfused tissues such as the brain, since blood MTBE concentrations and brain MTBE concentrations remained similar during the exposure. In contrast, MTBE accumulates in fat during the exposures as indicated by the about 10-fold higher concentrations in fatty tissues compared to blood at all time points. This is consistent with the logarithm of the partition coefficient between octanol and water, the log P_{ow} of 1.06 at 23 °C, and with the fat/blood partition coefficient of 10.05 measured by Borghoff *et al* (1996). Since the concentrations in fat after 2, 6, 10, and 15 weeks were similar, the removal of MTBE after the end of the 6-hour exposure time has to be rapid. Exposures longer than 6 hours should not cause an MTBE accumulation in fat exceeding the 10-fold accumulation observed.

In study C the total radioactivity in tissues and carcasses of the rats 48 hours after dosing was 0.28% to 2.14% of the administered dose, indicating MTBE and/or its metabolites did not accumulate in the tissues. Analysis of the individual tissues (brain, fat, heart, kidneys, liver, lungs, muscle, gonads, bone, and skin) showed only marginal levels of radioactivity and no specific association of radioactivity with one of these tissues that may be regarded as biologically significant. The liver showed the highest levels but this amounted to <0.2% of the received dose.

Table 4-4 MTBE and TBA Burden of Rats Exposed to MTBE Vapour (from Savolainen *et al*, 1985, see table 4-1)

Vapour (mg/m^3)	Exposure Time							
	2 weeks		6 weeks		10 weeks		15 weeks	
	MTBE	TBA	MTBE	TBA	MTBE	TBA	MTBE	TBA
				Blood				
180	9 ± 1	5 ± 3	11 ± 2	38 ± 3	5 ± 2	11 ± 3	7 ± 2	15 ± 7
360	18 ± 4	17 ± 9	24 ± 2	82 ± 5	20 ± 2	34 ± 9	23 ± 4	42 ± 10
1080	67 ± 18	28 ± 8	66 ± 18	151 ± 24	55 ± 14	89 ± 32	72 ± 19	127 ± 39
				Brain				
180	11 ± 3	trace	trace	trace	trace	trace	trace	trace
360	27 ± 5	trace	28 ± 5	77 ± 3	23 ± 5	66 ± 5	trace	53 ± 16
1080	87 ± 20	24 ± 10	78 ± 8	212 ± 24	44 ± 19	135 ± 24	56 ± 18	147 ± 35
				Perirenal fat				
180	184 ± 13	trace	91 ± 14	trace	90 ± 19	trace	81 ± 25	trace
360	245 ± 20	trace	236 ± 38	trace	194 ± 30	trace	210 ± 45	trace
1080	642 ± 119	trace	610 ± 28	trace	643 ± 39	trace	698 ± 67	trace

Each figure (nmol/g) is the mean of five rats (± SD). The detection limit for MTBE was 1.5 nmol/g and for TBA 2 nmol/g, respectively.

In study D, after a single inhalation exposure to 1,440 mg/m^3, about 5% of the total radioactivity was recovered in tissues and carcasses. At a concentration 20 times higher, about 15% was recovered. Animals exposed to 28,800 mg/m^3 showed a lower relative tissue-radioactivity than animals exposed to 1,440 mg/m^3, reflecting a higher proportion of MTBE eliminated via exhalation at higher concentrations and concurrent saturation of other elimination pathways. Determination of the radioactivity in individual tissues, as mentioned above, did not reveal any specific accumulation of radioactivity. Besides the skin (probably due to surface contamination), highest values were found in liver, but only amounting to <0.3% of total radioactivity recovered.

Similar results were obtained in study E after *ip* administration (table 4-1). Total radioactivity found in tissues averaged 3.39, 1.94, and 1.14% of the administered dose for the 15 min, 6 and 24-hour sacrifice intervals, respectively. The majority of the radioactivity present in tissues was found in the liver. In another *ip* study with rats the amount of radioactivity remaining in the body 96 hours after treatment (study F) ranged from 8 to 16%. All tissues and organs analysed contained radioactive material; however muscle (3.47 to 8.89%), skin (1.77 to 3.58%) and fat (0.59 to 1.56%) contained the greatest amounts. In the intraperitoneal study with monkeys approximately 7 to 8% of the radioactivity was retained in the body 96 h after dosing (study G). All tissues and organs were analysed for radioactivity; muscle (3.18 or 3.06%), skin (1.24 or 2.07%) and fat (1.29 or 1.08%) contained the greatest amounts.

Borghoff *et al* (1995) determined tissue/blood partition coefficients for male F-344 rats and found values of 1.18 for liver, 0.56 for muscle, 3.11 for kidney and 10.05 for fat. In their PBPK model the liver value was also used for the rapidly perfused tissue compartment and the muscle value was used for the slowly perfused tissue compartment. The partition coefficients measured are in good agreement with the observations made in the toxicokinetic studies with regard to distribution: MTBE is distributed uniformly in most tissues whereas in fat there is a 10-fold higher MTBE concentration compared to blood at steady state. Using these values, the model successfully predicted MTBE dosimetry in the rat after iv, oral and inhalation administration as described in studies A, B, C and D (table 4-1). Borghoff *et al* (1995) also reported the tissue blood partition coefficient for TBA. The blood/air partition coefficient is 481 indicating high solubility in blood. The liver/blood, kidney/blood, muscle/blood and fat/blood partition coefficients were 0.83, 1.13, 1.02 and 0.40, respectively. These values indicate a high solubility of TBA in all tissues and no tendency for TBA to accumulate in fat.

Evaluation

The distribution of MTBE and its metabolites has been investigated in a number of well designed studies conducted in rats. Limited additional information is also available from monkeys. The data indicate that the ^{14}C-label derived from MTBE is uniformly distributed throughout the body in rats and primates. Although in the mass balance studies the radioactive label was on different carbons, the

principal results of the experiments were similar. In inhalation studies with unlabelled MTBE, a higher concentration of MTBE in fat tissue has been observed when steady state had been reached and when compared to other tissues. This is due to the fat solubility of MTBE characterised by the low log P_{ow} of MTBE and the fat/blood partition coefficient of 10.05 measured for rats. Repeated daily inhalation did not cause a further increase in the fat concentration. Although experimental data are lacking, there is no reason to believe that MTBE distribution in humans would differ from the distribution as observed in experimental animals.

4.4 METABOLISM

Data on the metabolism of MTBE and TBA are mainly available from studies conducted with MTBE. The metabolic fate of the other principle MTBE metabolite, i.e. formaldehyde, was not specifically investigated in most MTBE studies. Although a number of reviews are available for formaldehyde, these reviews do not address the specific issue of the relationship between formaldehyde formation rates from MTBE and the formaldehyde detoxification rates. Accordingly, formaldehyde is discussed here in the detail necessary to address this issue.

MTBE and TBA

MTBE is metabolised by cytochrome P450-dependent monooxygenases via O-demethylation yielding formaldehyde and *tert*-butanol (TBA) as the main metabolites. A flowchart of the metabolic pathways of MTBE is provided in figure 4-1 (see page 11).

Investigations on the contribution of cytochrome P450-monooxygenase (CYP) isoenzymes to MTBE metabolism were performed by Brady *et al* (1990). They found that TBA and formaldehyde are produced in equimolar amounts in microsomal incubations with MTBE. Microsomal preparations from control, acetone- or phenobarbitone-treated rats were incubated with MTBE and the formation of formaldehyde and TBA was measured. In the microsomes from control animals, a Km of 0.67 mM and a Vmax of 1.22 nmol/min/mg protein for formaldehyde formation was calculated. The Km was slightly lower (0.39 mM) in microsomes from acetone-treated rats and about twice as high in microsomes from phenobarbitone-treated rats (similar results were reported by Snyder, 1979). The Vmax was increased by a factor of 4 by acetone treatment, and by a factor of 5.5 by phenobarbitone treatment. Pre-incubation of microsomes with monoclonal antibodies against CYPIIE1 inhibited about 35% of the MTBE demethylation, suggesting that other P450 isoenzymes also contribute to MTBE metabolism. A single 18-hour pre-treatment of rats with 740 or 3,700 mg/kg MTBE via *ip* administration resulted in a 50 fold induction of liver microsomal pentoxyresorufin dealkylase activity accompanied by an elevation of CYPIIB1 levels. CYPIIE1 activity was not increased. Vmax and Km were not determined in microsomes from MTBE-treated animals. In summary, these results demonstrate involvement of

CYPIIB1 and CYPIIE1 in the O-demethylation of MTBE by rat hepatic microsomes and also that MTBE is able to induce its own metabolism.

In the inhalation study H, an approximately linear relationship between MTBE dose and TBA concentration in blood was observed (see table 4-4). This relationship did not change after prolonged exposures, however, the TBA levels increased by a factor of about 5, from week 2 to week 6, and subsequently decreased in week 15. This may indicate an induction of the MTBE metabolism after repeated MTBE-exposure and subsequent enhanced TBA elimination. Whereas no TBA was detected in fat, this metabolite was found in the brain after the highest MTBE exposure. The values are comparable with the TBA concentrations determined in blood at the individual time points. The data indicate that TBA is not accumulated in fat and rapidly perfused tissues. MTBE exposure caused a transient increase in UDP-glucuronosyltransferase activities in liver and kidney microsomes, but had almost no effect on hepatic microsomal cytochrome P450 content and only a slight inducing effect on kidney microsomal cytochrome P450 content.

In study A, TBA was formed rapidly from MTBE and was detected in the blood 15 minutes after intravenous and oral administration, approaching its Cmax within 1 to 4 hours. After dermal administration, TBA was detected 1 hour (first sampling point) after the start of the MTBE administration, approaching Cmax within 4 to 7 hours.

In the inhalation experiments of study B, TBA was detected 10 min after the start of exposure approaching steady state and Cmax within the 6-hour exposure period. The Cmax-values for TBA were of the same order of magnitude for all dose groups, indicating similar transformation rates for MTBE to TBA. In oral experiments with male and female rats, the increase in MTBE and TBA plasma AUC was not proportional to the increased dose: The MTBE-AUC was higher than expected whereas the TBA-AUC was proportionally lower than expected. Similar results were observed with inhalation: The MTBE plasma AUC was much higher than expected and the increase in TBA-AUC plasma ratios was less than expected. These results suggest that the MTBE metabolising enzymes are saturated at the high oral dose of 400 mg/kg and at the high inhalation exposure of 28.800 mg/m^3. The apparent half life of TBA was slightly longer than that of MTBE, ranging from 1 to 3.4 hours depending on administration routes and dose. After 14 days of repeated inhalation exposure to 1.440 mg/m^3 the apparent half lives in males of both MTBE and TBA were shorter than after a single exposure to 1.440 mg/m^3; in females, in contrast, only the half life of TBA was decreased. Since an additional decrease in MTBE-AUC and an increase in TBA-AUC was observed after repeated inhalation exposure, it appears that MTBE exposure exerts a slight induction of its own metabolism. This is in agreement with the data reported by Savolainen *et al* (1985) and Brady *et al* (1990).

The formation of TBA from MTBE was also observed in the mass balance study C. TBA was found in expired air at the first sampling time point (3 hours) after intravenous, oral and dermal administration

of MTBE. However, the amount of TBA eliminated via this route was only 1 to 6% of the administered dose. HPLC-analysis of the urine showed two major and two minor peaks of radioactivity. The two major peaks were identified as 2-methyl-1,2-propanediol and 2-hydroxyisobutyric acid. The two minor peaks were not identified. The metabolite profile of radioactivity in the urine was similar for both male and female rats for all routes of exposure, indicating that metabolic pathways are not sex- or route-dependent. The proportion of 2-hydroxyisobutyric acid increased with time after dosing, while the proportion of 2-methyl-1,2-propanediol and of one of the minor peaks decreased, suggesting the latter were intermediates in the formation of 2-hydroxyisobutyric acid.

In study E, radiolabelled formic acid accounted for 96.6% of the urinary radioactivity. The differences in metabolites found by Zacharias and Eschbach (1984) compared to those found by Bio-Research Laboratories (1990c, 1990d, 1991) in studies C and D respectively and may partly be due to differences in the labelled material used (the methyl carbon was labelled in addition to the central butyl carbon). Different methods for trapping volatile compounds from expired air and other analytical methods may be other reasons.

The authors of study J analysed the blood of rats for MTBE and methanol after intragastric administration of MTBE. MTBE appeared in the first blood sample taken after the administration (15 min). After reaching the peak concentration after 0.9 hours, the MTBE concentrations decreased gradually to almost zero at 6 hours resulting in a half life of 1.26 hours when analysed by a one compartment model. Methanol also appeared in the first blood sample taken after the administration (15 min) and methanol concentrations increased for the next 3 hours. On a molar basis the ratio of methanol to MTBE increased over time from 11 to 16 to 25 at 15 minutes, 1 hour and 2 hours respectively, reaching 262 at 6 hours. The results are difficult to understand with regard to methanol. Methanol is formed from MTBE via formaldehyde (see figure 4-1) and current knowledge (Horton *et al*, 1992; Perkins *et al*, 1995) would predict that the small amounts of methanol formed from a dose of 0.379 mg/kg MTBE are removed very rapidly, which apparently is not the case.

Borghoff *et al* (1995) described a physiologically based pharmacokinetic model that predicted successfully the MTBE dosimetry after *iv*, oral and inhalation administration of MTBE as described in studies A, B, C and D (table 4-1). They optimised Vmax and Km for MTBE metabolism from gas uptake data and found that two saturable pathways were necessary to adequately model the measured data. The first pathway had high capacity and low affinity with a Vmax of 0.104 mmol/(h*kg) and a Km of 0.264 mmol/L. The second pathway had low capacity and high affinity with a Vmax of 0.008 mmol/(h*kg) and a Km of 0.001 mmol/l.

Quantitative Aspects of Formaldehyde Formation from MTBE

Although formaldehyde metabolism was not specifically investigated in the context of MTBE metabolism, plenty of information is available from other investigations (Heck *et al*, 1990). Formaldehyde is a physiological intermediate in mammalian systems that is generated intra-cellularly during steroid biosynthesis and the degradation of choline, glycine, and serine. Furthermore, it occurs as an intermediate in cellular metabolism required for the biosynthesis of purines, thymidine, and certain amino acids, referred to as the one-carbon pool. The most important pathway in formaldehyde metabolism appears to be oxidation to formic acid (see figure 4-1) catalysed by formaldehyde dehydrogenase (FDH). The true substrate for FDH is S-hydroxymethyl-glutathione, i.e. formaldehyde reversibly conjugated with glutathione (GSH). This conjugate is oxidised to formyl-glutathione with NAD^+ as cofactor and subsequently hydrolysed to formic acid. Formic acid as well as formaldehyde itself is incorporated in the one-carbon pool via different forms of tetrahydrofolic acid (IPCS, 1989).

Quantitative information on the probable fate of formaldehyde formed from MTBE can be obtained from other compounds that are also metabolised to formaldehyde. A physiologically-based pharmacokinetic (PBPK) model for methanol deposition in the rat, the monkey, and man used two enzymatic pathways to describe the *in vivo* metabolism of methanol to formaldehyde (Horton *et al*, 1992). The high affinity pathway had a Km of 1.06 mmol/l (33.92 mg/l) and a Vmax of 0.48 mmol/(h*kg) (15.41 mg/(h*kg)). The corresponding values for formaldehyde detoxification were 0.127 mmol/l and 7.69 mmol/(h*kg). Consequently, formaldehyde increases have not been observed in animals treated with methanol (Tephly 1991, Liesivouri and Savolainen, 1991). This is in agreement with findings by Lutz (1986) who investigated the effect of large doses of methanol and aminopurine (precursors of endogenous formaldehyde) but did not observe any significant increase in hepatic DNA-protein cross-links, although a 60-fold higher rate of formaldehyde production was calculated in comparison with endogenous production.

The Km and Vmax values for formaldehyde formation from MTBE determined for rat liver microsomes by Brady *et al*, (1990) were 0.67 mmol/l and 1.22 nmol/(min*mg), respectively. Assuming 10 g liver and 20 mg microsomal protein/g liver for a 250 g rat, this value can be scaled to a 1 kg rat ($BW^{0.74}$) and a Vmax of about 0.041 mmol/(h*kg) can be calculated. Such recalculated values from Brady *et al*, (1990) have recently been used in a PBPK model that successfully described the uptake kinetics of MTBE in rats (Charest-Tardif *et al*, 1995). The PBPK model by Borghoff *et al* (1995) used two saturable pathways to describe MTBE metabolism to TBA and formaldehyde. The Vmax values were 0.104 and 0.008 mmol/(h*kg), the Km values were 0.264 and 0.001 mmol/l. The comparison of the enzymatic constants for MTBE metabolism to formaldehyde with those for formaldehyde detoxification (Km 0.127 mmol/L and Vmax 7.69 mmol/(h*kg)) reveals that the likelihood of an increased formaldehyde concentration following MTBE exposure is much smaller than for methanol.

Human data (see 4.6) also indicate rapid metabolism of MTBE to TBA and formaldehyde. There is no indication that the principal pathways of biotransformation for TBA and formaldehyde in humans differ from those in experimental animals.

Evaluation

Metabolism of MTBE has been investigated in a number of well designed *in vitro* and *in vivo* studies. Although only the rat has been investigated in any detail, the findings are considered representative of mammalian metabolism in general. They demonstrate that MTBE is metabolised to formaldehyde and TBA via O-demethylation of MTBE. CYPIIE1 and CYPIIB1 appear to be the isoenzymes responsible for this reaction. Subsequently formaldehyde is rapidly metabolised to methanol, formic acid and/or CO_2. Formic acid as well as formaldehyde itself enter the physiological one-carbon pool. TBA is oxidised to 2-methyl-1,2-propanediol and subsequently to 2-hydroxyisobutyric acid. Prolonged MTBE exposure appears to induce additional MTBE metabolism however, the magnitude of this induction is regarded as being too small to influence significantly the toxicity of MTBE. Even at high MTBE doses, no increase in intracellular formaldehyde concentration is expected, since the pathways for formaldehyde metabolism have a very high capacity.

4.5 ELIMINATION

The apparent half life for MTBE was not significantly different in male and female rats and was in the range of 0.45 to 2.3 hours (studies A and B). Although some differences were observed in the total plasma clearance between high- and low-dose groups, the values were generally similar in all dose groups, and ranged from 273 to 573 ml/h. These results indicate that the general kinetics of MTBE elimination are not route or sex dependent.

Mass balance studies C and D confirmed the results obtained with unlabelled MTBE. Most of the radioactivity administered via the intravenous, oral, dermal, or inhalation route was excreted via urine and expired air whereas elimination via the faeces was only a minor excretion pathway. The excretion of radioactivity via expired air was virtually complete 3 hours after treatment, and consisted mainly of unchanged MTBE. Exhaled TBA represented only 1 to 6% of the administered radioactivity which is consistent with the blood/air partition coefficient of 481 measured by Borghoff *et al* (1995). Urinary excretion of metabolites (see above) was slow compared to exhalation and recovery of radioactivity continued up to 36 hours after treatment. A shift of excretion route from urine to expired air was observed with increasing dose. This indicates that metabolic pathways may become saturated at high-doses, leading to the appearance of increased amounts of unchanged MTBE in exhaled air. Excretion percentages were similar after single inhalation and repeated inhalation exposure.

Urinary radioactivity accounted for an average of about 3% of the administered *ip* dose in study E (table 1). A total of 92% of the administered radioactive dose was eliminated in expired air, 99.1% of which was attributed to MTBE, and 7.38% of the administered dose was eliminated as radiolabelled carbon dioxide. Mass balance was complete 48 hours after treatment.

In study F the bulk of the injected radioactive material was recovered in the expired air from rats. The greatest portion was recovered within 12 hours of treatment. Total ^{14}C recoveries within 96 hours of 67 to 82% were obtained in the expired air samples. Small amounts were recovered in the urine (3 to 5%) and faeces (0.5 to 1.5%) 48 hours following administration.

In study G in monkeys, the bulk of the expired radioactivity was recovered within 8 hours of *ip* dosing. Total ^{14}C recoveries within 96 hours of 69 to 72% were obtained in the expired air samples. About 5% appeared in the urine, less than 0.5% was excreted via the faeces.

Pulmonary elimination after intraperitoneal administration of MTBE was investigated in male ddY mice at 50, 100, and 500 mg/kg (Yoshikawa *et al*, 1994). The calculated half lives for the two observed elimination phases were 45 and 80 min. The pulmonary elimination ranged from 23.2 to 69% of the total dose. Most of the exhaled MTBE was eliminated within 3 hours.

Human experimental data (see 4.6) indicate that elimination kinetics of MTBE and TBA do not differ from the data obtained in animal experiments.

Evaluation

Elimination of MTBE has been investigated in a number of well designed studies conducted in rats. Supporting information is also available from mice and monkeys. Elimination of MTBE and MTBE metabolites is rapid and is completed to a large extent within 24 hours after administration of MTBE. Elimination proceeds mainly via exhalation or metabolism to TBA and formaldehyde with subsequent excretion of metabolites in urine. Faecal excretion is only a minor pathway. The proportion of MTBE eliminated via these pathways is dose dependent. At higher doses a higher proportion of MTBE is exhaled indicating saturation of the metabolic pathways. Neither human nor animal data indicate that MTBE or MTBE metabolites accumulate in the body.

4.6 HUMAN DATA

Data from Nihlén *et al* (1995) and Johanson *et al* (1995) provide important information on the toxicokinetics of MTBE. Blood analysis was conducted on 10 human volunteers exposed in a chamber to 18, 90 and 180 mg/m^3 with concurrent light exercise (50 W). The authors observed a rapid increase

in MTBE blood concentrations and a steady state (about 110 µg/l) was reached after about 1 hour in the low exposure group. From the data it is not clear whether steady state was already achieved in the 90 and 180 mg/m^3 groups after 2 hours of exposure, but maximum concentrations were about 550 and 1,100 µg/l, respectively, and thus linearly related to the exposure concentration. MTBE-AUC and TBA-AUC were also linearly related to the MTBE exposure concentrations. The relative respiratory uptake of MTBE averaged 38% (range 32 - 42%) of the amount inhaled. MTBE elimination was complete about 12 hours after the start of the 2-hour exposure and blood decay curves were described mathematically using three half lives (7 min, 47 min, 6.6 hours). MTBE blood clearance was calculated to be 0.5 l/h/kg at all MTBE concentrations. The half-life of TBA elimination was 8 hours.

Pekari *et al* (1996) corroborated the findings of Nihlén *et al* (1995) and Johanson *et al* (1995). Four healthy volunteers were exposed to 0, 90 and 270 mg/m^3 for 4 hours. MTBE and TBA were monitored from blood, urine and exhaled air during and after exposure. During the last hour of exposure the lung retention was about 40% for MTBE and MTBE blood levels reached 11 µmol/l (970 µg/l) at 90 mg/m^3 and 29 µmol/l (2556 µg/l) at 270 mg/m^3 towards the end of exposure. Peak blood levels of TBA were found 15 to 45 min after cessation of exposure reaching 16 and 34 µmol/l (1,419 and 2,997 µg/l) at 90 and 270 mg/m^3 , respectively. Of the amount of MTBE taken up in the lungs, 58% was excreted unchanged in exhaled air and 1.4% in urine. Only 1.2% was excreted as TBA in urine. The blood elimination curve for MTBE showed a rapid and a slow phase with a half life for the slow phase of 5 hours. TBA showed a one-phasic elimination in blood with a mean terminal half life of 11.9 hours.

Two men and two women were exposed to 6.1 mg/m^3 MTBE in climate-controlled environmental chambers for one hour (Cain *et al*, 1994; 1996). Blood samples were obtained pre-exposure, during exposure at 2, 5, 10, 20, 30 and 60 min, and post-exposure at 2, 5, 10, 20, 40, 60, and 90 min. The blood was analysed by GC-MS for MTBE and TBA concentrations. The MTBE concentration rose steeply from 0.83 µg/l pre-exposure to 17.1 µg/l at the end of exposure, followed by a decline to a concentration of 6.32 µg/l at 90 min post-exposure. The TBA concentrations were highly variable. A mean value of 2.79 µg/l was measured pre-exposure , with values between 10 and 15 µg/l recorded from 30 min into the study and up to 90 min post-exposure. This is consistent with the continuous metabolism of MTBE to TBA. These data imply both a rapid uptake and rapid elimination of MTBE as was also observed in animal studies. The decay of MTBE concentrations in blood to half the maximum concentration took approximately 40 min. This is comparable with the half life of MTBE observed in rats.

A similar study was performed with one man and one woman exposed to 5 mg/m^3 MTBE for 60 min (Prah *et al*, 1994). The concentration of MTBE in blood rose rapidly during exposure to a peak level of 8 and 14 µg/l respectively and MTBE was rapidly metabolised to TBA. The TBA level in blood increased gradually to concentrations similar to the MTBE concentrations and reached a plateau for the duration of the sampling period of 7 hour. The clearance half life of MTBE was about 34 min.

Limited information on the distribution and elimination of MTBE was obtained from 113 patients receiving MTBE via intra-cystic infusion for dissolution of gallstones (Leuschner *et al*, 1991). The dose infused varied between 1 and 15 ml MTBE. In 27 patients, MTBE and TBA blood and urine concentrations were determined by headspace gas chromatography. After an average treatment time of 5.1 hours the mean MTBE blood concentration was 40 µg/ml. Five hours later, the concentration was 20 µg/ml, and 12 to 18 hours later, MTBE was only detected at trace levels. The TBA blood concentration at the end of treatment also had a mean value of 40 µg/ml and decreased to 25 µg/ml after 12 to 18 hours. In urine, the MTBE concentration was 18 µg/ml 5 hours after the start of treatment and was not detectable 12 to 18 hours later. The TBA urinary concentration 5 hours after beginning of the treatment was about 40 µg/ml and TBA was still detectable 12 to 18 hours later at about the same level. Methanol was found in traces in three patients, but no formaldehyde or formic acid were detected. Fatty tissue from the abdominal wall was sampled from 9 patients at the end of treatment and was analysed for MTBE. The mean MTBE concentration determined in fatty tissue was 0.135 mg/g after a mean treatment time of 9.5 hours. In a lactating patient, the MTBE and TBA concentration was determined in the milk after the end of a 4-hour treatment and 24 and 48 hours after the treatment. At 4 hours, the MTBE concentration was about 25 µg/ml, the TBA concentration 30 µg/ml. At 24 hours the MTBE concentration had decreased to about 3 µg/ml whereas the TBA concentration was still 20 µg/ml. At 48 hours both MTBE and TBA concentrations had reached the pre-treatment level.

Evaluation

The toxicokinetics of MTBE in humans have been investigated in a number of studies. Although limited in design and number of participants, these provide important information on the disposition of MTBE in man. The data from Nihlén *et al* (1995), Johanson *et al* (1995) and Pekari *et al* (1996) are considered particularly critical to this assessment. As with rodents and monkeys, MTBE is readily absorbed following inhalation exposure (average relative respiratory uptake of 38%), rapidly metabolised and completely excreted within 24-48 hours.

4.7 CONCLUSIONS

Animal studies provide extensive information on the toxicokinetics of MTBE and there is adequate supplementary information available from humans. Most of the toxicokinetic data were obtained from the rat. The limited data available from the mouse, the monkey and humans are consistent with the general toxicokinetic profile of MTBE observed for the rat and described above. Other studies are in progress to expand this data base (see chapter 9).

MTBE is rapidly absorbed from the gastrointestinal and respiratory tract. Absorption from the gastrointestinal tract is complete whereas bioavailability of MTBE after dermal administration in

occluded chambers is below 40% of the oral bioavailability. The absorbed MTBE is distributed uniformly, and at steady state fat contains a tenfold greater concentration than blood due to the lipid solubility of MTBE. Even with prolonged repeated exposures higher fat concentrations relative to other tissues are not expected on the basis of the fat/blood partition coefficient of about 10.

The absorbed MTBE is eliminated mainly via two pathways: exhalation of unchanged MTBE in the expired air and O-demethylation of MTBE catalysed by cytochrome P450 enzymes (CYPIIE1/CYPIIB1) resulting in the formation of *tert*-butanol (TBA) and formaldehyde. Some TBA is exhaled in the expired air but most TBA is further oxidised to 2-methyl-1,2-propanediol and subsequently to 2-hydroxyisobutyric acid which are both excreted in the urine. Faeces are only a very minor excretion pathway for MTBE or MTBE metabolites. Formaldehyde is rapidly metabolised to methanol, formic acid or CO_2. Formic acid as well as formaldehyde enter the physiological C_1-carbon pool. Elimination of MTBE and TBA are rapid processes leading in rats to elimination half lives of < 1 hour for MTBE and 1 to 3 hours for TBA following either oral or inhalation exposure.

With increasing oral or inhaled doses, a shift in the elimination pathways from urine to exhalation was observed, probably due to saturation of metabolising enzymes at high-doses. Therefore, effect data from animals following exposures to high concentrations are not necessarily predictive for exposure to lower concentrations.

Calculation of retained doses for inhalation exposures

In humans the retained percentage (net uptake rate) of the inhaled concentration has been determined for concentrations from 18 mg/m^3 to 180 mg/m^3 and has been averaged at 38% (see chapter 4.6). In another study 40% lung retention has been determined for exposures up to 270 mg/m^3 . Since this range of exposure concentrations is relevant for the human exposure situation (see chapter 7), a retained percentage of 40% has been used by the Task Force in this assessment to calculate the retained dose for human inhalation exposure.

For rats and mice it has not yet been possible to derive the retained percentage of inhaled MTBE from the available data. However, the blood/air partition coefficient of 11.5 may serve as a basis to estimate the retention of MTBE from a comparison with the retention of compounds with a similar blood/air and tissue/blood partition coefficients such as diethyl ether and benzene (EEC, 1993c; Medinsky *et al*, 1989). The uptake behaviour of the three compounds should be comparable and is modified only by metabolic processes after the uptake.

Dose dependent retained percentages have been estimated for diethyl ether and benzene. For diethyl ether in the rat these values range from 33% (at 150 mg/m^3), 33% (at 600 mg/m^3), 13.3% (at 3,000 mg/m^3) to 6.7% (at 6,300 mg/m^3) (EEC, 1993b), for benzene in the rat from 33% (at 33 mg/m^3),

44% (at 75 mg/m^3), 22% (at 680 mg/m^3) to 15% (at 2,260 mg/m^3) and for benzene in the mouse from 50% (at 33 mg/m^3), 52% (at 75 mg/m^3), 38% (at 340 mg/m^3) and 9.7% (at 2,260 mg/m^3) (Sabourin *et al*, 1987). Since the metabolic rates for diethyl ether and benzene are lower when compared with MTBE, a higher retention of MTBE is expected at concentrations not saturating the metabolic pathways. At saturating concentrations a comparable retention is expected.

On the basis of the comparisons made with benzene and diethyl ether the Task Force estimated the percentage of MTBE retained in the rat and mouse to be 15% for exposure concentrations at or above 3,600 mg/m^3. This value has been used to calculate the retained doses after inhalation exposures. At concentrations between 360 and 3,600 mg/m^3, a retained percentage of 30%, and for the range form 3.6 to 360 mg/m^3 a retained percentage of 60% is expected. PBPK models currently under development will refine the estimates of the retention values used here (see also chapter 9).

5. ANIMAL TOXICITY

Toxicity has been determined in several acute and repeat-dose studies in laboratory rodents and in one case in rhesus monkeys. The results are described below. The details of the experiments are contained in Appendix II.

5.1 ACUTE TOXICITY

The following LD_{50} and LC_{50} values are representative:

Oral	rat	3,800 mg/kg	(Hathaway *et al*, 1970a)
		3,866 mg/kg	(ARCO, 1980)
	mouse	4,000 mg/kg	(Little *et al*, 1979)
Dermal	rat	> 6,800 mg/kg	(Shell, 1971)
	rabbit	>10,000 mg/kg	(ARCO, 1980)
	rabbit	>10,200 mg/kg	(Hathaway *et al*, 1970a)
Inhalation (4h)	rat	85,000 mg/m^3	(Hathaway *et al*, 1970a)
	rat	120,300 mg/m^3	(ARCO, 1980)
	rat	142,000 mg/m^3	(ARCO, 1980)
Intravenous	rat	0.41 g/kg	(Snamprogetti 1980)
Subcutaneous	rat	4.96 g/kg	(Snamprogetti 1980)
	mouse	2.67 g/kg	(" ")
	mouse	1.01 g/kg	(" ")

Effects of non-lethal doses following oral exposure included diarrhoea, slight CNS effects, transient anaesthesia, ataxia, laboured breathing and tremors.

In the dermal studies no deaths were reported at any dose. Skin irritation was reported at the site of application (erythema, blanching, epidermal thickening, acanthosis, focal necrosis and hyperexcitability).

Clinical signs in inhalation studies were typical of a volatile, low molecular weight organic solvent and included mucous membrane irritation, irregular laboured breathing, ataxia, inco-ordination, loss of righting reflex, prostration and death depending on the concentration. Survivors were fully recovered a

few hours after cessation of the treatment. Inhalation studies of shorter duration have also been conducted in the mouse.

In the intravenous studies clinical changes were reported to be transient with animals returning to normal within 20 minutes .

Evaluation

Although the acute studies with MTBE were carried out before EU test guidelines were established, several are judged to be of sufficient quality for the determination of acute effects.MTBE has a low order of acute toxicity with the most immediate effect at high exposures being anaesthesia (CNS depression). Clinical signs at non-lethal concentrations have been local irritation (typical of organic solvents) and transient behavioural effects.

5.2 IRRITATION

This section covers local effects from exposure to MTBE liquids and vapours.

Skin irritation

Undiluted MTBE (0.5 ml) was applied to abraded and non-abraded skin of six rabbits for 24 hours under occluded conditions (Cuthbert, 1979). Moderate erythema and oedema was noted in all animals at 24 and 72 hours (primary irritation index (PII) = 3.36). Effects were slightly more pronounced on abraded skin than on non-abraded skin.

Two samples of MTBE were tested on abraded and intact rabbit skin (ARCO,1980) using a 24 hour occluded exposure period. Neither was considered a primary skin irritant, although one of the samples induced slight to severe erythema and blanching in two of six animals. No oedema was noted. Histological effects ranged from normal to slight acanthosis or slight focal epidermal necrosis of the abraded site but these were concluded to be due to a parasitic infection or trauma rather than chemical irritation.

A later skin irritation study was conducted according to OECD guidelines (Murmann, 1985a). Moderate erythema and moderate to severe oedema occurred in all 6 rabbits from 1 hour to day 8 of the 14 day observation period. On day 14 desquamation and flaking was observed at the treatment sites. The Primary Irritation Index (PII) was 5.0 while the means of the scores at 24, 48 and 72 hours were 3.0 for erythema and 2.3 for oedema.

Eye irritation

Snamprogetti (1980) tested MTBE for eye irritation in rabbits. Instillation of 0.05 ml into one eye caused congestion of the conjunctivae, thickening and hypersecretion, all of which were reversible. Two samples were tested for eye irritation (ARCO, 1980). Each sample (0.1 ml) was instilled into one eye of 9 rabbits. The treated eye of 3 of the rabbits was washed immediately after treatment, the remaining eyes were not. Draize scores were determined at 24, 48 and 72h and 7d. One sample was practically non irritating to washed and unwashed eyes. Erythema was the only reaction, reaching a maximum score of 1.0 in 3/6 animals, 24h after instillation and declining to zero by 72h (mean of the 24, 48 and 72h scores was 0.2). The other sample was slightly irritating to unwashed eyes, producing slight erythema (mean score 1.0), slight chemosis (mean score 0.4) and slight corneal effects (mean score 0.1) at 24 hours. All effects had resolved within 7 days. With washed eyes the effects were slightly more pronounced. Mean 24, 48 and 72h scores were 1.4 (erythema), 0.6 (chemosis) and 0.3 (corneal opacity).

Industrial Biotest (1969) reported transient eye irritation after 0.1 ml of MTBE was instilled into the eyes of 5 rabbits. Effects appeared within 1 min after instillation and were limited to the iris and conjunctivae. Eyes had returned to normal after one week.

Two eye irritation studies were conducted according to OECD guidelines (Cuthbert, 1979, Murmann,1985b). In both studies, moderate erythema and slight chemosis and discharge were observed at 1 hour after instillation, declining to normal by 7 days. There were no effects on iris or cornea and mean scores at 24, 48, 72 hours did not exceed current EU values for classification.

Respiratory and sensory irritation

Respiratory and sensory irritation was measured using an Alarie bioassay in Swiss-Webster mice. A series of short-term inhalation studies was conducted (Tepper *et al*, 1994) with exposures up to 1 hour and vapour concentrations of 300-30,000 mg/m^3. Sensory irritation ranging from slight to severe (expressed as reduced breathing rate and changes to the pattern of tidal breathing) was recorded at all concentrations. No respiratory irritation was observed up to 10,000 mg/m^3. Exposure to 30,000 mg/m^3 produced both sensory and respiratory irritation (the breathing rate was reduced by 52%). At 300 mg/m^3, the breathing rate was reduced by 13% only. This was regarded by the authors as the threshold of sensory irritation. The effects observed are similar to those seen with known respiratory irritants, such as ozone, however, there was no evidence of lung injury as determined by measurements of total protein and lactate dehydrogenase (LDH) activity in broncho-alveolar lavage fluid. Recovery to normal respiration patterns was almost complete, even at the highest dose level, within 15 min after removal of the animals from the exposure chamber. The authors calculated an

RD_{50} concentration of 16,600 mg/m^3 (the exposure concentration at which the respiratory rate is decreased by 50%, as predicted by linear extrapolation).

Other local effects from vapour exposure

Ocular effects from exposure to MTBE vapours have been reported (ARCO,1980) such as ocular discharge and early irritation at ≥ 68,000 mg/m^3. Lachrymation has occasionally been described in repeated exposure studies with rat and mouse (e.g. Biles *et al*, 1987). Effects from vapour contact on skin and eyes have been observed in several inhalation studies. Clinical observations from whole body inhalation studies and histological findings from exposed skin sections after up to 13 weeks exposure to ≥ 28,800 mg/m^3 have not revealed treatment-related local effects (Dodd and Kintigh, 1989; Biles *et al*, 1987, see also para 5.4).

Evaluation

One skin irritation and two eye irritation studies have been conducted to current OECD guidelines. MTBE is an irritant to rabbit skin. It caused moderate to severe erythema and moderate oedema in a study conducted to OECD guidelines. These effects resolved after two weeks. MTBE is slightly irritant to rabbit eyes.

MTBE vapour causes slight to transient irritation to the respiratory system of laboratory animals. A sensory irritation threshold of 300 mg/m^3 and an RD_{50} concentration of 16,600 mg/m^3 were reported. For sensory irritants Alarie (1981) recommended that a provisional threshold limit value could be set at 3% of the RD_{50} to protect workers for respiratory irritation. If this procedure is applied to MTBE 500 mg/m^3 may be regarded as provisional threshold limit (occupational exposure limit).

5.3 SENSITISATION

Two animal studies have been conducted to determine skin sensitisation. Although no systematic survey on sensitisation in humans has been conducted, cases of human skin or respiratory sensitisation have not been reported.

MTBE was tested in a Magnusson and Kligman maximization test in guinea pigs (Cuthbert, 1979). Twenty test and 10 control animals were used and MTBE was applied at a concentration of 1% in water. There were no positive reactions in the test group.

In the second study (ARCO, 1980) using the Landsteiner technique, the MTBE group received initially intradermal injections of 0.5 ml of a 0.1% MTBE solution in water, followed every other day, for

3 weeks, by injections of 0.1 ml (a total of 10 injections per animal). Two weeks later they received a challenge injection of 0.05 ml of a 0.01% MTBE solution. No sensitisation reactions were recorded.

Evaluation

The conduct of these animal studies was adequate though not entirely consistent with current guidelines. Neither test indicated MTBE is a potential skin sensitiser. Furthermore, there are no indications that MTBE can act as a skin or respiratory sensitiser in humans.

5.4 REPEAT-DOSE TOXICITY

Discussed below are repeat-dose studies in rat and mouse, up to 90 days duration via oral (gavage) and inhalation (see Appendix I, Table 5.2 for details.). These studies were conducted to determine whether protracted exposure to MTBE may cause toxicity. The studies are presented in order of increasing exposure period.

Oral studies

Groups of ten male and ten female Sprague-Dawley rats were administered MTBE by gavage in corn oil at 0, 357, 714, 1,071 and 1,428 mg/kg/d, daily for 14 days (Robinson *et al*, 1990). There were no treatment-related deaths. There were occasional clinical signs in males and females at all dose-levels but, they were considered incidental to treatment, except for the local irritation of the upper gastrointestinal tract (sub-mucosal oedema, sub-acute inflammation, epithelial hyperplasia) at ≥ 357 mg/kg/d. Significant systemic effects in both sexes were confined to the highest dose groups. These included profound transient anaesthesia immediately after dosing, reduced food consumption, reduced body-weight gain, increased blood Hct and Hb levels (males), decreased monocyte numbers (males), increased relative kidney weights in males at ≥ 1,071 mg/kg/d and decreased absolute and relative lung weights (females). Significant changes in several clinical chemistry values were also observed, blood urea nitrogen (BUN) was reduced in males and females and creatine levels were reduced at the highest dose in females. In males, aspartate amino transferase (AST) and LDH were reduced at 1,071 and 1,428 mg/kg and cholesterol was increased at 1,428 mg/kg. There were no gross changes at necropsy. Histopathologic examination of the male kidneys revealed increased hyaline droplets in proximal renal tubules at the highest dose. The authors concluded that MTBE had low toxicity with significant treatment-related effects occurring only at the highest dose (1,428 mg/kg/d). The most significant effects were anaesthesia and renal changes. The NOAEL derived from this study is 714 mg/kg/d.

Groups of 10 Sprague-Dawley rats per sex were dosed by oral gavage 5 d/wk over 4 weeks with MTBE in water at 0, 90, 440 and 1,750 mg/kg/d (IIT Research Institute, 1992). There were no treatment-related deaths. Mean body-weight gains and final body weights were similar in control and MTBE-treated groups. Clinical findings in MTBE-treated animals in all dose groups were limited to salivation, hypoactivity and ataxia which occurred immediately after dosing, particularly in the high- and mid-dose groups. These effects were transient and the animals returned to normal promptly after dosing. Gross necropsy findings in untreated and treated groups were similar. Occasional differences in haematology and clinical chemistry values between the groups were observed, but the only statistically-significant finding was an increase in cholesterol in high-dose animals (both sexes). Robinson *et al* (1990) observed a similar effect, but this was not dose-related. No significant increase in absolute organ weights were noted although absolute kidney and liver weights were increased. Mean kidney weights relative to mean terminal body weights were increased in mid- and high-dose males and low- and high-dose females, as were relative liver weight and adrenal weight in high-dose males and relative liver weight in high-dose females. There were no gross changes observed at necropsy. Microscopic examination revealed hyaline droplets in proximal convoluted tubules of mid- and high-dose male kidneys. Microscopic lesions, considered to be due to local irritation, were seen in the forestomach of most high-dose animals. The LOAEL in this study was 440 mg/kg/d and the NOAEL 90 mg/kg/d.

In a 90-day study, groups of 10 male and 10 female Sprague-Dawley rats were administered by gavage 0, 100, 300, 900 and 1,200 mg/kg/d MTBE in corn oil (Robinson *et al*, 1990). Profound narcosis occurred at 1,200 mg/kg/d immediately after dosing (with recovery within 2 hours) and all MTBE-treated animals had diarrhoea from day 3 onwards. Body weight gain was significantly reduced at the top dose in females (9%) and there were minor variations observed in the haematological parameters in the 300 mg/kg (males) and 1,200 mg/kg (males and females) dose groups (e.g. small increases in RBC, HB and Hct and decreases in WBC in females and increased monocyte numbers in males). Clinical changes were similar to those seen in the 14-day study described previously. None incriminates MTBE as a significant systemic or target-organ toxin but noteworthy is the finding that BUN levels were statistically-reduced in all treatment groups when compared with controls. Additionally, there was a trend towards elevated cholesterol levels in males. Absolute and relative kidney and relative liver weights were significantly increased in males at ≥ 900 mg/kg/d. Relative kidney weights were also significantly higher in females at ≥ 300 mg/kg/d but this effect was not dose-related. Kidney changes in males were reflected, histologically, by tubular degeneration, accumulation of hyaline droplets and increased a2u-globulin. Some degeneration was present in all groups including the controls but effects were most apparent in the highest dose group. Absolute and relative lung weights in males were increased at 1,200 mg/kg/d. MTBE exhibited low toxicity and significant effects were limited to doses ≥ 900 mg/kg/d. As in the 14-day and 28-day studies, treatment-related effects included profound transient anaesthesia, slightly reduced food intake and correspondingly lower body-

weight gain, minor haematological and clinical chemistry changes and increased weight of some organs, principally kidney (with associated histopathological changes), liver and lung. On the basis of kidney and liver effects in males the authors concluded that the NOAEL was 300 mg/kg/d. For other effects the NOAEL was 900 mg/kg/d.

Inhalation Studies

Inhalation studies were conducted in the rat and the mouse and observations at exposures in excess of 3,600 mg/m^3 were similar to those reported in the studies using oral gavage. Significant toxicity was not noted at lower exposures.

A 9-day inhalation study was conducted with groups of 20 male and 20 female Sprague-Dawley rats in order to set dose levels for a teratology study. MTBE vapour concentrations were 0, 360, 1080, 3,600 and 10,800 mg/m^3 for 6 h/d (Terrill and Daly, 1984). Clinical signs were monitored daily. There were no treatment-related deaths or changes in clinical signs. Effects observed at 3,600 and 10,800 mg/m^3 included chronic inflammation of the nasal mucosa and trachea (in 27/40 exposed animals at ≥ 3,600 mg/m^3) increased serum phosphatase levels (females at 1080 and 3,600 mg/m^3) and increased relative liver weights (both sexes, 10,800 mg/m^3, only). There were no effects on kidney or other major organs, blood, immune system, bone and muscle and no histopathological changes. The authors commented that during exposure no effects on behaviour were noted.

A two-week vapour inhalation study was carried out by Hathaway *et al* (1970a). Groups of 5 male and 5 female albino rats were exposed to 0, 10,000 or 30,000 mg/m^3 MTBE vapours in air for 6h/d, 5d/wk. All animals were observed for body weight, mortality and clinical changes. At termination, haematology, clinical chemistry and urinalysis were performed. Histopathology of tissues obtained from top-dose and control animals was conducted. No adverse effects were reported.

Three separate short-term, repeat-dose inhalation studies with rats and mice were reported by Snamprogetti (1980). In the first, groups of 20 Wistar rats were exposed for 30 days (10 min/d, 5 d/wk) to MTBE concentrations of 0, 180,000 and 288,000 mg/m^3. In-life clinical signs and food consumption were monitored and at termination, body weights and a range of organ weights were measured. Clinical chemistry, haematology, liver function measurements and urinalysis were also carried out. Findings were similar in both treated and control groups.

In the second study, groups of 30 Swiss mice were exposed for 30 days (5 or 10 min/d, 5 d/wk) as above to 180,000 or 288,000 mg/m3 of MTBE vapour. Motor activity and co-ordination were monitored in this study. Treatment-related effects were not observed.

In the third study, groups of Wistar rats (25 animals/sex/dose) were exposed for 120 days (10 min/d, 5d/wk) to approximately 180,000 mg/m^3 of MTBE vapour. In-life and terminal examinations were similar to those undertaken in the previous studies. No treatment-related effects were observed.

Dodd and Kintigh (1989) conducted two 13-day studies in rodents (CD-1 mice and Fischer 344 rats). Little evidence of toxicity was noted in either study and it was generally limited to transient effects on the nervous system. Relative liver, kidney and adrenal weights were increased in the rat at the two highest dose levels but there were no accompanying histopathological changes. NOAEL's were 14,400 mg/m^3 (mouse) and 7,800 mg/m^3 (rat).

In a subsequent study, groups of 25 male and 25 female Fischer 344 rats were exposed to vapour concentrations of 0, 2,880, 14,400 and 28,800 mg/m^3, for 6h/d, 5d/wk for 13 weeks (Dodd and Kintigh, 1989). Survival in treated and control groups was similar and the only clinical finding of note was ataxia which was observed each day immediately after exposure in animals exposed to 28,800 mg/m^3, during the first four weeks of the study. Occasionally minor changes were recorded at 14,400 and 28,800 mg/m^3 in the functional observation battery (e.g. elevated body temperature - see also section 5.5) and a slight reduction in weight gain during the first three weeks of the study, corresponding to reduced food intake. There were no treatment-related gross lesions at termination. Statistically significant dose-related increases in mean absolute and relative weights of liver, kidneys and adrenals were observed in males. At 2,880 mg/m^3 these increases were 8% (liver), and 4% (kidney), at 28,800 mg/m^3 39% (liver), 49% (kidney) and 55% (adrenals). There were similar increases in females at 14,400 and 28,800 mg/m^3. No treatment-related histopathological changes were observed in these organs, in tissues of the nervous system or in other visceral organs. A slightly higher incidence of lymphoid hyperplasia, spleen haemosiderosis and hyaline droplets in kidney tubules was noted in males exposed to 28,800 mg/m^3 MTBE. Mild haematological changes (decreased erythrocyte counts and increased reticulocyte counts) were present in MTBE-exposed animals. In males, effects were noted at all dose levels and were not treatment-related. In females, changes were noted only at 28,800 mg/m^3. The small alterations in serum chemistry included elevated calcium and protein values, decreased levels of aspartate and alanine transferases and decreased glucose. In males, serum corticosterone was increased at 28,800 mg/m^3. This finding agrees well with the increase in adrenal gland weight and provides a possible explanation for some of the other reported changes, e.g. elevated serum corticosterone is often associated with increased liver size. In conclusion, the findings in this study suggest that exposure to MTBE causes only mild toxicity in rats. The NOAEL in this study was 2,880 mg/m^3.

In a sub-chronic study in Sprague-Dawley rats, groups of 10 males and 10 females were exposed to MTBE vapours at 0, 900, 1,800 and 3,600 mg/m^3 for 6h/d, 5d/wk for 13 weeks (Greenough *et al*, 1980). There were no deaths. Transient dose-related anaesthesia was reported as was lower weight gain at the top dose. Male rats showed significant increases in blood cell haemoglobin at 3,600 mg/m^3

which the authors considered to be incidental to the treatment. Mean absolute and relative lung weights were reduced in females at the top dose. The authors judged this to be of no toxicological significance. There were no treatment-related effects on haematology, clinical chemistry, urinalysis and on gross and histopathological changes at termination. In this study MTBE vapours at up to 3,600 mg/m^3 caused no marked toxicity. The NOAEL was 3,600 mg/m^3.

In another series of studies Chun and Kintigh (1993) studied the effects of repeat inhalation exposure of Fischer 344 rats (4 and 13 weeks) and CD-1 mice (4 weeks) to MTBE. The study designs were similar and only the 4-week studies are summarised here. Groups of 10 animals per sex were exposed either to filtered air (control) or to MTBE vapour. Target concentrations were 0, 1,440, 10,800 or 28,800 mg/m^3 and exposure conditions were 6h/d, 5d/wk. Additional animals (5/sex/group) were included in the control and high-dose groups to be sacrificed after an additional 16-day recovery period. In addition to the standard end-points, cell proliferation in rat kidney and mouse liver was investigated using a BrdU (bromodeoxyuridine) immunohistochemical technique. No exposure-related mortality was observed in any of the groups. The principle findings were confined to the mid- and high-dose groups, and included clinical signs (e.g. ataxia, blepharospasm, etc.), increased kidney, liver and adrenal weight in male and female rats and increased liver weights in mice of both sexes. Microscopic evaluation of the proximal convoluted tubules in the kidneys of male rats from the 10,800 mg/m^3 and 28,800 mg/m^3 groups showed greater protein accumulation compared with the controls. In addition, increased cell proliferation was observed in the kidneys of male rats from the 10,800 and 28,800 mg/m^3 groups. In mice the only microscopic lesion that could be attributed to MTBE exposure was a hepatocellular hypertrophy of the centrilobular area, which was present in male and in female mice of the 28,800 mg/m^3 group. The lesions were slightly more severe in males. Cell proliferation was increased in livers of mice exposed to 28,800 mg/m^3 at day 5 of the evaluation (increased 12-fold in males and 16-fold in females). This finding was only significant in females.

Swenberg and Dietrich (1991) stained kidney sections of the animals of the Dodd and Kintigh (1989) study with a special staining technique in order to detect α_{2u}-globulin. They observed no α_{2u}-globulin stain in controls and treated female rats. In treated males an increase in staining was observed but this was not dose-related. The staining was diffuse and present in the proximal tubules rather than distinct and localised within the lysosomes, as is normally the case with α_{2u}-globulin induced by exposure to d-limonene.

Fowler and Chun (1993) examined kidney sections from the same study and Fowler and Martin (1994) additionally examined sections from the 28-day study (Chun and Kintigh, 1993). Both used a Mallary-Heidenhain stain and both provided conclusions that were consistent with those of Swenberg and Dietrich (1991), i.e. no evidence for the presence of α_{2u}-globulin in female rat kidneys and no

compelling evidence for its presence in the male kidneys. In males exposed to ≥ 10,800 mg/m^3 increased staining was evident but not typical of a classical α_{2u}-globulin inducer.

Evaluation

MTBE has been studied in the rat and mouse in well-conducted studies that have involved multiple exposures by ingestion (gavage) or inhalation. Findings have been similar in all studies. In general, the findings show that MTBE posesses a low order of toxicity in rodents.

Few adverse effects have been reported and they have been largely confined to animals at the high-dose levels where CNS impairment was the most immediate consequence of treatment (see chapter 5.5 for detailed information). Other treatment-related findings at high-doses were irritation of the respiratory and gastrointestinal tracts, enlargement of the liver and associated minor variations in certain serum enzymes, reduced food intake and body weight gain as well as kidney enlargement, particularly in male rats, possibly associated with increased presence of hyaline droplets and α_{2u}-globulin

Whilst the reported kidney changes may be specific to the male rat and, therefore, not relevant in assessing the hazards of MTBE for man (Borghoff, 1993), there does, however, appear to be some evidence that the mechanism leading to this effect may not be entirely consistent with other agents e.g. gasoline and d-limonene that operate via a "classical" α_{2u}-globulin mechanism. This aspect will require further specific study.

From a 28-day oral study in rats, a NOAEL of 90 mg/kg bw (LOAEL 440 mg/kg bw) was determined and in a 90-day study a NOAEL of 300 mg/kg bw was established. This apparent anomaly arises from the different spacing of dose levels used. It is concluded that the NOAEL for oral repeated dose studies up to 90 days is 300 mg/kg bw in rats.

The effects of inhalation exposure to MTBE were examined in a number of studies, generally of good quality, of up to 13 weeks duration using rats and mice. They differ in range of concentrations and spacings between the exposure levels but in spite of this, effects of treatment were similar and the derived NOAEL's are of the same order (1,440; 2,880 and 3,600 mg/m^3). The LOAEL for these studies were 10,800, 14,400 and >3,600 (highest dose level) mg/m^3. For the purpose of this assessment 2,880 mg/m^3 is taken to be the NOAEL in rats and mice for repeat inhalation studies up to 90 days.

The daily retained dose per animal can be estimated using standard body weights and inhalation rates. For the Fisher 344 rat the US-EPA has compiled reference body weights and inhalation rates (EPA, 1988). For subchronic studies these values are 180 g and 0.19 m^3/d for male rats and 124 g and 0.14 m^3/d for female rats. Using these values together with an exposure value of 2,880 mg/m^3 and a

retention of 30% (see chapter 4.7), a daily retained dose of 228 and 244 mg/kg/d is obtained for male and female rats respectively. The US-EPA does not list values for CD-1 mice but has considered figures for B6C3F1 mice. Body weights and inhalation rates for this strain are 31,6 and 24,6 g, and 0.053 m^3/d and 0.040 m^3/d for males and females respectively. Using these values, the daily retained doses following exposure to 2,880 mg/m^3 are 362 and 351 mg/kg/d for males and females respectively.

5.5 NEUROTOXICITY

Neurobehavioural effects have been reported in several studies that were designed to measure these effects and also in conventional repeated dose studies discussed in section 5.4; specific neurotoxicity studies are listed in table 5-3 (see Appendix II).

Oral studies

Of the 3 acute LD_{50} study reports available, only one describes clinical effects as well as fatalities (ARCO, 1980). Some degree of central nervous system depression occurred at all dose levels (1,900-6,810 mg/kg) ranging from slight at the low doses to marked at ≥ 4,080 mg/kg at which lethality was greater than 50%. Other effects included ataxia, tremors, laboured breathing and loss of righting reflex in rats at doses ≥ 4,080 mg/kg/day, loss of righting reflex, ataxia at 3,160 mg/kg and 2,450 mg/kg/day and central nervous system depression at 1,900 mg/kg. Onset of these effects was rapid but they were transient, with animals returning to normal within a few hours.

In a conventional repeated gavage study (14 days) in Sprague-Dawley rats large daily oral doses of ≥ 1,200 mg/kg MTBE in corn oil caused profound anaesthesia immediately after treatment (Robinson *et al*, 1990). This effect lasted for about 2 hours with subsequent full recovery of motor and sensory functions within 6 - 12 hours. Males given 1,428 mg/kg/day (the highest dose) for 14 days had reduced absolute brain weights ($P = \leq 0.05$) compared with controls. In contrast, relative brain weights were significantly increased in females at the same dose. The significance of this finding is not clear to the task force and was not commented upon by the original authors.

Oral gavage administration at 90, 440, and 1,750 mg/kg/day for 28 days, caused occasional salivation in all groups and transient ataxia and/or hypoactivity at the two highest doses shortly after dosing (IIT Research Institute, 1992). No histopathological lesions were observed in brain, spinal cord, or sciatic nerve at the termination of this study.

Anaesthesia was also reported in rats at the highest dose level after daily gavage dosing for 90 days at up to 1,200 mg/kg/day but there was full recovery within 2 hours (Robinson *et al*, 1990). There were no significant effects on brain weight and, histologically, there were no brain lesions.

Inhalation studies

Effects on neurobehavioural functions have been observed in rat, mouse and rhesus monkey following acute exposure by inhalation. Rats exposed to a non-lethal concentration of around 70,000 mg/m^3 for 4 h exhibited lachrymation within 3 minutes. During the 4 hours of exposure, signs progressed to ataxia, loss of righting reflex, hyperpnoea, inco-ordination, and prostration. In groups exposed to higher concentrations behavioural effects were more marked and more immediate in onset with fatalities occurring at > 120,000 mg/m^3 (ARCO, 1980). Rats exposed to 28,800 mg/m^3 MTBE for 0.5 hour and 1.5 hours showed ataxia and drowsiness, respectively (Bio-Research Laboratories, 1990b).

A study in the mouse determined the anaesthetic effects of high MTBE exposures over periods of 5 minutes (Hathaway *et al*, 1970b). Anaesthesia (loss of righting reflex) occurred at all doses between 125,000-800,000 mg/m^3, but all animals recovered except at 400,000 and 800,000 mg/m^3 (see table 5-1) in Appendix II. The authors reported that inhalation of MTBE was accompanied by a slight increase in respiratory rate accompanied by deep respiration (convulsive hyperventilation). Unconsciousness was preceded by hypoactivity, ataxia and sporadic convulsive seizures.

Gill (1989) studied neurobehavioural effects in groups of 22 male and 22 female F344 rats exposed to 2,880, 14,400 and 28,800 mg/m^3 MTBE vapours for 6 hours. Motor activity was recorded and animals were subjected to a functional observation battery of tests (FOB). Changes in behaviour were not observed at 2,880 mg/m^3 but there were concentration-related increases in ataxia and duck-walk gait at 14,400 and 28,800 mg/m3, indicative of transient CNS sedation. At the highest exposure, males had laboured respiration, decreased muscle tone, decreased performance on a treadmill, and increased hind limb splay; females had decreased hind limb grip strength, laboured respiration and increased latency to rotate on an inclined screen. These effects were transient, first seen at 1 hour but absent 4 hours after cessation of exposure. Alterations in motor activity corresponded with changes in the functional observation battery, and suggested exposure-related but reversible CNS sedation. Because of FOB changes at the mid- and high-dose levels and motor activity changes at the high-dose the authors concluded a NOAEL of 2,880 mg/m^3.

An acute inhalation study was conducted with 2 (1 male and 1 female) rhesus monkeys (Hathaway *et al*, 1970c). The animals were exposed to periods up to 6h/d and the concentrations of MTBE in air were increased successively over a 5 day period from 12,400 to 341,000 mg/m^3. Only behavioural effects were noted. No effects were seen at 12,400 and 17,400 mg/m^3. At 32,000 mg/m^3 both animals became ataxic within 65 minutes, but neither lost consciousness and recovered promptly at the end of the exposure period. All higher exposures concentrations (68,400, 110,000, 175,000 and 341,000 mg/m^3) caused effects on the CNS (ataxia, prostration, tremors, etc.) and unconsciousness at an increasingly early time after the start of the exposures (80, 50, 43 and 24 min, respectively). At

the highest dose ataxia was observed at 8 minutes and the respiration rate dropped to zero at 85 minutes. Upon return to normal atmosphere, consciousness was regained after 17 minutes of artificial respiration and recovery was complete within 2h. From this study the NOAEL for neurological effects is 17,400 mg/m^3.

Mice exposed to 28,800 mg/m^3 of MTBE vapour for 1 - 2 days did not show clinical signs (Vergnes and Kintigh 1993). Inhalation of MTBE vapour at ≥ 10,800 mg/m^3 for 9 days was not associated with histological brain lesions in rat (Terrill and Daly, 1984).

Mild neurological effects were reported in a 13 day range-finding inhalation study in Fischer 344 rats and CD-1 mice, preparatory to a 13 week study (Dodd and Kintigh, 1989). Behavioural effects were seen at all exposure levels, but principally at 28,800 mg/m^3. They included hypoactivity and periocular irritation. Reversible behavioural effects (ataxia, decreased startle and pain reflexes and decreased muscle tone) were noted in rats of both sexes at 28,800 mg/m^3. The NOAEL for neurological effects was 14.400 mg/m^3.

In the 13 week study, the only finding of note was transient ataxia which occurred at the highest exposure concentration (28,800 mg/m^3) immediately following daily exposure during the first 4 weeks of the study. Minor changes in the functional observation battery (e.g. elevated body temperatures) in males (28,800 mg/m^3) at week 1 and females (14,400 and 28,800 mg/m^3) at week 13; decreased hind limb grip strength in males at 14,400 mg/m^3. Motor activity was decreased in males at week 8 at 28,800 mg/m^3 but in females activity was increased at week 8 (2,880-14,400 mg/m^3) and week 13 (14,400 mg/m^3).

In rats and mice exposed for 28 days to MTBE vapours at 1,440, 10,800 and 28,800 mg/m^3 (6 h/d, 5 d/wk) the daily neurological effects at ≥ 10,800 mg/m^3 were transient ataxia, hypoactivity, and lack of startle (Chun and Kintigh 1993).

Snamprogetti (1980) exposed groups of Swiss mice for 5 or 10 min/d, 5d/wk for 30 days at 288,000 mg/m^3 but was unable to detect changes in motor activity or co-ordination at termination of the exposures.

Several developmental and reproduction studies have been conducted on mice, rats and rabbits with MTBE vapour concentrations of up to 28,800 mg/m^3. (Conaway *et al*, 1985; Biles *et al*, 1987; Tyl and Neeper-Bradley, 1989; Tyl 1989; Myhr *et al*, 1991). Neurological effects, similar to those described previously were seen in all studies at the highest dose level (28,800 mg/m^3).

Evaluation

Neurobehavioural effects of exposure to MTBE and MTBE vapours have been observed in several acute and repeated dose oral and inhalation studies in laboratory animals. MTBE has caused transient anaesthesia at 28,800 mg/m^3 in all laboratory animal strains. Higher exposures cause respiratory cessation which can result in death (Hathaway *et al*, 1970b).

At lower exposures, a variety of subtle dose-dependent, transient behavioural changes (typical of ether anaesthesia and alcohol intoxication) have been described. Principal among these effects were lachrymation, hypoactivity, ataxia, loss of righting and startle reflexes, hyperpnoea, lack of co-ordination, prostration, and drowsiness. Functional changes include duck-walk gait, laboured respiration, decreased muscle tone, decreased performance on a treadmill, increased hind limb splay, decreased hind limb grip strength and increased latency to rotate on an inclined screen (Gill, 1989). Hyperactivity has also been reported at below 28,800 mg/m^3 (Gill, 1989).

All of these behavioural effects are reversible when exposure is stopped and they have not been associated with gross structural changes to the nervous system. Animal studies have not provided evidence of overt neurotoxicity due to exposure to MTBE at concentrations up to 10,800 mg/m^3 over 30 days (6h/d, 5d/wk). For the purpose of this assessment 2,880 mg/m^3 could be considered the NOAEL for transient functional CNS effects from a specifically designed inhalation study in rats (Gill, 1989).

5.6 TOXICITY FOR REPRODUCTION

The studies on developmental and reproductive toxicity are summarised in table 5-4 (see Appendix II).

Effects on fertility

The following studies were conducted to evaluate the effects of almost continuous exposure to MTBE on fertility in rodents.

A two litter, single generation inhalation study was carried out with Sprague-Dawley rats (Biles *et al*, 1987). To provide two litters per female at each treatment level, fifteen F_0 males, pre-exposed to MTBE vapours for 6 h/d, 5 d/wk, for 12 weeks were mated with thirty F_0 females exposed for 3 weeks prior to mating. MTBE vapour concentrations were 0, 900, 3600 and 9,000 mg/m^3. Exposures were continued throughout a 15 day mating period, gestation (days 0 - 20) and through days 5-21 of lactation. There was no exposure between day 21 of gestation and day 4 of lactation.

There were no treatment-related deaths or other signs of treatment as assessed by in-life observations, body weights, male and female mating indices for both mating intervals and reproduction data. No significant effect on pregnancy rates were noted (although exposed groups in the second interval were slightly smaller) and gonad and organ weights of the males were unaffected. Overall litter sizes, gestation times, pup survival and pup weights were not significantly affected by treatment and there were no gross or histopathological changes in adults or pups at necropsy at any exposure level up to 9,000 mg/m^3.

A two generation reproduction study was conducted in which groups of 25 male and 25 female Sprague-Dawley rats were exposed to MTBE vapours for 6 h/d, 5 d/wk at target concentrations of 0, 1,440, 10,800, or 28,800 mg/m^3 (Myhr *et al*, 1991). As with the previous study, animals were exposed during the pre-mating, mating, gestation and postnatal periods. With both generations exposures were started 10 weeks before mating and were continued through mating until day 19 of gestation and then during lactation, days 5 to 28.

Parental animals were monitored throughout for clinical signs of toxicity, food consumption and body weight and for gross lesions at necropsy. Organs were weighed at necropsy. All tissues with gross lesions, upper and lower respiratory tracts and selected reproductive tissues from high-dose and control groups were examined histologically. Offspring were evaluated for viability, survival, body weights and sex distribution.

There were no treatment-related deaths in this study. During certain periods of the pre-breed exposure at 28,800 mg/m^3, food consumption, body weight gain and body weights were reduced in males, but not in females. Clinical signs immediately following exposure at ≥ 10,800 mg/m^3 included hypoactivity, lack of startle reflex, ataxia and blepharospasm. Reproductive parameters were unaffected by treatment and there were no significant gross or histopathological changes in F_0 parents at necropsy. At the mid and high-dose levels the F_1 litter body weights were significantly lower than those of the control animals on lactation days 14 and 28 (10,800 mg/m^3), and day 14 (28,800 mg/m^3), respectively. F_1 pup body weight gains were reduced on lactation days 7 and 21 at 10,800 and between days 7 and 14 at 28,800 mg/m^3 . There were no treatment-related lesions at necropsy of the F_1 pups.

In the eight week pre-breeding exposure clinical signs observed in the F_1 parents included hypoactivity and lack of startle reflex (10,800 mg/m^3) and ataxia (28,800 mg/m^3). Reduced body weight gain was frequently noted in males at 10,800 and 28,800 mg/m^3 but this was only statistically significant at 28,800 mg/m^3 during the first two weeks of exposure. At 10,800 mg/m^3, weight gain in males was faster than that of controls so that final body weights were equivalent to controls. In F_1 females, final body weight of the 28,800 mg/m^3 group was lower than controls throughout pre-breeding exposure and body weight gain in the 10,800 mg/m^3 group was equivalent to that of the control group. Food consumption was reduced in F_1 males at 2,880 mg/m^3 only for the first two weeks of exposure. There

were some transient clinical effects during exposure but these were limited to hypoactivity, blepharospasm and lack of startle reflex at 10,800 and 28,800 mg/m^3, and ataxia at 28,800 mg/m^3. No exposure-related gross lesions were noted at necropsy in F_1 adults. A statistical increase in absolute liver weight occurred in both sexes at 28,800 mg/m^3 and relative liver weights were increased at 10,800 and 28,800 mg/m^3, but there were no corresponding histopathological changes.

MTBE did not adversely affect reproductive parameters for production of F_2 litters at any exposure level. Maternal body weights were unaffected during gestation and lactation. Food consumption during gestation (except for a small reduction at 28,800 mg/m^3 between days 7 and 14) was equivalent across groups. The total number of F_2 pups born was equivalent in control and treated groups. Body weights in F_2 litters exposed at 28,800 mg/m^3 MTBE vapour were reduced on lactation days 7 and 28 and at 10,800 mg/m^3 on days 14 and 28. Pup weight gains were also reduced at 28,800 mg/m^3 on lactation days 1- 4 and 4-28. Pup weight gains at 10,800 mg/m^3 were reduced on lactation days 7-14 and on 14-21. Perinatal deaths were increased on postnatal day 4 at 28,800 mg/m^3, but survival at lactation from day 4 to day 28 was unaffected by treatment. There were no treatment-related gross lesions observed at necropsy of F_2 pups that died during lactation.

In conclusion, maternal and foetal toxicity in rats was noted at 28,800 mg/m^3 and maternal toxicity at 10,800 mg/m^3. The main effect in animals exposed to 10,800 and 28,800 mg/m^3 was CNS depression (hypoactivity, lack of startle reflex). Other effects, e.g. reduced food intake, reduced body weight gain may have been secondary to this. There were no adverse effects at 1,440 mg/m^3 and the NOAEL was, therefore, 1,440 mg/m^3.

Effects on development

The following studies were conducted to evaluate the embryotoxic and/or teratogenic effects of exposure to MTBE vapours during organogenesis.

In the first of these studies, groups of 25 pregnant CD-1 mice and Sprague-Dawley rats were exposed to MTBE vapours at target concentrations of 0, 900, 3,600 and 9,000 mg/m^3 for 6 h/d on days 6-15 of gestation (Schroeder and Daly, 1984a and b; Conaway *et al*, 1985). Food and water consumption were recorded and the dams were sacrificed on day 18 (mouse) or 20 (rat) of gestation. At necropsy, uterine and liver weights and uterine implantation data were recorded. Foetuses were evaluated for external, soft tissue and skeletal malformations.

There were no mortalities in either study and there were no treatment-related effects on final body weight, body weight gain, water consumption, organ weights, pregnancy rate, the number of implants, resorption and live foetuses, sex ratios or gross pathology. Increased lachrymation was noted occasionally in mice of all MTBE-treated groups and food consumption was reduced in rats between

day 9-12 of gestation and in mice on days 12-15. In rats, there were no significant differences in the number of corpora lutea and no significant effects on mean foetal weight and crown-rump length. No external, skeletal or soft tissue anomalies were noted in either species.

The authors concluded that, at exposures of up to 9,000 mg/m^3, MTBE was not maternally toxic, embryotoxic or teratogenic in rats or mice. The NOAEL for both species for maternal and developmental toxicity was at least 9,000 mg/m^3.

In a second study (Tyl, 1989), groups of 15 pregnant albino rabbits were exposed to MTBE vapour at target concentrations of 0, 3,600, 14,400 and 28,800 mg/m^3 for 6 h/d on days 6-18 of gestation. Maternal clinical signs were recorded daily, body weights and food consumption were measured between days 0 - 29 of gestation. At necropsy on gestation day 29, maternal body weight, gravid uterine weights and liver weights were recorded. Ovarian corpora lutea were counted and all uterine implantation sites were identified and recorded for early and late resorptions, dead foetuses and live foetuses. Foetuses recovered were evaluated for external, soft tissue and skeletal malformations.

The two highest exposure levels were maternally toxic, as judged by a statistically significant decrease in food consumption and body weight gain during the exposure period. At 28,800 mg/m^3, ataxia and hypoactivity were observed immediately after exposure on 6 of the 13 exposure days. A significant increase in relative liver weight was also noted at 28,800 mg/m^3. There were no treatment related effects on gestation parameters including the number of corpora lutea, total non-viable implantations per litter, sex ratio, pre- or post-implantation loss and foetal body weights per litter.

The author concluded that the NOAEL for maternal toxicity was 3,600 mg/m^3 and the NOEL for developmental toxicity was at least 28,800 mg/m^3. No developmental toxicity, including teratogenicity was observed at any exposure concentration.

A similar study was conducted using the same dose levels with groups of 30 pregnant CD-1 mice (Tyl and Neeper-Bradley, 1989). Animals were exposed for 6h/d on gestation days 6-15 and they were sacrificed on day 18. Maternal clinical signs were recorded daily and body weights were measured between days 0 - 18 of gestation. Maternal food consumption was measured throughout gestation. At scheduled sacrifice, maternal body weight, gravid uterine weights and liver weights were recorded. Ovarian corpora lutea were counted and all uterine implantation sites were identified and assessed for early and late resorptions, dead foetuses and live foetuses. Foetuses were evaluated for external, soft tissue and skeletal malformations.

As with the rabbit, maternal toxicity was seen in mice exposed at 14,400 and 28,800 mg/m^3. Significant effects were reduced body weight gain, reduced final body weight and reduced food consumption. During exposure at 28,800 mg/m^3, treatment-related clinical signs of toxicity were

hypoactivity, ataxia, prostration, laboured breathing, lachrymation and periocular encrustation and at 14,400 mg/m^3, they were hypoactivity and ataxia. Gestation parameters were affected at 28,800 mg/m^3 (reduced numbers of viable implantation's per litter and altered sex ratio) and foetal body weights (totals, males or females) per litter were significantly reduced at 14,400 and 28,800 mg/m^3. At 28,800 mg/m^3, there was a significant increase in the incidence of cleft palate which lead to an increase in the overall visceral malformations. There were also treatment-related increases in the incidence of individual skeletal variations at 14,400 and 28,800 mg/m^3, consistent with developmental toxicity. These changes were probably secondary to maternal toxicity.

The authors concluded that MTBE was maternally toxic at 14,400 and 28,800 mg/m^3 and also caused developmental toxicity at these concentrations. There was an increased incidence of cleft palate at 28,800 mg/m^3 concomitant with profound maternal toxicity. The NOAEL for maternal and developmental toxicity was 3,600 mg/m^3.

Evaluation

Effects of the inhalation of MTBE vapours on reproduction and development have been evaluated in well conducted inhalation studies with rats, mice or rabbits. In general, effects have been the same as those described in repeated dose sub-chronic studies (see para 5.4).

Maternal and foetal toxicity in rats was noted at 28,800 mg/m^3 and maternal toxicity at 10,800 mg/m^3. The main effect in animals exposed to 10,800 and 28,800 mg/m^3 was CNS depression (hypoactivity and lack of startle reflex). Other effects, e.g. reduced food intake, reduced body weight may have been secondary. Effects on fertility of parent and F_1 generation were not observed and the NOAEL for fertility is 28,800 mg/m^3.

MTBE was not maternally toxic, embryotoxic or teratogenic in the rat in an inhalation study conducted to evaluate developmental effects at concentrations up to 9,000 mg/m^3. In a similar study with rabbits, MTBE vapour was non-toxic up to 3,600 mg/m^3 but was maternally toxic during organo-genesis at 14,400 and 28,800 mg/m^3. It was not a developmental toxicant at any concentration examined up to 28,800 mg/m^3. Exposure of pregnant mice to MTBE during organogenesis was maternally toxic at 14,400 and 28,800 mg/m^3 and caused developmental toxicity at these concentrations. At 3,600 mg/m^3 MTBE did not cause developmental toxicity. There was an increased incidence of cleft palate at 28,800 mg/m^3 concomitant with profound maternal toxicity. The NOAEL for maternal and developmental toxicity was 3,600 mg/m^3.

The task force concludes that these studies provide no indication that MTBE can adversely influence reproductive performance or foetal development at exposure levels not causing maternal toxicity.

5.7 GENOTOXICITY

5.7.1 MTBE

MTBE has been tested extensively for genotoxicity in a variety of *in vitro* and *in vivo* systems, using scientifically acceptable standard protocols. MTBE does not provide structural alerts for genotoxic or mutagenic activity (Rosenkranz and Klopman, 1991).

Genotoxicity in vitro

Studies in Bacteria and Yeast

The mutagenic potential of MTBE has been tested in several bacterial and yeast mutation assays (summarised in Table 5-5). The results showed no induction of base pair substitution or frameshift mutations in bacterial cells, or mitotic gene conversion in a yeast, under the conditions of these studies.

Studies with Mammalian Cells

MTBE has been tested for mutagenic potential using five end points in four mammalian cell systems (summarised in Table 5-5). It did not induce chromosome aberrations (CA) or sister chromatid exchanges (SCEs) in Chinese Hamster Ovary (CHO) cells, gene mutation in V79 cells or Unscheduled DNA Synthesis (UDS) in rat hepatocytes, respectively, although equivocal results were obtained in two other assays.

Evidence of a weak positive result was obtained when mouse lymphoma L5178Y TK+/- cells were exposed to 0.115-4.44 mg/ml MTBE, in the presence of Arochlor 1245-induced rat S9. Concentrations > 2.22 mg/ml were toxic to the cells (detected as a >90% reduction in growth relative to the solvent controls during cloning), hence any increase in mutant frequency under these conditions is not considered reliable. At lower concentrations (with acceptable growth), increases in mutant frequencies relative to controls were observed although this was not always clearly dose-related. Overall, the findings are suggestive of a weak, variable increase in reversion at the TK +/- locus caused by a metabolite of MTBE, since the results were clearly negative in the absence of S9. While this result may be explainable by the production of the metabolite formaldehyde, it should be noted that no free formaldehyde was detectable in a follow up study when MTBE was incubated with L5178Y TK +/- cells.

Table 5-5: Genotoxicity of MTBE *in vitro*

Test system	End point	Concentration	Results -S9	Results +S9	Reference
Salmonella typhimurium (TA 1535, 1537, 1538, 98, 100)	Reverse mutation	0.007-7.40 mg/plate (Two samples)	-	-	ARCO, 1980; Litton Bionetics Inc., 1978
Salmonella typhimurium (TA 1535, 1537, 1538, 98, 100)	Reverse mutation	0.341-5.41 mg/plate	-	-	Seeberg and Cinelli, 1989
Salmonella typhimurium (TA 1535, 1537, 1538, 98, 100)	Reverse mutation	0.004-2.66 mg/plate	-	-	Hüls AG, 1991
Saccharomyces cerevisiae	Gene conversion	0.007-7.40 mg/plate	-	-	ARCO, 1980
Mouse lymphoma cells L5178Y TK +/-	Forward mutation	0.229-7.40 mg/ml	-	ND	ARCO, 1980; Litton
		0.118-4.44 mg/ml (Two samples)	ND	+/ -	Bionetics Inc., 1979a
Chinese Hamster Ovary cells	SCE; chromosome aberrations	0.003-0.740 mg/ml	-	ND	ARCO, 1980; Litton
		0.007-3.70 mg/ml	-	+/ -	Bionetics Inc., 1980
Chinese Hamster V69 cells	Forward mutation	0.003-5.40 mg/ml	-	ND	Seeberg, 1989a
		0.148-1.33 mg/ml	ND	+/-	
Rat hepatocytes	Unscheduled DNA synthesis	0.148-5.40 mg/plate	-	ND	Seeberg, 1989b

- S9 or + S9 = assayed in the absence or presence of S9 activation

\- = no mutagenic activity

+/- = equivocal mutagenic activity (see text)

ND = test not performed

MTBE has also been reported to cause a variable increase in SCE in CHO cells in the presence, but not in the absence, of rat S9. Two samples of MTBE were tested in these studies. One sample gave a slight increase in SCE (i.e. 15-16 SCE per cell with 12.5 per cell for the solvent control), in the presence of S9, following incubation with 0.148-0.740 mg/ml. The magnitude of the change was independent of the concentration (i.e. no dose response) and could not be reproduced with the other sample. Furthermore, neither sample of MTBE showed evidence of clastogenic activity in a chromosome aberration assay in the same cell line, either in the presence or absence of S9.

Genotoxicity in vivo

MTBE has been tested in the micronucleus assay for chromosome aberrations (CA) in rats and mice after gavage or inhalation exposure, for any potential to induce Unscheduled DNA Synthesis (UDS) in rat hepatocytes (*in vivo/in vitro* assay), and also in the *Drosophila* sex-linked recessive lethal test. The results are summarised in Table 5-6 and discussed below in more detail. These studies are considered to give a realistic assessment of the genotoxic potential of MTBE, which is readily diffusing into all body compartments, since they integrate absorption, metabolism and excretion with *in vivo* end-points of DNA damage.

Rodent Bone Marrow Cytogenetic Assay

The clastogenic potential of MTBE was evaluated *in vivo* using a rat bone marrow cytogenetic assay following acute or repeated oral exposure. In the acute study, male Sprague-Dawley rats were given a single dose of 30, 96 and 296 mg/kg, and groups of animals were sacrificed 6, 24 or 48 hours later. In the repeat-dose study, identical dosages of MTBE were administered daily over 5 days, and the animals were sacrificed 6 hours after the final treatment. No increase in CA in tibial bone marrow was seen at any dose level following single or repeated administration of MTBE. A normal response was obtained with triethylene melamine, the positive control material. It was concluded that MTBE did not cause clastogenic damage to rat bone marrow under the conditions of the study.

Table 5-6: Genotoxicity of MTBE *in vivo*

Species	End point	Route	Dose	Result	Reference
Rat (male, Sprague-Dawley)	Chromosome aberrations (tibial bone marrow)	oral (gavage)	30, 96 and 296 mg/kg (as a single dose or five consecutive daily doses)	No clastogenic activity	ARCO Chemical Company, 1980 Litton Bionetics Inc., 1979b
Rat (male and female, F344)	Chromosome aberrations (femoral bone marrow)	inhalation	0, 2,880, 14,400, 28,800 mg/m^3, (6h/d on 5 consecutive days)	No clastogenic activity	Vergnes and Morabit 1989
Mouse (male and female, CD-1)	Chromosome aberration (femoral bone marrow)	inhalation	0, 1440, 10,800, 28,800 mg/m^3, (6h/d on 2 consecutive days)	No clastogenic activity	Vergnes and Kintigh, 1993
Mouse (male and female, CD-1)	Unscheduled DNA synthesis in hepatocytes (*in vivo/in vitro*)	inhalation	0, 1440, 10,800, 28,800 mg/m^3, (6h/d on 2 consecutive days)	No increase in UDS	Vergnes and Chun, 1994
Drosophila melanogaster	Sex-linked dominant lethal	ingestion	0.3% in sucrose	No increase in recessive lethal events	Hazleton Laboratories America Inc., 1989

In another investigation, male and female F344 rats were exposed to MTBE vapour at concentrations of 0, 2,880, 14,400 and 28,800 mg/m^3 for 6 hours per day on 5 consecutive days. Animals were sacrificed 6 or 24 hours after the final exposure, and femoral bone marrow smears prepared and stained. Five hundred cells per animal were scored under the microscope to determine the number of cells in mitosis. Low frequencies of simple chromatid breaks and chromosome fragments were the predominant types of damage observed at both time points in all animals. No statistically significant or exposure-related increase in CA was seen in either male or female rats, although a satisfactory response was obtained with the positive control material (cyclophosphamide). It was concluded that MTBE was not clastogenic in rat bone marrow under the conditions of the investigation.

The effect of inhalation of MTBE vapour (0, 1,440, 10,800 and 28,800 mg/m^3, 6 hours per day for 2 consecutive days) on the occurrence of micronuclei in bone marrow was investigated in male and female CD-1 mice. Animals were sacrificed approximately 24 and 48 hours after the final exposure. There was a significant increase in the ratio of polychromatic erythrocytes (PCE) over normal chromic erythrocytes (NCE) in femoral bone marrow of the males from the 1,440 mg/m^3 exposure group at the 48 hour sampling point, but this appeared an isolated finding within the control range, and not of biological significance. No significant increase in the incidence of micronucleated PCE was seen in any treatment group. The positive control, cyclophosphamide, gave a satisfactory response. The authors concluded that MTBE did not induce clastogenic damage in mouse bone marrow following inhalation exposure under the conditions of the study.

These studies show the absence of clastogenic effects in several rodent bone marrow assays following single or repeated exposure to MTBE by ingestion or inhalation.

***In vivo/In vitro* Unscheduled DNA Synthesis (UDS) Assay**

Male and female CD-1 mice (10 per group) were exposed to 0, 1,440, 10,800, 28,800 mg/m3 MTBE by inhalation, 6 hours per day on two consecutive days. The animals were sacrificed approximately 18 hours after the final exposure, hepatocytes isolated and pulse labelled with ^{3}H-thymidine. The proportion of cells undergoing repair did not differ between the control or the MTBE-exposed animals for either sex. A satisfactory response was obtained for the positive control groups (dimethylnitrosamine). It was concluded that MTBE did not induce DNA repair in mouse hepatocytes in this *in vivo/in vitro* assay.

Sex-linked recessive lethal (SLRL) test in Drosophila

MTBE was tested for the ability to induce forward mutation on the X chromosome of male *Drosophila melanogaster*. Treated males were mated with virgin females using a three brood mating sequence in

order to assess the impact of MTBE exposure on different stages of the sperm cycle. The results show that MTBE has no effect on fertility of male *Drosophila* at any dose level tested (0.01-0.3%), nor does it induce any increase in the SLRL mutation frequency (0.03-0.3%). A satisfactory result was obtained with the positive control material (ethyl methanesulfonate). The authors concluded that MTBE was not mutagenic to *Drosophila melanogaster* under the conditions of the test.

Genotoxicity - evaluation

MTBE has been tested for its genotoxic potential in a range of *in vitro* and *in vivo* test systems and with different end points.

The studies in *Salmonella typhimurium* and *Saccharomyces cerevisiae* appear slightly deficient in design in terms of the likely loss of MTBE from the assay as a result of evaporation. Nonetheless, the absence of any revertants supports the conclusion that MTBE is not mutagenic to bacterial or yeast indicator strains (both in the absence or presence of exogenous hepatic activation) under the conditions employed.

No evidence of genotoxicity was seen in *in vitro* tests using mammalian cell line cultures or isolated cells in the absence of S9 activation. In the presence of S9, a weak response for mammalian gene mutation was reported in mouse lymphoma L5178Y TK+/- cells but not in Chinese Hamster V79 cells. It has been suggested that formaldehyde may have been responsible for the findings in L5178Y TK+/- cells, but no free formaldehyde was detected under the conditions of the assay . This indicates that factors other than or in addition to formaldehyde may cause these effects. The lack of dose response and the large degree of inter-experimental variability make the interpretation of the results difficult.

A weak response was also reported for the induction of SCE *in vitro* in CHO cells exposed to one sample of MTBE *in vitro* but not to another (Litton Bionetics, 1980). This lack of consistency combined with the absence of a dose-response relationship suggests that this SCE result is probably due to chance and not indicative of mutagenicity.

MTBE was not genotoxic *in vivo*. The results from the rat bone marrow study (Vergnes and Morabit, 1989) appear critical, since the strain of animals and the exposure conditions were identical to those used in a subsequent toxicokinetic investigation (Bio-Research Laboratories, 1990b), thereby allowing an estimate of the maximum concentration achieved. The internal dose may be calculated using reference values for body weight and ventilation rate published by the US-EPA (EPA, 1988, see also chapter 5.4) and a retention of 15% (see chapter 4.7). The animals from the highest treatment group (28,800 mg/m^3) received a daily dose of over 1000 mg/kg/bw. The maximum concentration in blood (and other freely perfused tissues such as bone marrow) was around 560 mg/ml blood. Toxicokinetic data indicate that MTBE and its metabolite TBA are distributed uniformly within the body and it can be

assumed that MTBE and TBA will reach the target tissues used in the *in vivo* genotoxicity assays. The other metabolite, formaldehyde, is transformed very rapidly. The unequivocal absence of chromosome aberrations, coupled with the highly exaggerated exposure conditions and large delivered dose in the target tissue, provide strong evidence that neither MTBE nor its metabolites are genotoxic to rat bone marrow *in vivo*.

The weak and inconsistent response in the *in vitro* SCE with CHO cells and the variable response in the presence of S9 in the *in vitro* mammalian gene mutation test with L5178Y TK+/- cells are outweighed by, in particular , the evidence from *in vivo* mammalian systems. The *in vivo* data suggest that MTBE is not genotoxic.

5.7.2 MTBE metabolites

A summary of available information on the genotoxic activity of *tert*-butyl alcohol and formaldehyde is presented below.

Formaldehyde

Formaldehyde is mutagenic in a number of experimental systems *in vitro*, and induces gene mutation, chromosomal aberrations and sister chromatid exchanges in cells, especially after exposure to high concentrations (IPCS, 1989). This activity is decreased, however, by addition of S9 fraction indicating rapid conversion of formaldehyde to non-genotoxic products by mammalian enzymes (reviewed in chapter 4).

Studies of genotoxicity *in vivo* have revealed equivocal results for the induction of SCE in mouse bone marrow, whereas no activity was seen in the micronucleus test in rats or mice. Both positive and negative results have been reported in the mouse dominant lethal assay. A mouse somatic cell mutation test ('spot test') was negative.

Evaluation

Formaldehyde is a normal cell constituent and plays a pivotal role in the one-carbon pool and its intermediary metabolism. It is highly probable that the capacity of these processes would effectively trap and utilise the formaldehyde released during catabolism of MTBE, and in this way prevent genotoxicity or cell damage. This hypothesis is supported by *in vivo* studies following repeated administration of MTBE, where no evidence of cytogenetic damage in bone marrow or blood cells in rodents and induction of UDS in rat liver was seen. On this basis, production of formaldehyde from MTBE in the body is unlikely to cause genotoxic damage.

Tert-butyl alcohol (TBA)

Tests on the mutagenicity of *tert*-butyl alcohol *in vitro* are reviewed in table 5-7. No mutagenic potential was observed in *Salmonella* strains after exposure to up to 10 mg/plate both in the presence and absence of S9 fraction (Hüls 1979; Zeiger *et al*, 1987; NTP, 1995).

Table 5-7 : Mutagenicity of *tert*-butyl alcohol *in vitro*

Test system	End point	Concentration	Results -S9	Results +S9	Reference
Salmonella typhimurium (TA 1535, 1537, TA 1538, 98, 100)	Reverse Mutation	up to 1 mg/plate	-	-	Hüls, 1979
Salmonella typhimurium (TA 1535, 1537, 98, 100)	Reverse Mutation	0.1-10 mg/plate	-	-	Zeiger *et al*, 1987; NTP, 1995
Mouse lymphoma cells L5178Y TK +/-	Forward Mutation	trial 1: 0.625-5.0 mg/plate;	+/-	ND	McGregor *et al*, 1988; NTP, 1995
		and 1.0 - 5.0 mg/plate	ND	-	
		trial 2: 1.0 -5.0 mg/plate;	-	ND	
		and 2.0 -5.0 mg/plate	ND	-	
Chinese Hamster Ovary cells	Chromosome Aberration	trial 1: 0.16 - 5.0 mg/plate			NTP, 1995
		trial 2: 2,0 - 5,0 mg/plate	-	+/-	
			-	-	
Chinese Hamster Ovary cells	Sister Chromatid Exchange	trial 1 : 0.16 - 5,0 mg/plate	+/-	-	NTP, 1995
		trial 2 : 2,0 - 5,0 mg/plate	-	-	

- S9 or + S9 = assayed in the absence or presence of S9 activation
- = no mutagenic activity
+/- = equivocal weak mutagenic activity (see text)
ND = Not Determined

Results from a mouse lymphoma assay were considered negative by the authors, although a small increase in mutant colonies was seen in a single trial at the highest dose tested (5 mg/plate) in the absence of S9 (McGregor *et al*, 1988; NTP, 1995). Two trials conducted in the presence of S9 were clearly negative.

In cytogenetic tests TBA did not induce SCE or CA in CHO cells at doses up to 5 mg/plate, both in the presence and absence of S9. A weak SCE response seen in one trial without S9 was not reproducible, and considered a chance finding by NTP (1995).

TBA has been tested *in vivo* for its potential to increase the frequency of micronucleated monochromic erythrocytes (NCE) in male and female mice administered drinking water containing 9,000 - 120,000 mg/m^3 TBA for 13 weeks (NTP, 1995). No evidence of genotoxicity was found.

Evaluation

It is concluded on the basis of the above studies that TBA is not genotoxic to bacterial or mammalian cells *in vitro* or in the mouse micronucleus assay *in vivo*.

5. 8 CHRONIC TOXICITY AND NEOPLASTIC EFFECTS

The chronic toxicity and potential carcinogenicity of MTBE has been investigated in two well conducted long-term inhalation studies in CD-1 mice (Burleigh-Flayer *et al*, 1992) and Fischer 344 rats (Chun *et al*, 1992). These studies were designed in consultation with the US-EPA (40 CFR 799.5000) following conventional experimental protocols and conducted in accordance with the principles of Good Laboratory Practice. Results are also available from a rat oral intubation study (Belpoggi *et al*, 1995). The three studies are summarised below separately.

5.8.1 Mouse inhalation study

Groups of male and female CD-1 mice (50 per sex per treatment level) were exposed to 0, 1,440, 10,800 and 28,800 mg/m^3 MTBE vapour for 6h/d, 5d/w for 18 months (Burleigh-Flayer *et al*, 1992). In-life observations included clinical condition, water consumption, body weight, haematological parameters, blood corticosterone levels and urine chemistry/urine analysis. The animals were subject to a full post-mortem examination at the end of the study and organ weight data collected. A comprehensive range of tissues from control and high-dose animals, as well as from animals that died or were sacrificed moribund were sampled and subjected to microscopic histopathological examination.

Non-neoplastic effects

A slight increase in mortality and decrease in survival time was seen in male mice exposed to 28,800 mg/m^3 (49% mortality versus 33% in controls). Survival in all other groups was unaffected by treatment. Clinical signs were commonly noted in male and female mice from the intermediate and the high exposed groups, and included blepharospasm, hypoactivity, ataxia, prostration and lack of

startle reflex. No clinical signs were observed in animals exposed to 1,440 mg/m^3 MTBE vapour. Body weight and body weight gain were decreased in the 28,800 mg/m^3 exposed groups, for both sexes, with a 15-24% reduction in terminal weights. Water consumption was unaffected by treatment. There were no exposure-related changes in the haematological parameters. Increased corticosterone values were observed in the high exposed groups, although this was significant only for males. Urine pH was generally decreased in males and females exposed to 28,800 mg/m^3 MTBE, although the changes were rarely significant. Increased urine γ-globulin was also occasionally seen in males from the high exposure group.

An exposure-related increase in liver weight (absolute and relative to body weight) was noted for male mice at all dose levels (11%, 14% and 22%, respectively, relative to body weight). A small (approx. 10%) increase in kidney weight (absolute as well as relative) was also observed in male mice, but this was unrelated to the exposure concentration. Absolute and relative adrenal weights were increased, and absolute brain weight reduced in males from the high exposure group. Female mice showed an exposure-related increase in absolute or relative liver weight (3%, 9% and 39% respectively). Kidney weight (relative to body weight) was increased, and absolute spleen and brain weights decreased, in females from the high exposure group.

Macroscopic findings at necropsy included an increase in the number of masses in the livers of both sexes exposed to 28,800 mg/m^3 MTBE. These were noted in 26% of males (14% for control) and 18% of females (0% for control). The authors did not provide an interpretation for these findings. A slightly increased frequency of urinary bladder dilation/distension was recorded in male mice found dead from the high exposure group. There were no other treatment-related gross lesions in either sex.

Microscopic examination revealed an exposure-related increase in hepatocellular hypertrophy in male mice exposed to 28,800 mg/m^3 (15/49; controls 5/49), in males exposed to 10,800 mg/m^3 MTBE (10/50) and in females exposed to 28,800 mg/m^3 (9/50; controls 4/50) although neither value was statistically significant. A high incidence of tubular proteinosis, amyloidosis and interstitial nephritis was seen in kidneys from both sexes, however the lesions were distributed among the control and treated animals and not considered related to treatment (although there was an indication that changes were more extensive in treated animals versus controls, but no formal grading was conducted). A decrease in mineralisation within the brain was noted in both sexes exposed to 28,800 mg/m^3 MTBE. Female mice showed an exposure-related decrease in the incidence of cystic endometrial cell hyperplasia (Table 5-8).

Exposures of mice to MTBE vapour for 18 months produced various signs of toxicity at 10,800 mg/m^3 and higher. The NOAEL was 1,440 mg/m^3.

Table 5-8: Incidence of Endometrial Cell Hyperplasia in Female Mice Exposed to MTBE Vapour

	Dose (mg/m^3)			
Group	Control (0)	1,440	10,800	28,800
Number of animals per group	50	50	50	50
Number of animals examined	50	50	50	50
Microscopic diagnosis :				
endometrial cell hyperplasia	3	3	3	0
endometrial cell hyperplasia, cystic	26	17	15*	6**
endometrial cell hyperplasia, polypoid	7	1	0*	3

* $p < 0.05$ ** $p < 0.01$

Neoplastic effects

Neoplastic lesions were significantly increased in the livers of female mice, with an increase in the incidence of hepatocellular adenoma in the high exposure group only (20%; controls 4%), with no dose-response relationship present in the lower exposure groups (Table 5-9). No increase in malignant tumours was reported in females. The incidence of liver tumours (both adenoma and carcinoma) in male mice was slightly increased (16/49; controls 12/49), but this was not dose-related nor statistically significant (Table 5-10). The study report noted that the combined tumour incidence for males was also in the range of the historic control data for CD-1 mice at 24 month, indicating that these findings are probably unrelated to MTBE exposure. Liver tumour incidence in the intermediate and low exposure groups was unaltered by treatment. The NOAEL for tumour induction was 10,800 mg/m^3 in female mice and at least 28,800 mg/m^3 in male mice based on the occurrence of benign tumours.

Table 5-9: Incidence of Liver Tumours in Female CD-1 Mice Exposed to MTBE Vapour

	Dose (mg/m^3)			
Group	Control (0)	1,440	10,800	28,800
Number of animals per group	50	50	50	50
Number of animals examined	50	50	50	50
Microscopic diagnosis :				
hepatocellular adenoma	2	1	2	10**
carcinoma	0	1	0	1
adenoma and carcinoma	0	0	0	0
adenoma and/or carcinoma	2	2	2	11

** $p < 0.01$

Table 5-10: Incidence of Liver Tumours in Male CD-1 Mice Exposed to MTBE Vapour

	Dose (mg/m^3)			
Group	Control (0)	1,440	10,800	28,800
Number of animals per group	50	50	50	50
Number of animals examined	49	50	50	49
Microscopic diagnosis :				
hepatocellular adenoma	11	11	9	12
carcinoma	2	4	3	8
adenoma and carcinoma	1	3	0	4
adenoma and/or carcinoma	12	12	12	16

Evaluation

In this chronic study, the mice were exposed to 1,440, 10,800 or 28,800 mg/m^3 MTBE vapour. Using the EPA reference body weight and inhalation volume for male B6C3F1 mice in chronic studies (37.3 g and 63 l/d respectively), and a retention of 30 or 15%, depending on the exposure concentration (see section 4.7), an equivalent dose of 182, 648 and 1,824 mg/kg/day is obtained. The corresponding doses for female mice (35.3 g and 60 l/d respectively) are 184, 669 and 1,836 mg/kg/day.

Treatment at the highest dose level was associated with a significant decrease in body weight gain (15% for males, 24% for females) demonstrating that the maximum tolerated dose (MTD) was exceeded in this study. Findings seen under these conditions, particularly tumour data, require careful interpretation since they are likely to arise from generalised disruption of internal biological homeostasis rather than due to chemical-specific mechanisms (Haseman and Lockhart, 1994; Swenberg, 1995, ECETOC, 1996a and b).

Although the study design allowed for only 18 months exposure, and hence cannot be considered to represent a full lifetime exposure, the design and duration were considered adequate to assess the chronic toxicity and carcinogenicity of the MTBE in the CD-1 mouse.

Results indicate that the liver is a primary target for MTBE toxicity, based upon an increased occurrence of hepatocellular hypertrophy in intermediate and high-dose males and high-dose females, associated with an increase in liver weight. These changes may represent a metabolic adaptation to MTBE, and correlate with the increased cytochrome P-450 activity noted in other studies (see section 4.4).

The weight of evidence from *in vivo* as well as *in vitro* genotoxicity studies suggests that the increased incidence of hepatocellular adenoma, a benign liver tumour common in the mouse, in female mice

exposed to 28,800 mg/m^3 MTBE is probably not due to a genotoxic action of MTBE. Several non-genotoxic mechanisms have been postulated for this phenomenon.

1. The induction of liver tumours could be explained through changes in the rate of cell turnover and/or alterations to hepatic growth control that may be related to the very high MTBE exposures (Grasso and Hinton, 1991). Cell proliferation measurements demonstrated a transient mitotic stimulation of hepatocytes in female (but not male) mice following exposure to the high (but not lower) level of MTBE (see section 5.4). It is unlikely, however, that this short-lived phenomenon can be held responsible directly for the subsequent appearance of liver tumours since hepatocyte proliferation had returned to normal within one month after the start of treatment. Nonetheless it is possible that an enlarged liver cell population was retained during subsequent exposure to MTBE, since liver weight was still increased at termination of the long term study.

2. Another mechanism proposed to explain the sensitivity of the mouse liver to induction of tumours by non-genotoxic mechanisms involves increased damage due to free radical production and uncoupling of microsomal cytochrome P-450 activity (Parke and Ioannides, 1990). This mechanism may be operative in the case of MTBE. Enzymology studies have shown induction of cytochrome P-450 oxygenase IIB1 (CYPIIB1) in rats. Although definitive information for the mouse is missing.

3. The sex-specific nature of the mouse liver tumour response suggests a hormonal involvement. In this respect MTBE shares several of the characteristics of unleaded gasoline (without MTBE), which also causes liver tumours in female (but not male) mice following long term, high level exposure (reviewed by Standeven et al, 1994). These similarities include the induction of cytochrome P-450 enzyme activity, a transient increase in hepatocyte proliferation and decreased endometrial/uterine cystic hyperplasia. Changes in hormonal homeostasis (possibly coupled to the altered hepatic cytochrome P-450 activity) may be responsible. The decrease in endometrial cystic hyperplasia noted in female mice exposed to MTBE also points to a hormonal perturbation. Studies are in progress to clarify whether changes in hormone balance play a role in MTBE-induced liver tumour formation in female mice (see chapter 9). Recent findings (Moser et al, 1996) have confirmed the impact of MTBE on the B6C3F1 mouse reproductive system (decreased relative uterine and ovarian weight) after exposure to 28,800 mg/m^3 (nominal) MTBE vapour, and shown an increased rate of metabolism of 17-β-oestradiol by freshly isolated hepatocytes from females pretreated with MTBE (1,800 mg/kg/d for 3d). MTBE also significantly increased hepatic microsomal cytochrome P-450 content and the ethoxy resorption O-dealkylate (EROD) activity. The CYP 1A family, for which EROD is a marker, is one of the P-450 isozymes that metabolises oestrogens. Since oestrogens antagonise (androgens promote) the expression of spontaneously arising hepatic tumours in female mice (Kemp and Drinkwater, 1990), these observations support a potential role for a sex/species-specific hormonal modulation in the genesis of liver tumours in female mice following long-term, high level exposure to MTBE vapour.

Conclusion

MTBE causes an increase of benign liver tumours in female CD-1 mice after long term exposure to 28,800 mg/m^3 MTBE vapour, a level in excess of the MTD.

5.8.2 Rat inhalation study

Groups of male and female Fischer 344 rats (50 per sex per treatment level) were exposed to 1,440, 10,800 and 28,800 mg/m^3 MTBE vapour 6 h/d, 5 d/wk for up to 105 weeks (Chun et al, 1992). Parameters recorded during the in-life phase included body weight, clinical condition, haematology and blood corticosterone levels. All animals were subjected to full necropsy, including macroscopic examination and weighing of the major organs. A comprehensive range of tissues from control and high-dose animals, as well as those from animals that died or were sacrificed moribund were sampled and subjected to microscopic histopathological examination. In addition, the liver, kidneys, testes and all gross lesions from low and intermediate dose level exposed males, together with the liver and gross lesions from females from the other treatment groups, were examined microscopically.

Non-neoplastic effects

Increased mortality was observed in all groups of male rats, but was most common in the intermediate (88% mortality at week 97) and high (82% mortality at week 82) exposure groups. As a result, males from the 10,800 and 28,800 mg/m^3 groups were sacrificed early (weeks 97 and 82, respectively). Mortality in the control and low dose males was 73% and 88%, respectively, at week 104. Female survival was unaffected by treatment.

Clinical signs recorded in intermediate and high-dose males and females included eye and respiratory tract irritation, hypoactivity and ataxia; the severity of these effects increased in a dose-related manner. No clinical signs were recorded in the low exposure groups. Absolute body weight and body weight gain was decreased in both males and females exposed to 28,800 mg/m^3 throughout the study, but no consistent effect was recorded at the lower exposures. Haematological parameters were similar to control for all MTBE-exposed animals. Blood corticosterone concentrations were significantly increased in males exposed to 10,800 mg/m^3, and decreased in (surviving) males exposed to 28,800 mg/m^3, but unchanged in females.

An exposure-related increase in liver weight (absolute and relative to body weight or brain weight) was recorded for female rats from the intermediate and high exposure groups (20 and 42% respectively). Relative kidney weight increases were 18% and 29%, respectively.

Interpretation of liver and kidney weight findings from males was hindered by the absence of contemporaneous control data due to the early sacrifice of the intermediate and high-exposed animals. Nonetheless, kidney and liver weights from male rats exposed to 10,800 mg/m^3 appeared increased, with a probable increase in adrenal weight for high-dose group males. Other minor changes were considered to be unrelated to treatment.

The predominant finding at necropsy in treated males was an increased frequency of chronic nephropathy, as determined microscopically from an increased incidence and/or severity of glomerulosclerosis, tubular proteinosis, interstitial nephritis and interstitial fibrosis (Table 5-11). Secondary (i.e. nephropathy-dependent) changes, such as increased weight of the parathyroid glands, stomach thickening, hyperinflation of the lungs and mineralisation, were also observed. A slight increase in nephropathy was diagnosed microscopically in females from the 10,800 and 28,800 mg/m3 exposed groups, its magnitude and severity being much less than seen in males.

Table 5-11: Summary of Selected Microscopic Findings for Kidney from Rats Exposed to MTBE by Inhalation

Microscopic findings :	M1	M2	M3	M4	F1	F2	F3	F4
Tubular proteinosis								
minimal	49	49	50	50	47	23	9	49
mild	3	0	1	0	5	0	0	1
moderate	6	9	1	0	14	11	9	5
marked	18	18	14	5	20	9	15	23
severe	21	9	10	12	8	3	8	14
	1	13	14	33	0	0	7	6
Glomerulo-sclerosis								
minimal	43	46	48	50	37	17	34	45
mild	3	1	1	0	9	1	4	1
moderate	19	19	9	4	22	11	15	26
marked	17	14	12	11	5	5	8	14
severe	4	10	19	33	1	0	7	4
	0	2	7	2	0	0	0	0
Interstitial nephrosis								
minimal	42	44	45	50	29	14	31	37
mild	7	3	0	0	9	2	3	7
moderate	15	17	15	3	17	12	19	15
marked	19	24	30	47	3	0	9	15
	1	0	0	0	0	0	0	0
Interstitial fibrosis								
minimal	27	35	43	48	13	7	25	23
mild	2	3	0	0	8	1	3	5
moderate	20	11	12	5	3	6	12	11
marked	5	20	29	40	2	0	10	7
	0	1	2	3	0	0	0	0

M = male rats (50/group) F = female rats (50/group);

1 = control ; 2 = (1440 mg/m^3); 3 = (10,800 mg/m^3); 4 = (28,800 mg/m^3)

Kidney sections from all exposed female rats were re-examined subsequently (Busey, 1993) and the initial diagnosis confirmed. It appeared that MTBE exposure exacerbated the severity of this normally occurring event in both sexes, but that the magnitude of response was greatest in males.There were no exposure-related microscopic findings in the livers from either sex. All other tissues from treated animals of both sexes were unaffected.

The decreased survival seen in intermediate and high exposed males suggests that the MTD had been exceeded. Based on a slight increase in severity of nephropathy following exposure to 1,440 mg/m^3 MTBE, the NOEL could not be determined for male rats. In females, 1,440 mg/m^3 was considered to be the NOEL (based upon a slight increase in nephropathy seen at 10,800 mg/m^3). [Note: Since these renal changes appeared to be 'adverse', the NOEL and NOAEL were identical in this study).

Neoplastic effects

The kidney was the location of the principle neoplastic lesion reported in male rats, with increased renal tubular cell tumours present in 16% of the animals exposed to 10,800 mg/m^3 (carcinomas, at week 97) and 6% of the animals exposed to 28,800 mg/m^3 (carcinomas and adenomas, at week 82) relative to a control incidence of 2% (for adenomas, at week 104) (see Table 5-12). No tubular cell tumours occurred in males from the low treatment group. Historic data for male F344 rats (Haseman *et al*, 1990) indicate the spontaneous occurrence of kidney tumours is in the range 0 - 4% (mean 0.4%). The combined incidence of renal tubular cell adenomas and carcinomas for the intermediate dose group (16%) was significantly different from the concurrent controls and was also outside the historical control range. The incidence of adenomas in the high-dose group (6%) was not significantly different from concurrent control, but did lie outside the historical control range. There is a strong presumption, that the high degree of early mortality seen among the high-dose males has influenced this incidence.

Table 5-12: Incidence of Renal Cell Tumours in Male Rats Exposed to MTBE Vapour

	Dose (mg/m^3)			
Group	Control (0)	1,440	10,800	28,800[a]
Number of animals per group	50	50	50	50
Number of animals examined	50	50	50	50
Microscopic diagnosis :				
renal tubular adenoma	1	0	5	3
renal tubular carcinoma	0	0	3	0
renal tubular adenoma and/or carcinoma	1	0	8*	3

* $p < 0.05$

[a] early mortality may have influenced tumour incidence

A single renal cell adenoma in one female from the 10,800 mg/m^3 dose group (2% incidence), was the only kidney tumour to affect females. This incidence is within the 0 - 2% historic range (mean 0.1%) for this lesion in female F344 rats (Haseman *et al*, 1990) and is therefore considered unrelated to the treatment.

The incidence of interstitial adenomas of the testes (Leydig cell tumours) increased in an apparent dose-related manner, affecting 64%, 70%, 82% and 94% of male rats from the control, low, intermediate and high-dose groups, respectively (Table 5-13).

Table 5-13: Incidence of Interstitial Cell Adenomas in Male Rats Exposed to MTBE Vapour (Leydig cell tumours)

	Dose (mg/m^3)			
Group	Control (0)	1,440	10,800	28,800[a]
Number of animals per group	50	50	50	50
Number of animals examined	50	50	50	50
Microscopic diagnosis - interstitial cell adenoma :				
mild	0	3	3	2
moderate	6	9	4	14
marked	22	20	28	27
severe	4	3	6	2
total (%)	32 (64%)	35 (70%)	41 (82%)	47 (94%)

The NOEL for neoplastic effects was 1,440 mg/m^3 in males (renal tumours) and at least 28,800 mg/m^3 in females.

Evaluation

In this chronic study, the rats were exposed to 1,440, 10,800 or 28,800 mg/m^3 MTBE vapour. Using the EPA reference body weight and inhalation volume for male F344 rats in chronic studies (380 g, 360 l/d respectively), and a retention 30 to 15%, respectively (see chapter 4.7), an equivalent dose of 102, 384 and 1,023 mg/kg/day is obtained. The corresponding doses for female rats (229 g, 240 l/d) are 113, 425 and 1,132 mg/kg/day.

The highest treatment resulted in a 20-30% reduction in body weight gain, indicating that this was in excess of the MTD in both sexes. As noted before in the evaluation of the mouse bioassay, non-specific disruption of normal biological processes may occur under such conditions, leading to a tumour response, that requires careful interpretation.

Overall, the report concluded that nephropathy was the major cause of death in males from the intermediate and high exposure groups. The principle neoplastic change reported in male rats was an increase in renal tubular adenoma and carcinoma. These lesions are commonly seen in male F344 rats following exposure to a range of chemicals, and are associated with a well defined mechanism involving the excessive accumulation of hyaline (protein) droplets in lysosomes of kidney proximal epithelial cells (Borghoff *et al*, 1990; Baetcke *et al*, 1991). This causes chronic inflammation, necrosis and cellular proliferation resulting in the production of renal tumours through a non-genotoxic mechanism. The α_{2u}-globulin protein is highly specific to the male rat, hence this mechanism for induction of renal tumours is considered irrelevant to human risk assessment (Borghoff *et al*, 1990; Baetcke *et al*, 1991).

The microscopic appearance and localisation of the kidney lesions in this study are highly suggestive that a mechanism involving protein accumulation and chronic inflammation is operative in male rats exposed to MTBE. This is supported by cell proliferation studies showing increased cell turnover in proximal, but not distal, tubular epithelium after exposure to 10,800 and 28,800 mg/m^3 MTBE (Chun and Kintigh, 1993). Involvement of an α_{2u}-globulin dependent mechanism is also supported by the sex difference in tumour incidence and the cell proliferation data. These observations lend strong support to a non-genotoxic mechanism which is consistent with the assessment of the genotoxicity data.

Interpretation of these data is complicated by other results which do not fully support a 'classical' α_{2u}-globulin-related mechanism. Thus it has not been possible to demonstrate a dose-related increase in immunostaining for α_{2u}-globulin in kidney tissues from male rats exposed to up to 28,800 mg/m^3 MTBE vapour for 28 days (Chun and Kintigh, 1993) or for 13 weeks (Swenberg and Dietrich, 1991). In addition, no α_{2u}-globulin proteinaceous casts were detected at the junction of the proximal tubules and the thin loop of Henle in a 13 week exposure study (see section 5-4). Overall, the observations suggest that MTBE-induced nephropathy and renal tumours exhibit some, but not all the symptoms of the classical α_{2u}-globulin -related renal cancer. An additional, so far uncharacterised, protein accumulation mechanism may also be involved. Further research is underway to investigate these aspects (see section 9). Preliminary results indicate that MTBE appears to be a very mild inducer of α_{2u}-globulin nephropathy in male rats and does not cause nephrotic effects in the female rat (Bond, 1996, personal communication).

Histopathological changes seen in the testes were suggestive of a dose-related increase in benign Leydig cell tumours. However, the biological significance of these findings is questionable since Leydig cell tumours commonly occur in untreated F344 rats (as evidenced from concurrent controls included in this investigation) with a spontaneous incidence of approx. 83% reported for the historical control data base (Haseman *et al*, 1990). This suggests that the incidence seen in control animals from this study was below that expected, while that of the treated groups was within the historic control

range. On this basis, biological chance variation in the background rate appears responsible for the trend seen in this study rather than any treatment-related effect due to MTBE (see, for instance, Prentice and Meikle, 1995).

Conclusion

It is concluded that MTBE is a sex- and tissue-specific carcinogen in F344 rats, causing kidney tumours in males after long term inhalation exposure to 10,800 or 28,800 mg/m^3; the highest treatment level was in excess of the MTD. Evidence from short-term tests indicate that this cannot be due to a mutagenic mechanism, hence a non-genetic mechanism appears responsible for these renal effects. The underlying histopathology exhibits many of the features of an α_{2u}-globulin -induced kidney cancer. Further studies are in progress to clarify the aetiology of these changes. No increase in tumours was seen in female rats at any exposure level (up to 28,800 mg/m^3 MTBE).

5.8.3 Rat oral intubation study

The carcinogenic potential of MTBE following ingestion was investigated by Belpoggi *et al* (1995). Male and female Sprague Dawley rats (in-bred, Bentivoglio Castle colony) were administered MTBE by gavage in extra virgin olive oil at dose levels of 0, 250 or 1,000 mg/kg/d. Each group comprised 60 rats. The dosing solutions were administered once daily on four days per week (Monday, Tuesday, Thursday, Friday) for 104 weeks (This dosing regime was chosen because the highest dose was not tolerated by the rats when administered daily.). The animals were observed until natural death. Food and water intake, body weight and daily observations were recorded during the course of the investigation. At necropsy a range of tissues were sampled, processed and subjected to a microscopic histopathological examination. The study ended after 166 weeks with the death of the last surviving animal at an age of 174 weeks.

Non-neoplastic effects

For male animals survival in all groups was similar up to 80 weeks of treatment, after which the mortality was lower in rats given 1,000 mg/kg/d relative to the control and low dose groups. Treated female rats, in contrast, showed a dose-related decrease in survival after approximately 32 weeks of treatment. No statistical analysis is presented in the report, hence it is unclear if any of these effects were significant. No differences in water, food intake, body weight gain, or behaviour, were reported. The report noted that non-neoplastic changes were not detected in any of the tissues from the treated animals.

These results support a no-observed effect level for MTBE of at least 1,000 mg/kg/d in male rats. For female rats, however, given the uncertainty over the significance of the decreased survival such a conclusion cannot be reached, although the NOEL for organ toxicity was at least 1,000 mg/kg/d.

Neoplastic effects

The incidence of Leydig cell tumours was increased significantly in male rats from the 1,000 mg/kg/d dose group (34% incidence) relative to the control group (8% incidence), but unaffected by treatment in animals from the low dose group.

A significant, dose-related increase in combined lymphomas and leukaemias was also reported among female (but not male) rats, affecting 3%, 12% and 25% of animals from the control, low and high-dose groups, respectively. (The report noted that historic control incidences of these changes in female rats is "in a range below 10%".) The incidence of all other tumours was reported to be within the expected range.

On the basis of the above findings, the study supports a NOAEL of 250 mg/kg/d in males (Leydig cell tumours), and a LOAEL in females (lymphomas and leukaemias) of 250 mg/kg/d.

Evaluation

Due to deficiencies in the design and reporting of this study, the Task Force considered the NOAEL's for non-neoplastic end points questionable and in conflict with findings from good quality subchronic studies. The absence of CNS depression, together with no specific effect on liver and kidney, was also unexpected (see section 5.4).

The only significant treatment-related lesion noted in male rats after oral administration of MTBE for 104 weeks was an increase in benign Leydig cell tumours of the testes. However, interpretation of these findings is hampered since no diagnostic criteria were presented to allow the Task Force to distinguish small Leydig cell tumours from focal hyperplasia: this may have lead to an overestimation of the incidence of this tumour type. In any event, it has been noted that this type of tumour is frequently increased in rat cancer screening studies following treatment with pharmaceutical agents or dietary materials, yet is rarely reported in man, even after long-term exposure (Bär, 1992; Prentice and Meikle, 1995). The relative ease with which Leydig cell tumours may be induced in rats is strongly suggestive of a species-specific effect that is not predictive of any hazard to man.

In combination, the number of lymphomas and leukaemias in female rats was considered a significant tumour effect following oral administration of MTBE. Evaluation of these findings is complicated, however, by the apparently low control incidence recorded in the study (3.4%, relative to historical

control data "in a range below 10%"), making their biological significance questionable. The biological relevance of the findings is also confounded, since only 'combined' results are presented and analysed in the report, and no data or analysis of the individual occurrences of different types of leukaemias and lymphomas is given. A biological justification of such an assessment is required. In the criteria and guidelines for the analysis of tumour data from rodent carcinogenesis studies, developed by the US National Toxicology Programme (McConnell *et al*, 1986), it is indicated that tumours of different cellular origin should not be combined for the purpose of statistical analysis. On this basis it is probable that the approach of Belpoggi *et al* (1995), leads to an overestimation of the significance of the findings.

It is relevant to note that there was no evidence of genotoxicity for MTBE when tested *in vivo* (rat bone marrow cytogenetic assay, see section 5.7), nor were any comparable effects seen following chronic inhalation exposure, at an equivalent retained dose (see section 5.8.2). Overall, evaluation of the oncological findings from this life-time study is complicated by the limited reporting of the findings making a detailed analysis impossible. In particular, information on the historical control incidence for the principle lesions described by the authors is missing. The criteria used in diagnosis of the lesions by the study pathologists are also not stated.

Conclusion

This study was considered inadequate due to deficiencies in design and reporting. The results reported do not give sufficiently clear indications to require them to be taken into consideration in a risk assessment.

5.8.4 MTBE metabolites

Formaldehyde

Chronic and subchronic exposures to high formaldehyde concentrations have resulted in the induction of squamous cell carcinomas in the nasal cavity of rats and to a minor extent mice (Kerns *et al*, 1983; Albert *et al*, 1982; Feron *et al*, 1988). Mechanistic and molecular dosimetry studies demonstrated an association between local cytotoxicity and carcinogenic effects in the respiratory epithelium. The cytotoxicity is based on the local irritating properties of formaldehyde. Exposures to relatively low, non cytotoxic levels of formaldehyde are assumed to represent a negligible risk, since the nasal tissue is not sufficiently injured under these conditions (Feron *et al* 1991). Whether formaldehyde is a human carcinogen is still a matter of scientific debate (ECETOC, 1995; IARC, 1995).

Three long-term drinking water studies did not provide any evidence that formaldehyde is a systemic carcinogen (Takahashi *et al* 1986, Tobe *et al* 1989, Til *et al* 1989). However, another study described haemolymphoreticular neoplasms in male and female rats after oral administration in drinking water (10-2,500 ppm) (Soffritti *et al*, 1989). On the basis of this latter investigation it has been suggested that formaldehyde may have been responsible for the increase in combined leukemias/lymphomas seen in female rats following oral administration of MTBE (Belpoggi *et al*, 1995). The findings in the formaldehyde study and their interpretation by Soffritti *et al* (1989) have been put into question (Feron *et al*, 1990, 1991) and were considered not scientifically robust by the Task Force.

Evaluation

Overall, the evidence from rodent drinking water studies is not consistent and does not indicate that formaldehyde is a systemic carcinogen. The Task Force concluded that there is no evidence that formaldehyde formed in the body from MTBE plays a role in tumour induction.

tert-Butyl alcohol (TBA)

Mouse ingestion study

Groups of 60 B6C3F1 mice were given TBA in drinking water for 103 weeks at dose levels of 0, 5, 10 or 20 mg/ml, approximately equivalent to 0, 535, 1,035 or 2,065 mg/kg /day in males or 0, 510, 1,015 or 2,105 mg/kg /day in females (NTP, 1995). Non-neoplastic lesions were characterised by a significant increase in the incidence of follicular cell hyperplasia of the thyroid in all male treatment groups and in female mice from the intermediate and high-dose groups. The thyroid was also the only site of neoplastic lesions (follicular cell adenoma and/or carcinoma). In male mice these lesions showed a marginal, non-significant increase. In females, follicular cell adenoma was increased significantly in the high-dose group (9/59 versus 2/58 in control). The NOAEL for carcinogenicity (thyroid adenoma) was greater than 2,065 mg/kg/day in males, and 1,015 mg/kg/day in females.

Rat ingestion study

TBA was administered to F344 rats (60 per group) in drinking water at doses of 0, 1.25, 2.5 or 5 mg/ml (approximately equivalent to 0, 85, 195 or 410 mg/kg bw/day) in males and 0, 2.5, 5 or 10 mg/ml (approximately equivalent to 0, 175, 330 or 650 mg/kg bw/day) in females. Ten animals per sex from each treatment group were subject to an interim examination 15 months after the start of treatment, while treatment of the remainder continued for up to two years. Survival was reduced significantly in males.

Findings at autopsy, both during the interim and final examinations, were limited to increased relative and /or absolute kidney weight in mid- and high-dose animals from both sexes. Nephropathy was commonly present in treated animals (both sexes) at the interim and terminal examinations. Mineralisation of the kidney was increased in incidence and severity in males after two years of treatment (significant in the top-dose group), but females were not affected.

At termination of the study, focal renal tubule hyperplasia and adenoma were increased in exposed males, and a carcinoma was found in one high-dose male. In the light of these observations, additional kidney sections were prepared and a more detailed examination carried out. Additional lesions were detected as a result of this extended evaluation, and a significantly increased incidence of hyperplasia was seen in males from the 5 mg/ml group (25/50 versus 14/50 for controls). Significant increases in multiple tubular adenoma (10/50 versus 1/50 for controls) and in combined adenoma or carcinoma (19/50 versus 17/50 in controls) in males from the 2.5 mg/ml group were also noted.

In females, renal tubular lesions were limited to hyperplasia in a single animal from the high-dose group. Transitional epithelial hyperplasia was also increased significantly in this group (affecting 17/50 versus 0/50 in controls), together with significantly increased inflammatory changes in mid-(13/50) and high (17/50) dose animals.

The NOAEL for carcinogenicity was equivalent to 85 mg/kg/day in males (renal tubular adenoma/carcinoma) and at least 650 mg/kg/day in females (no increased tumour response noted).

Evaluation

Results from genotoxicity studies showed no mutagenic activity in bacterial- (*Salmonella typhimurium*, 4 strains) or mammalian- (L5178 mouse lymphoma line) cells *in vitro*, both in the absence or presence of S9 fraction. This suggests that tumours seen in the rat and mouse bioassays probably occurred via a non-genotoxic mechanism. In rodents, the thyroid (mouse) and kidney (rat) appeared to be the target tissues for TBA after chronic oral administration.

Increased cell proliferation in thyroid tissue was considered to be relevant for TBA in male and female mice, and appeared causally-related to the increased incidence of follicular cell adenoma/carcinoma seen in female (but not male) mice. Enzyme induction, leading to decreased levels of thyroid hormone and a compensatory increase in thyroid activity, was one possible explanation for these changes (NTP, 1995), although no supporting data were available.

In rats, the nephropathy seen in males showed features consistent with hyaline droplet accumulation and α_{2u}-globulin was the presumed causative agent (although no biochemical characterisation was carried out). Earlier studies, conducted during the pre-chronic investigation, had also demonstrated increased renal cell proliferation.

The Task Force concluded that TBA produced tumours in male rat kidney and female mouse thyroid through a non-genotoxic mechanism.

5.8.5 Overall evaluation of chronic studies

The genotoxicity and carcinogenicity of MTBE and its metabolites has been assessed in a comprehensive range of test systems.

The weight of evidence indicates that MTBE and TBA are not genotoxic *in vivo*. Formaldehyde, in contrast, has a proven potential to damage DNA, but when formed from MTBE in the body it will be detoxified very rapidly, minimising its operational genotoxic potential. The Task Force concluded that genotoxicity is unlikely to play a role in neoplastic findings reported in chronic studies with MTBE.

Animal carcinogenicity studies with MTBE vapour show an increased tumour incidence in male rat kidney and female mouse liver. The effect on rat testicular tumour incidence after inhalation and oral treatment is considered by the Task Force of doubtful significance. An effect on combined lymphoma/leukaemia incidence in female rats has been reported in an oral study, but deficiencies in the report preclude meaningful interpretation of the results, hence their importance is unclear.

With respect to the primary metabolites chronic ingestion of TBA produced tumours in male rat kidney and female mouse thyroid. The Task Force noted that both MTBE and TBA caused similar effects in male rat kidney, suggesting that a common mechanism involving TBA and α_{2u}-globulin is operative in both instances. In contrast, formaldehyde caused tumours only at the site of contact following inhalation or ingestion. Although a genotoxic mechanism cannot be excluded in the carcinogenicity of formaldehyde, the Task Force believes that this plays no role in the induction of tumours seen following long term exposure to MTBE.

Overall, these studies demonstrate that MTBE has some potential to increase the occurrence of certain tumours in female mice or male rats after chronic, high-dose inhalation exposure. It is probable that these effects are secondary to very high-dose-induced toxicity, occur through a species specific, non-genotoxic, process and are therefore not predictive of a hazard to humans under normal exposure conditions.

6. HUMAN HEALTH EFFECTS

The information discussed in this chapter is divided into three parts according to the type of information/studies discussed:

1. Medical use and possible side effects;
2. Population studies related to the introduction of MTBE as oxygenate in gasoline;
3. Human volunteer studies with controlled exposures to MTBE.

6.1 MEDICAL USE AND SIDE EFFECTS

MTBE has been successfully used in clinical practice to dissolve gallstones *in situ*. In this procedure, MTBE is introduced into the gallbladder through a catheter and removed by suction after some time along with dissolved gallstones. The procedure must be repeated several times exposing patients to an amount as high as approx. 500 ml of MTBE. There are some 30 references describing this clinical use of MTBE including its side effects. The primary side effects reported were nausea, vomiting and mild drowsiness. These side effects were reported for about 30% of the patients. The symptoms disappeared quickly and completely and no lasting side effects were observed. Clinicians consider the side effects to be not seriously disadvantageous. Evidence of some systematic absorption is obtained from the detection of MTBE in blood (discussed in Section 4.6) and from the odour of MTBE in exhaled air.

During the introduction of MTBE in the biliary tract spillages may occur and subsequent absorptions have been reported. For instance in one patient 21 ml MTBE passed into the duodenum and was absorbed with a resulting renal failure (Ponchon et al, 1988). In another instance haemolysis occurred after an inadvertent injection of a large quantity of MTBE into the blood stream. From the standard application to several hundred patients clinically significant haemolysis and renal failures have not been reported.

Evaluation

The clinical use of MTBE as an *in situ* solvent for gallstones is rapid and safe with only minor side effects. The case reports on accidental exposures that occurred during this treatment provided additional information on the fate of MTBE in the human body (see chapter 4).

Table 6-1: Study Design

Ref.	General	Population		Exposure Assessment	Effects Assessment
		Exposed	**Reference**		
Moolenaar *et al*, 1994. (Fairbanks)	Questionnaire survey of a convenience sample of two groups of volunteers considered to be exposed at work to gasoline; one group had exposure to oxygenated fuel (Phase I, Nov. 1992), the other was surveyed after suspension of the oxygenated fuel programme (Phase II, Feb. 1993)	10 service station/ garage employees, plus 8 individuals who spent most of their workday with motor vehicles (Phase I). This group was considered exposed to oxygenated fuel.	12 of the original participants (from Phase I), plus 16 additional volunteers (service station/garage employees). This group was not considered to be exposed to oxygenated fuel.	The MTBE concentration in work place air was determined during Phases I and II. MTBE levels in blood were measured before and after a workshift during both Phases in individuals, considered to be exposed to gasoline. Additional blood measurements were also obtained from commuters believed not occupationally exposed during Phases I and II.	Occurrence of 15 symptoms, 7 of which were considered "key" symptoms and believed to be related to MTBE exposure.
CDC, 1993b (Stamford)	Questionnaire survey of a convenience sample of volunteers conducted in Stamford, Connecticut during the first two weeks of April 1993.	120 individuals with presumed exposure to oxygenated fuel, including 50 service station employees/mechanics, 58 professional (e.g. taxi) drivers, 12 others (e.g. meter readers)	101 commuters not occupationally exposed to oxygenated fuel.	Personal breathing zone samples were taken from 37 volunteers with potential exposure to oxygenated fuel. Blood samples from 27 of these subjects were also analysed for MTBE concentration.	Occurrence of "key" symptoms.
CDC, 1993c (Albany)	Questionnaire survey of a convenience sample of volunteers conducted in Albany, New York during the first week of May, 1993. No oxygenated fuel programme was in place during the period of the study.	82 individuals with presumed exposure to non-oxygenated fuel including 34 service station employees /mechanics, and 48 others (policemen, toll booth operators etc.).	182 commuters (office workers/students) not occupationally exposed to gasoline.	Personal breathing zone samples collected from 24 individuals occupationally exposed to gasoline. Blood analyses were carried out on 18 commuters and 20 subjects from the service station/mechanics group.	Occurrence of "key" symptoms.

Table 6-1: Study Design (continued)

Ref	General	Population		Exposure Assessment	Effect Assessment
		Exposed	Reference		
Mohr *et al*, 1994 (New Jersey)	Cross sectional cohort study of self-reported symptoms of garage workers in the state of New Jersey presumed to be exposed either to oxygenated fuel or traditional gasoline.	115 garage workers in the northern part of New Jersey with an oxyfuel programme from November 15, 1992 to April 30, 1993. Data were collected in April 1993.	122 garage workers in the southern part of New Jersey with an oxyfuel programme from November 15, 1992 to February 28, 1993. Data were collected in May 1993.	Active sampling was done for one hour at one garage in northern New Jersey and at all the garages included in the study of southern New Jersey. In addition passive samplers were given to 20 subjects; 14 in the northern and 6 in the southern part of the state. Blood levels of MTBE were not measured.	Symptoms ("key" and "control") over the last 30 days among the study populations. A separate questionnaire was used to establish pre- and post-shift symptoms.
Anderson *et al*, 1995 (Wisconsin)	Telephone questionnaire distributed to three randomly selected groups; two from areas using oxygenated fuel (OF) and one using traditional gasoline.	527 telephone responders from the Milwaukee metropolitan area and 485 from the Chicago metropolitan area, both with a oxygenated fuel programme (OFP) in place during the study.	501 individuals from areas in the state of Wisconsin not having a OFP-programme.	MTBE was monitored in air from different, selected points; from 24 hour samples in presumably high and low-level areas; 15 min. personal samples were also collected during refuelling. The questionnaire also included inquiries about type and brand of gasoline usually purchased and also where it had been bought.	Symptom-prevalence ("key" and "control") was compared between the three groups.

6.2 POPULATION STUDIES

In 1992 the use of gasoline with MTBE at relative high concentrations (10-15%, called oxygenated fuels, or simply oxyfuels) became mandatory in 37 areas of the USA as a result of the US Clean Air Act of 1990. Following the introduction of this new type of gasoline a number of health complaints were heard from its users. As a result various USA Federal and State Health and Environmental Authorities commissioned a survey of the potential health effects related to the introduction of the oxygenated fuel programme in Fairbanks, Alaska. This study, and follow up studies in Stamford, Albany, New Jersey and Wisconsin, are summarised in Table 6-1. All studies used questionnaires which asked for health symptoms that could be related to exposure to MTBE containing gasoline, so-called 'key' symptoms, together with other symptoms considered unrelated to MTBE exposures (see Table 6-2 for further detail). Exposures were determined from MTBE concentrations in air at the workplace or from personal breathing zone samples.

Table 6-2: Summary of Symptoms Commonly Assessed in Health Survey Questionnaires Related to Oxygenated Fuel

"Key" symptoms (considered related to MTBE)	"Other" symptoms (considered unrelated to MTBE)
headache	fatigue
eye irritation	fever
burning of the nose and throat	sweats and chills
cough	diarrhoea
nausea and vomiting	fainting or black' outs
dizziness	skin irritation or redness
"spaciness" or disorientation	muscle aches
	difficult breathing

(From Moolenaar *et al*, 1994)

'spaciness' is an American term to indicate disorientation, related to 'space cake'.

6.2.1 The Fairbanks (Alaska) Study

Moolenaar *et al* (1994) studied 18 individuals exposed to gasoline with MTBE during work in phase I of the study and 28 individuals, 12 of them participating also in phase I, exposed to gasoline without MTBE during phase II. Phase I occurred in November 1992 after the introduction of the oxygenated fuel programme and phase II in February 1993 after the suspension of the programme. The MTBE-blood levels were determined for the individuals, before and after their workshifts.

Results

Table 6-3 presents the scores of health complaints obtained from two groups of garage workers and service station attendants. The prevalence of 'key' symptoms, especially headache, eye irritation, burning sensation and dizziness, was greater during Phase I as was the concentration of MTBE in the blood (see Table 6-4). The median exposure concentration during phase I was 0.37 mg/m^3.

Table 6-3: Health Complaints Reported by Individuals Occupationally Exposed to Gasoline (Fairbanks Alaska)

HEALTH COMPLAINTS	Phase I * (14-10-92) (n = 18)	Phase II ** (01-01-93) (n = 28)
Headache	13 (72%)	1 (4%)
Eye irritation	12 (67%)	2 (7%)
Burning sensation of nose or throat	9 (50%)	0 (0%)
Cough	5 (28%)	0 (0%)
Nausea or vomiting	6 (33%)	1 (4%)
Dizziness	8 (44%)	0 (0%)
"Spaciness" or disorientation	6 (33%)	0 (0%)

* Phase I - Individuals exposed to oxygenated fuel

** Phase II - Individuals considered not exposed to oxygenated fuel

(Ref. Moolenaar *et al*, 1994).

Table 6-4 : Pre-shift and Post-shift Blood MTBE Concentrations in Workers during Phase I and Phase II (Fairbanks, Alaska)

	BLOOD MTBE concentrations (µg/l)			
	Phase I * (n = 18)		Phase II ** (n = 28)	
	Median	**Range**	**Median**	**Range**
Preshift MTBE	1.15	0.1 - 27.8	0.21	<0.05-4.35
Postshift MTBE	1.80	0.2-37.0	0.25	<0.05-1.44

Approximate detection limit = 0.05 µg/l.

Maximum linear standard = 37 µg/l.

Evaluation

The authors concluded that in Phase I there existed a correlation between the highest post-shift quartile in MTBE-blood concentrations and the 'key' symptoms reported on the day of testing. This finding was not statistically significant. Some of the limitations of the study are apparent and were reported by the authors:

- The authors consider the survey a pilot study as the number of subjects was small;
- The subject selection procedure could not be arranged without excluding individual beliefs concerning the possible health consequences from exposure to the newly introduced oxygenated gasoline;
- Widespread media coverage reporting the public opposition to the oxygenated gasoline programme took place during Phase I of the survey;
- The estimates of MTBE exposures have limited value as personal monitoring measurements were not carried out;

The relatively high pre-shift MTBE-blood concentration given for phase II may be due to problems of sampling and analysis.

Other comments to this study include:

- The median exposure concentrations reported for phase I (0.37 mg/m^3) appears to be insignificant in the light of subsequent chamber studies (see section 6.3);
- Similar symptoms are well known to be caused by exposure to gasoline.

In view of the shortcomings of this survey and the related confounding factors, conclusions on a possible causal association between the introduction of oxygenated gasoline and specific health effects should not be drawn .

6.2.2 Stamford (Connecticut) and Albany (New York) Study

In view of the shortcomings in the Fairbanks study the USA Centre for Disease Control (CDC) decided on a follow up survey in two other regions, Stamford (Connecticut) and Albany (New York). In Stamford the oxygenated fuel programme had been introduced without any particular health complaints; in Albany oxygenated gasoline had not become mandatory and was not used. The data obtained with the survey in Albany were used as negative control. In both places a group exposed regularly to gasoline and a non-exposed group was formed. The questionnaires used were similar to those used in the Fairbanks survey. Personal breathing zone sampling was carried out with the exposed groups to determine the exposure to MTBE in addition to the determination of the concentrations of MTBE in blood. The exposed groups were composed of motor-car mechanics, professional drivers (e.g. taxi) and other professionals (e.g. meter readers) expected to be exposed

regularly to gasoline vapours, and the non-exposed groups were commuters with a profession not related to car driving or fuel distribution (e.g. school teachers, students). For further details see Table 6-1, references CDC, 1993b and 1993c.

Results

The results obtained with the questionnaire for the Stamford groups are reviewed in Table 6-5.

Table 6-5: Prevalence of Health Symptoms Potentially Related to MTBE Exposure Among Men by Job Category, Stamford, Connecticut, April 1993

(Symptom scores are given in percentage)

	Commuters (Controls)	Professional drivers	Motor Mechanics/ Service Station Attendants (Exposed)	Others (e.g. meter readers)
Number	59	57	48	12
Key symptoms				
Headache:				
1 or more times;	25.4	26.3	27.1	41.7
2 or more times;	23.7	21.1	15.0	41.7
3 or more times	5.1	8.8	8.3	8.3
Irritated eyes	18.6	7.0	20.8	16.7
Burning nose, throat	6.8	0.0	14.6	33.3
Cough	15.3	5.3	14.6	41.7
Dizziness	1.7	5.3	6.3	16.7
Spaciness (disorientated)	3.4	1.8	10.4	8.3
Nausea	0.0	0.0	2.1	8.3
One or more key symptoms	42.4	35.1	52.1	66.7
Two or more key symptoms	13.6	7.0	22.9	50.0

From CDC (1993b)

In Stamford the median MTBE-blood concentration in the non-exposed group was 0.12 µg/l, for motor mechanics 1.73 µg/l and for service station attendants 15.9 µg/l. The MTBE-blood levels were in good correlation with the personal sampling data. The median MTBE-blood levels were 0.42 µg/l for motor mechanics and service station attendants and 0.08 µg/l for others. No MTBE could be detected in the blood of individuals not exposed to gasoline.

Depending on the MTBE concentrations in blood, two groups were formed from the Stamford individuals. The group with high MTBE-blood concentrations (> 2.4 µg/l) scored significantly higher in 'key' symptoms of the questionnaire than the group with low MTBE blood concentrations.

Evaluation

Although there were slight differences in the selection of the subjects for the different groups, similarities in design and conduct allow a direct comparison between the reported data from Albany and Stamford. A highly similar prevalence was scored for "key" symptoms in the motor mechanics/service station attendants groups. These results suggest that these symptoms commonly occur in both occupations, but apparently also in the groups of commuters (see table 6-5). Together with the MTBE-blood concentrations and the information on exposure these data suggest there is no relationship between oxygenated gasoline and the scored "key" symptoms.

6.2.3 New Jersey Study

Beginning on November 15th 1992, an oxygenated fuel programme was launched in the southern part of New Jersey for 3.5 months and in the northern part for 5.5 months. The investigators compared two groups of garage workers differing in MTBE-exposure, timing the study so that sampling occurred when the northern workers were still exposed to MTBE-containing gasoline while their southern colleagues were not.

Mohr *et al* (1993) carried out a cross-sectional cohort study among gasoline-exposed garage workers in the state of New Jersey. Subjects involved in car maintenance and refuelling were asked to indicate the frequency of any symptoms over the past 30 days and to rank any discomfort experienced pre- and post-shift. Questionnaires took account of the symptoms reported previously in Fairbanks (Akaska) and those noted in human clinical studies and in animal studies. The exposure concentrations were monitored as a control. (For further details see Table 6-1)

Results

Exposure measurement over 1 hour revealed high concentrations in garages in the north (6-22 mg/m^3) and low concentrations in the south (1- 3 mg/m^3). A similar geographic pattern of high and low concentrations was observed after analysis of the passive personal sampling data. Some high figures were found in the south raising concerns about the accuracy of the sampling and analysis. No increase in the scores of symptom reporting was found among northern and southern garage workers, although both groups reported feeling worse by the end of the work day.

Included in the study was a sub-group described as 'fuellers' (13 from the North and 15 from the South) whose work meant that they routinely dispensed gasoline for at least 5 hours on each day. There were no differences in symptom reporting between these two groups.

Evaluation

In this study the presence of MTBE in gasoline did not result in differences in the scores of symptoms supposed to be related to exposure to oxygenated gasoline between the two study populations. The extent of exposure measurement was limited and did not provide insight into the actual individual exposures. Hence the results are still inconclusive.

6.2.4 Wisconsin Study

To meet public concerns over the introduction of oxygenated fuels the Department of Health and Social Services of the State of Wisconsin initiated a well designed study (Anderson *et al*, 1995; see Table 6-1 for details). The study was composed of distinct parts: (a) an air monitoring study, (b) a study on the composition of gasolines on the market in Milwaukee and Chicago, (c) a study on the health complaints received by the State Health Department and (d) a random telephone enquiry about health complaints using a standardised questionnaire. This review only deals with the results of the latter part (d) of this study. Anderson *et al* studied three distinct locations: South-eastern Wisconsin, an area with required use of oxygenated fuel, indicated as "Milwaukee" in their report; North-eastern Illinois, with required use of oxygenated fuel, indicated as "Chicago" and the rest of Wisconsin, with no required use of oxygenated fuel, indicated as "Wisconsin". Groups of approx. 500 individuals were selected from the three geographical locations using a 'random digit dial' procedure. Telephone interviewers were randomly assigned calls in all three areas to eliminate potential bias. Interviews were conducted by telephone between 24th February and 19th March 1995, the period coinciding with the introduction of the oxygenated fuels programme. The interview was structured to minimise bias and identify activities that might be associated with acute health effects deriving from exposure to oxygenated fuel.

At the start of the interview participants were told that the purpose of the investigation was to study the relationship between the environment, the use of gasoline and health. The questionnaire began with questions on the respondent's car use and type and brand of gasoline normally purchased. Subsequent questions related to health conditions over the last six months with particular reference to influenza. A further set of questions enquired about experience of unusual symptoms unrelated to cold or flu and whether such symptoms could be related to exposure to gasolines, for instance when filling the car or visiting the garage. Those who responded positively were further questioned about these specific symptoms. Smoking habits and general health status were recorded to take care of potential

confounders. Awareness of the existence of an oxygenated fuels programme in the Wisconsin area was scored separately.

Results

Measures of awareness were considered important and were highest in Milwaukee, followed by Wisconsin and then Chicago (table 6-6).

The prevalence of other symptoms, such as backaches and fever not previously associated with gasoline exposures were also higher in the Milwaukee region. The health symptoms were scored as "unexplained" health symptoms meaning symptoms unrelated to cold, flu or any other chronic health problem respondents were aware of.

Table 6-6: Awareness of the Existence of the 'Oxygenated Fuel Programme' (OFP), Scored by Region, Given in Number and (%)

	Region		
Question	Chicago	Milwaukee	Wisconsin
Do you live in an OFP area?	105 (21%)*	409 (78%)* #	13 (3%)
Purchased OFP since 11/1/94	51 (10%)	264 (50%)*#	13 (3%)
Heard of MTBE	113 (23%)*	283 (54%) *#	199 (40%)
Saw 1/19/95 "Day One" (49)	14 (3%)	29 (6%) #	25 (5%)
Saw week-long local TV series on MTBE	1 (0.2%)*	99 (19%)*	16 (3%)
Noticed different smells from gas	96 (20%)	275 (52%)*	77 (15%)
Saw other news stories about OFP	40 (8%)	187 (35%)*	106 (21%)

* Different from Wisconsin, p<0.05 # Different from Chicago, p<0.05
OFP = oxygenated fuel programme
Questions posed relate directly or indirectly to the introduction of the OFP or to media coverage of this programme

The definitions of the symptoms were similar to the ones in other studies (Table 6-2). The symptoms were further scored for potential correlation with exposure to gasoline (Table 6-7) and risk ratios for the individual symptoms were derived from the scores for the group of car owners. This risk ratio is the ratio of the perceived risk as indicated by the score of a particular symptom for a region divided by the corresponding score for the Wisconsin region.

A similar ratio was derived for the group of individuals from all regions reporting they had a cold, presented in the 'cold status' column of Table 6-8. This table indicates that Milwaukee residents were

between 3 and 35 times more likely to report "unexplained" symptoms that are supposedly related to exposure to oxygenated gasolines. Individuals with colds were also significantly more likely to report the 'unexplained' health symptoms but in all three regions. When analysed separately this relationship only holds for Milwaukee. Also the association between reporting purchasing oxygenated gasoline and specific "unexplained" symptoms was only evident in Milwaukee.

Table 6-7: Prevalence of "Unexplained" Health Symptoms by Region, Number and (%)

	Region					
Symptom	Chicago	Milwaukee	Wisconsin	Chicago	Milwaukee	Wisconsin
	Felt Symptoms since 11/1/94			Still Feeling Symptoms at Time of Interview		
Any Unexplained Symptom	30 (6%)+	119 (23%)*	32 (6%)	NA	NA	NA
Headache	13 (3%)+	67 (13%)*	9 (2%)	9 (2%)+	58 (11%)*	6 (1%)
Nausea	4 (1%)+	39 (7%)*	4 (1%)	2 (0.4%)+	27 (5%)*	2 (0.4%)
Eye Irritation	9 (2%)+	36 (7%)*	4 (1%)	6 (1%)	35 (7%)	3 (1%)
Dizziness	7 (1%)+	44 (8%)*	5 (1%)	6 (1%)+	34 (6%)*	1 (0.2%)
Diarrhoea	2 (0.5%)+	27 (5%)*	4 (1%)	0	20 (4%)*	3 (1%)
Rashes	3 (1%)+	23 (4%)*	2 (0.5%)	2 (0.4%)+	20 (4%)*	2 (0.4%)
Muscle Ache	2 (1%)+	19 (4%)*	5 (1%)	2 (0.4%)+	15 (3%)*	5 (1%)
Throat Irritation	8 (2%)+	47 (9%)*	4 (1%)	6 (1%)+	40 (8%)*	4 (1%)
Difficult Breathing	6 (1%)+	27 (5%)*	2 (0.5%)	3 (1%)+	20 (4%)*	2 (0.4%)
Back Pain	5 (1%)+	13 (2%)*	4 (1%)	2 (0.4%)+	10 (2%)*	4 (1%)
Fever	2 (0.5%)+	15 (3%)*	3 (1%)	2 (0.4%)+	7 (1%)*	0
Spaciness (disorientation)	5 (1%)+	35 (7%)*	1 (0.2%)	4 (1%)+	29 (6%)*	1 (0.2%)
Sinus Congestion	10 (2%)+	45 (9%)*	7 (1%)	7 (1%)+	40 (8%)*	6 (1%)
Funny Smells	9 (2%)+	50 (9%)*	4 (1%)	6 (1%)+	44 (8%)*	2 (0.4%)
Other symptom	3 (1%)+	8 (2%)*	1 (0.2%)+	2 (1%)+	6 (2%)*	0

* Different from Wisconsin, $p<0.05$ NA = Not asked

\+ Not different from Wisconsin, $p<0.05$

In conclusion, there were clear differences in symptom reporting between Milwaukee and Chicago, despite the similarity in gasoline composition and use of MTBE. The study demonstrated that individuals in the Milwaukee area reported an increased prevalence of symptoms. There was no difference in prevalence between Chicago and Wisconsin, despite the difference in gasoline composition in these areas (no MTBE in the gasoline marketed in Wisconsin). The study concluded that factors in addition to the use of oxygenated fuels obviously had contributed to the responses obtained. It was noted that a recent history of having a cold or 'flu' was a strong predictor for the reporting of unusual health effects in Milwaukee, leading the authors to suggest that recent illness rather than exposure to fuels was responsible for these findings. The authors also noted that respondents from Milwaukee were much more aware of the issues surrounding the use of oxygenated fuels than the respondents in the Chicago area (see Table 6-6). This heightened perception could have resulted in attributing any experienced unusual health symptom to exposure to oxygenated fuels.

The peer review panel concluded that the findings did not support a conclusion that exposure to oxygenated fuel was associated with widespread or serious acute adverse health effects.

Table 6-8: Health Scores (in percentage) for Individual Symptoms among Car Owners by Region and Respondents Reporting a Cold Status, Compared with the Scores Obtained for Wisconsin. Reference Group: Wisconsin Car Owners Without Colds

Symptom	Region			
	Chicago	Milwaukee	Wisconsin	Cold since 11/1/94
			Unadjusted	
Any Unusual Symptom	0.9+	4.5*	1	1.6*
Headache	1.5+	7.9*	1	2.2*
Nausea	1.0+	9.8*	1	3.0*
Diarrhoea	0.5+	6.5*	1	3.2*
Rashes	1.6+	11.2*	1	1.8+
Muscle Ache	0.6+	3.7*	1	1.7+
Throat Irritation	2.1+	12.0*	1	3.3*
Difficulty Breathing	3.1+	13.3*	1	12.0*
Back Pain	1.3+	3.1*	1	1.9+
Spaciness	5.3+	35.7*	1	5.3*
Sinus Congestion	1.5+	6.4*	1	2.5*
Funny Smells	2.2+	17.8*	1	2.4*
Other Symptoms	3.2+	7.5*	1	2.2+
			Age-Adjusted	
Eye Irritation	2.5+	7.9*	1	2.9*
Dizziness	1.5+	10.6*	1	1.9*
Fever	0.4+	4.0*	1	9.7*

* Different from Wisconsin, $p < 0.05$ + Not different from Wisconsin

Evaluation

This survey can be considered as a key study in design. Study and control populations have been selected at random and the populations were matched in terms of age, gender and commuting habits. Confounders were taken into account in advance and standard multivariate linear regression analyses were used throughout to adjust for possible additional confounders. Other limitations from the studies previously described were addressed, such as the subjectivity of self reporting of symptoms, recall bias on symptoms associated with type of gasoline purchased, cross-sectional nature of the study-design. The authors concluded that a prospective study including objective exposure measurement as well as an unbiased symptom reporting might overcome these limitations in a future study.

The survey reported high numbers of "key" symptoms for a population from Milwaukee living in an area required to use oxygenated fuels, but not for Chicago with the same requirement, or for Wisconsin with no requirement to use oxygenated fuel. The Milwaukee results differed significantly from both the Chicago and the Wisconsin ones. Therefore the authors concluded that other factors,

including awareness of supposed health effects due to MTBE in gasoline, had contributed to the findings of the Milwaukee area. In summary the overall study does not support an association between "unexplained" health symptoms (as reported by the people answering to the questionnaire) and the exposure to MTBE via oxygenated gasoline.

6.3 EXPERIMENTAL (VOLUNTEER) STUDIES

Four chamber studies (Cain *et al*, 1994; Prah *et al*, 1994, Johanson *et al*, 1995, Pekari *et al*, 1996, Riihimäki *et al*, 1996) have been reported in which healthy young adult volunteers were exposed to MTBE vapours under laboratory conditions. The purpose of these investigations was to examine the impact of MTBE exposure on selected objective and subjective end-points (see table 6-9).

Cain *et al*, 1994 exposed volunteers to 6.1 mg/m^3, for one hour, a simulated worst case consumer exposure at a fuelling station and introduced positive (VOC[a]) and negative (clean air) controls. The objective measurements included eye irritation, nasal lavage and CNS-function. Eye irritation criteria in the chamber suggested an increase but these effects were observed in both dose groups and are not related specifically to MTBE. Nasal lavage analysis revealed the polymorphonuclear cells to be unaffected by MTBE vapours. Standard neurobehavioural tests did not show differences between the exposed groups. The subjective responses obtained through a questionnaire similarly did not score differences between eye-, nose- or throat- irritation. The authors concluded that exposures of normal healthy young people to MTBE for one hour at a level of 6.1 mg/m^3, aside from the MTBE odour, did not induce any reaction.

Prah *et al*, 1994, exposed healthy volunteers to 5.0 mg/m^3 during one hour in a double-blind experiment. None of the tests revealed an increase in effects after exposure. Objective indicators for eye irritation and nasal inflammation were negative immediately after exposure and 20 hours later.

Johanson *et al*, 1995 (see also Nihlén *et al*, 1994), exposed volunteers to 18, 90 and 180 mg/m^3 during 2 hours to match the occupational exposure level. They observed a significant decrease in nasal peak expiratory flow in the MTBE exposed group, although the effect was not dose-related. No signs of eye or mucous membrane irritation were observed. Also the ratings for subjective effects did not reveal significant differences.

[a] VOC: Volatile Organic Chemicals mixture in air, not containing MTBE.

Table 6-9: Exposure Chamber Experimental Studies

Ref.	Exposure	Numbers Exposed	Questionnaire	Ocular measurements	Nasal measurements	Other End-points
Cain *et al*, 1994	6.1 mg/m^3 MTBE, 1 h, also to VOC and clean air	22 men 21 women	Three different questionnaires: - profile of mood states - air quality - symptom	Tear film break-up time scoring of the conjunctiva for epithelial damage, eye redness and presence of inflammatory cells in the tear fluid.	Inflammation markers (PMN's) in nasal lavage	Neurobehavioural testing; levels in blood
Prah *et al*, 1994	5.0 mg/m^3 MTBE, 1 h, clean air	19 men 18 women	Standardised 33 items/symptoms* in questionnaire	Pre- and post exposure tear film break-up time. Markers of inflammation form the conjunctiva.	Inflammatory markers from nasal lavage.	Levels in blood.
Johanson *et al*, 1995	18, 90 and 180 mg/m^3 MTBE, 2h. During light physical exercise.	10 men	Rating intensity of discomfort, irritation symptoms* and CNS-effects on a visual analogue scale.	Blinking frequency. Conjunctival epthelial damage. Eye redness. Tearfilm break-up time.	Acoustic rhinometry. Nasal and mouth peak expiratory flow. Inflammation markers in nasal lavage.	Levels in blood, TBA in blood.
Riihimäki *et al*, 1996	0, 90 and 270 mg/m^3, up to 3h.	13 men	Subjective symptoms and moods			Reaction time; Posturography

* Symptoms include those mentioned in the population studies; the so-called "key" symptoms.

Riihimaki *et al*, 1996, exposed thirteen healthy male volunteers to 0, 90 and 270 mg/m^3 MTBE in an exposure chamber for one and three hours. Subjects reported subjective symptoms and moods on a questionnaire using a rating of "not at all", "slightly" or 'clearly". Simple reaction time was measured with the computer-aided Swedish Performance Evaluation System (SPES). Posturography was performed with eyes open and eyes closed.

The authors concluded that only mild symptoms were reported by the exposed volunteers, mainly feeling of heaviness in the head and, to a smaller extent, mild mucous membrane irritation. The frequency of the symptom scores was related to the level of exposure to MTBE and reached statistical significance at 3 hours at 270 mg/m^3. No effects related to MTBE exposure were observed by measuring the reaction time or in body sway observed in posturography.

Joint evaluation of the human volunteer studies

The four reported studies were carried out under well controlled laboratory conditions. As such they eliminate many of the confounding factors from the population studies. Effects of exposure were assessed using objective and subjective measurements, the latter similar to the ones used in the population studies. Cain *et al* (1994), and Prah *et al* (1994) use exposure levels comparable to consumer exposures at fuel stations, the Johanson *et al* (1995) and Riihimäki *et al* (1996) studies are more relevant for occupational exposure setting. The latter focused on the toxicokinetic parameters. All four studies were well conducted and are acceptable for further assessment. It may be concluded on the basis of the available data that the studies do not reveal adverse effects of MTBE on humans for subjective or objective end-points. Though eye-irritation was reported at 6.1 mg/m^3 after one hour, it was not reported at 180 mg/m^3 after two hours exposure in a different study.

6.4 CONCLUSIONS

Information on the effects of human exposure to MTBE are available from clinical applications, population studies and laboratory experiments with volunteers.

The clinical data are of limited value as they describe effects observed using MTBE as a means to dissolve gallstones in situ. Accidental exposures (case reports) provide some information regarding the fate of MTBE in the human body. All this information relates to acute high level exposures. The quality of the data is limited.

The population studies mainly scored subjective information in relation to exposure data. The data may relate to acute as well as repeated exposures; it seems that a clear distinction between the two could not be made. The quality of the information is variable: from anecdotal to systematic. The New Jersey study, where the data on the group of professional workers is important, including some recordings on follow up several months after the exposure period, and the Wisconsin study are especially relevant for the assessment of the effects on the general population.

The laboratory studies with healthy adult volunteers have the advantage of providing objective data as well as subjective scores by the subjects which can be correlated with each other and with the results of the population studies. All exposures were acute at levels which can be considered relevant for consumer exposure at service stations (Cain *et al*, 1994; Prah *et al*, 1994) or for worker conditions (Nihlén *et al*, 1995; Riihimäki *et al*, 1996).

The first population studies (Fairbanks, Alaska) suggested a relationship between exposure to MTBE and health complaints. To test this hypothesis an objective correlation between exposure

concentration and subjective effects needed to be demonstrated but this could not be shown. Subsequent studies (New Jersey and Wisconsin) did not show an increase in the subjective effects which could be legitimately related to MTBE exposure either. This is in line with experimental studies showing no specific effects at similar exposure concentrations, and in two cases at concentrations approximately 30 times greater than that observed in the population studies. The only relationship demonstrated in the Wisconsin study was between the awareness of the introduction of MTBE in gasoline and effects hypothetically linked to MTBE.

One consistent finding in the laboratory studies was related to the odour of MTBE (and an odour threshold could be established - see chapter 2). Odour might have been the cause of the subjective responses in the population studies (see Appendix I). Eye and respiratory irritation has been a concern with regard to MTBE exposure and consequently this was investigated in volunteer studies dealing with concentrations occurring in the workplace. From the studies of Johanson *et al* (1995) and Riihimäki *et al* (1996) a NOAEL of 180 mg/m^3 is derived. At 270 mg/m^3 (3h) subjective symptoms were reported such as slight irritating effects on mucous membranes and feeling of heaviness in the head.

The volunteer studies have been conducted with young healthy adults. It is not known whether specific sensitivities with regard to MTBE exposure might exist in certain sections of the population like elderly people or children. Fiedler *et al* (1994) interviewed fourteen patients with multiple chemical sensitivities (MCS), five individuals with chronic fatigue syndrome (CFS) and six normal control subjects on situations related to gasoline with MTBE. Both the MCS and CFS subjects reported more symptoms than the normal controls across all situations, both MTBE- and non-MTBE-related. Their report is inconclusive with regard to any specific role for MTBE.

Published studies indicate that occupational and environmental exposures to MTBE, do not cause acute or medium-term health effects. Any relationship between MTBE exposures and subjective health complaints, as supposed before the population studies had been carried out, seem to be due to factors such as smell (also to the smell of gasoline in general), but also media coverage of the introduction of the oxygenated fuels programme, and seem therefore not directly related to the presence of MTBE in gasoline.

7. EXPOSURE ASSESSMENT

Data on worker exposures to MTBE are available for different phases of MTBE production, handling, distribution and use. Consumer exposure has been measured at gasoline stations. These data are reviewed in this chapter in relation to production and loading, blending with gasoline in the petroleum industry, handling of gasoline at service stations and garages, and consumer exposures at gasoline stations. The information related to production and loading are unpublished industry data, the information related to service stations, garages and gasoline stations are from the studies already reviewed in chapter 6. Recent data from a survey made by CONCAWE are also included.

7.1 PRODUCTION AND LOADING

Eight hour exposure measurements have been obtained for a number of job types and are reviewed in Table 7-1. They mainly relate to operations in the manufacturing plant except the jetty operation (loading/unloading of ships). For complete shift exposure measurements the operator was equipped, close to his breathing zone, with a small charcoal tube fixed to a pump to collect the MTBE (and other) contaminants in the air. The collected vapours were analysed subsequently according to the method described in NIOSH 1615.

Table 7-1 : Exposure measurements, Production (8 hr exposure period)

Job type	Duration (min) [a]	Number of Samples	Mean [b] (mg/m^3)	Range (mg/m^3)	Reference
Sample room operator	350	2	8.64	5.76-11.16	ARCO Chemical Company, 1995
Production operator	- [c]	7	0.86	0.04-3.71	
- emptying	- [c]	13	0.63	0.04-3.95	
- start-up	- [c]	6	2.12	0.14-7.92	
jetty operator	325-540	6	11.88	0.72-20.5	

[a] Time period over which the sampling was carried out.
[b] Value adjusted for the normal working day, 8h TWA.
[c] Exposure duration not known.

However, the duration times were much shorter. Exposure data from loading operations were obtained in the same way. The values were subsequently adjusted to a 15 minutes exposure period in order to provide for an estimate of short-term exposures (see Table 7-2). These values are considered short-term peak exposures.

Table 7-2: Exposure measurements, Loading operations (15 min peak exposure)

Activity	Duration (min)[a]	Number of samples	Peak exposure (mg/m^3)	Range (mg/m^3)	Reference
jetty operation	12-55	7	106.6	47.9-205.6	ARCO Chemical Company, 1995
other loading	14-25	7	119.5	48-153.8	
operations	3-56	5	100	62-140.1	
	17-40	5	122	62.1-228.5	
	2-13	3	13.0	1.4-31.8	

[a] Time period over which sampling was carried out.

Evaluation

A range of peak exposure values and 8-hour time weighted average data are available for MTBE. These data (ARCO Chemical Company, 1995) were collected according to recognised procedures. Production is associated with relatively low exposures (<3 mg/m^3 over 8 h), while sampling and loading may result in relatively high exposures (between 8 and 12 mg/m3). Peak exposures at loading, during periods ranging from 2 to 56 minutes, are in a range from 1 to around 228 mg/m^3. When adjusted to 15 minutes the maximum peak exposure is around 120 mg/m^3.

7.2 PETROLEUM INDUSTRY

Personal sampling data available from the petroleum industry include exposures to MTBE products and to MTBE in gasoline (CONCAWE, report in prep.). The data indicate that average personal exposures to MTBE products is in a range from 3.6 to 45.5 mg/m^3 over time periods of 100 to 390 minutes (equivalent to 9.5 mg/m^3 8h TWA), and to MTBE from gasoline from 0.3 to 85.6 mg/m^3 (maximum 162 mg/m^3) over time periods of 2 to 599 minutes. Fixed position sampling, measuring the concentration at the workplace for MTBE in gasoline, were in the range of 0.2 to 18 mg/m^3 over a period of 23 to 480 minutes.

Evaluation

Although limited details have been given about sampling and analysis these indicate maximal worker exposures of up to 9.5 mg/m^3 (8h TWA) during the handling and blending of MTBE in the European petroleum industries. Peak exposures up to 162 mg/m^3 (exposure time not known) have been observed.

7.3 HANDLING OF GASOLINE CONTAINING MTBE

Hakkola and Saarinen (1996) measured peak exposures to MTBE due to gasolines containing 10 to 15% MTBE in two Finnish gasoline depots, one located in the North and the other in the South of the country, and related service stations. At the Northern depot bottom loading road cars, equipped with a vapour recovery system were used whilst in the Southern depot, top loading road cars without vapour recovery were employed. Tanker drivers handling between one and four loads per day, more than half containing gasoline with MTBE, were monitored during loading and unloading operations. Exposure monitoring was carried out as described in 7.1. Exposures measured are given in Table 7-3.

CONCAWE (report in preparation) also reported personal sampling data on exposures to MTBE during loading operations of oxygenated gasoline at bulk storage, transport to gasoline stations and unloading at gasoline stations. Personal exposures were in the range of 0.3 to 2.8 mg/m^3 over 480 minutes, with a maximum of 20.2 mg/m^3 for one maintenance worker (equivalent to 5 mg/m^3 , 8h TWA).

Giacomello (1996) measured personal exposure to MTBE vapour in a group of "full service" attendants working in 58 Italian service stations. The study included a number of geographical locations and was conducted in summer 1991 , winter 1992 and summer 1995. The overall geometric mean concentrations were 0.71 mg/m^3, 0.37 mg/m^3 and 0.26 mg/m^3 respectively. Differences between the surveys were thought to reflect the effect of the season, variations in MTBE level in the blends and the introduction of Phase I vapour recovery systems. The lower level of exposure in 1995 could also reflect the aspect of training attendants to avoid unnecessary exposure and spillage.

Evaluation

Peak exposures were high for top loading road cars but relatively low for bottom loading road cars equipped with a vapour recovery system. Individual data for the service stations were not provided and further distinctions in the results cannot be made.

Extrapolation from these Finnish data to other types of gasoline (where MTBE is present at low concentrations, primarily as an octane booster) or other regions in Europe or different loading/unloading systems cannot be made. Considerably lower exposures were recorded for employees exposed to MTBE from oxygenated gasoline during bulk storage, maintenance and also during dispensing by service station attendants in Italy.

Table 7-3: Exposures to MTBE during loading/unloading of gasoline containing 10-15% MTBE.

Location	Duration [a] min	(range)	samples	Mean exposure [b] (mg/m^3)	(range)	Vapour recovery	Reference
Northern depot, Bottom loading, roadcars	28	(15-40)	6	13	(2.8-42.0)	yes	Hakkola Saarinen, 1996
Service station deliveries, North	33	(22-44)	5	16	(4.3-27.0)	not known	
Southern depot, Top loading, roadcars	20	(10-30)	4	91	(20-226)	no	
Service station deliveries, South	26	(10-37)	6	71	(10-98)	no	

[a] Time period over which sampling was carried out.
[b] Mean exposures not corrected for exposure duration.

7.4 GARAGES AND SERVICE STATIONS

Many measurements were collected in the course of the population studies described in Section 6.2. These measurements were carried out by the USA Centre for Disease Control and Prevention (CDC,1993b and 1993c). Representative data from these studies are summarised in Table 7-4. To simplify the presentation the data for job categories that could not be clearly defined in relation to possible exposures to gasoline and MTBE have been excluded, e.g. foremen, chauffeurs, part managers. Table 7-4 is limited to data for car mechanics and service station attendants.

Two of the studies reviewed measured exposures in regions of the US where MTBE was added to gasoline as an octane booster at levels < 1% (CDC, 1993a; Moolenaar *et al*, 1994; API, 1991). The remaining studies were carried out in regions of the US where MTBE was present in gasoline at levels > 11% (CDC, 1993b, 1993c; API, 1991). Personal breathing zone samples were collected over a normal day period and analysed as described (see 7.1). From these data 8h TWA exposures were calculated.

In regions where MTBE levels in gasoline were low, exposures for car mechanics were in the range of <0.1-1.62 mg/m^3, while in regions with high levels, exposures were in the range of <0.1-1.66 mg/m^3.

Table 7-4: Exposure Measurements, Service Station Attendants and Garage Workers

Job type	Samples	Mean, 8 h (TWA) (mg/m^3)	Range (mg/m^3)	Reference
Car Mechanics	7	0.14	< 0.14	Moolenaar *et al*, 1994
	3	0.45	0.36-0.54	
	4	0.10	<0.1-0.1	
	7	0.36	<0.1-0.47	
	4	1.08	0.65-1.62	
Car Mechanics	7	0.44	<0.1-1.62 *	CDC, 1993b
	5	0.96	0.43-1.66	
	3	0.35	<0.14-0.54	
Car Mechanics	5	0.11	<0.1-0.5	CDC, 1993c
	4	0.1	<0.1-0.1	
	3	0.1	<0.1-0.1	
Car Mechanics	4	0.45	<0.1--1.19	CDC, 1993b
	4	3.47	0.83-7.56	
Service station	10	2.98	0.5-6.82	HETA data, API, 1991
Attendant [a]	10	1.08	0.36-3.38	

[a] One high value (43.34 mg/kg) has been excluded.

Evaluation

These studies were carried out to a reasonable technical standard, although jobs were often poorly defined. It is also unclear to the Task Force whether exposures had actually occurred in many instances. In particular it is not clear why exposures in Fairbanks (CDC, 1993a) where the gasoline contained <1% MTBE, were similar to those from areas where gasoline with up to 15% was generally used. In all the facilities monitored the mean exposure to MTBE was 3.5 mg/m^3. The highest individual value obtained was 7.54 mg/m^3.

7.5 CONSUMERS

Car refilling at service stations appears to be the most likely source of consumer exposure to MTBE vapour arising from gasoline by evaporation. Information on this aspect is available from a single study recently conducted in Finland where MTBE is used in gasoline at approximately 11%. In a recent study (Vaimotalo *et al*, 1996), consumer exposure was measured at two self service stations in the Southwest of Finland (Table 7-5). To determine customer exposures during tank filling, vapours were collected by holding a sampling tube connected to a pump and a charcoal tube in their breathing zone for the duration of the filling period. Samples were collected during a period of four days at each location in summer and winter.

Evaluation

Although limited to a single report, the numbers of samples analysed provide a reasonable insight into probable consumer exposure during car refuelling. There was a wide range in the individual values obtained, but the overall mean short-term exposures were very similar (6.0 - 7.5 mg/m^3 for approximately 1 min).

Table 7-5: Exposure measurements, consumers at filling stations

Activity	Duration in seconds (range)	Samples	Short-term values (mg/m^3)	Range (mg/m^3)	Reference
Refuelling, summer	71(25-210)	77	7.5	<0.2-131	ARCO Chemical Company, 1995
	70 (25-242)	76	4.3	<0.2-203	
Refuelling, winter	58 (21-275)	80	7.4	<0.4-138	
	60 (23-150)	80	6.0	<0.2-245	

8. RISK CHARACTERISATION

8.1 SUMMARY OF TOXICOLOGICAL AND HUMAN HEALTH ASPECTS

Toxicokinetics

The toxicokinetic properties of MTBE have been studied extensively. It is absorbed readily by all routes of exposure but there are quantitative differences in the extent of absorption. The absorbed material is distributed uniformly in all tissues and shows no tendency to accumulate. This is due to rapid removal of MTBE via exhalation and metabolism. Metabolism leads to two principal metabolites, ie *tert*-butanol (TBA) and formaldehyde. These are further metabolized and show no tendency to accumulate. TBA excretion proceeds relatively slowly (half-life: 8 hours in humans). Elimination of the products resulting from TBA metabolism occurs mainly via urine. Formaldehyde and products resulting from formaldehyde metabolism enter physiological biochemical pathways. Formaldehyde may be viewed as potentially hazardous, but its rate of formation is considered to be too low relative to the detoxification rates to raise concerns about elevation of the natural formaldehyde levels in the body.

Conclusion

The biotransformation of MTBE leads to the formation of TBA and formaldehyde which in turn are further metabolised. The toxicokinetic data do not indicate reasons for concern with regard to bioaccumulation of MTBE or its metabolites.

Acute effects

MTBE is of low acute toxicity in experimental animals by oral, dermal and inhalation routes. LD_{50} values exceed 2000 mg/kg for oral and dermal exposure, and the inhalation LC_{50} value is 85,000 mg/m^3 for 4 hours. Sub-lethal acute exposure causes local irritation at the site of contact and transient clinical signs characteristic of CNS depression. Skin contact with MTBE causes reversible moderate to severe irritation in rabbits but it is only slightly irritant to the eye. MTBE vapour at concentrations above 300 mg/m^3 causes slight and transient irritation to the respiratory system of laboratory animals. An RD_{50} of 16,600 mg/m^3 was determined in the mouse for sensory and respiratory irritation. Animal tests have revealed no potential for skin sensitisation and there are no case reports of sensitisation in humans, although contact with neat MTBE as well as gasoline containing MTBE probably has occurred in the past. This suggests that MTBE is not a skin sensitiser.

Conclusion

Transient CNS depression and mortality occur at high-doses/concentrations only. Following acute exposures to MTBE skin and respiratory irritation are regarded as the primary concern. Labelling of MTBE as „irritant" (Xi) with the corresponding R-phrase 38 (irritating to skin) is proposed by the Task Force until formally reviewed by DG XI.

Neurotoxicity

MTBE caused loss of consciousness in experimental animals when inhaled at concentrations of 28,800 mg/m^3. Reversible functional CNS effects were detected in a rat study at 14,400 mg/m^3 (LOAEL) using a functional observation battery and 6 hours of exposure. The NOAEL in this study was 2,880 mg/m^3. Observations suggesting transient CNS depression were also consistently found in animal studies using repeated inhalation and oral exposure. However, all effects were reversed when exposure ended and repeated exposure did not lead to lower NOAELs in comparison with the single exposure.

Conclusion

MTBE causes loss of consciousness when inhaled at exposure concentration of 28,800 mg/m^3 and above. At lower exposures transient behavioural changes have been described in animal studies. The NOAEL for these reversible functional CNS effects observed after 6 hours of exposure is 2,880 mg/m^3.

Repeat-dose Effects

MTBE is of low toxicity following repeated oral exposure in the rat, mouse and the monkey. Whereas the mouse and the monkey studies are of limited value, a NOAEL of 300 mg/kg was determined for the rat in an oral sub-chronic study conducted according to GLP. A chronic oral gavage study in rats is of limited value due to reporting deficiencies, but the results do not contradict the sub-chronic NOAEL. Target organs for MTBE toxicity at higher doses have consistently been the liver and the kidney. Similar results were obtained in rats and mice after inhalation exposure. The NOAEL's for sub-chronic inhalation exposure in the rat and mouse were 2,880 mg/m^3. Reversible changes in behaviour and CNS depression were also seen. Chronic inhalation studies have also demonstrated low toxicity of MTBE in mice and rats, and a NOAEL of 1,440 mg/m^3 has been determined for non-neoplastic effects. Kidney effects (nephropathy) have been seen in male rats at lower concentrations, but these occur probably via a mechanism not relevant to humans. Higher concentrations also caused reversible CNS depression, but no structural damage to the nervous system. The effects are of a transient nature.

Conclusion

Principle effects identified for MTBE following repeat oral or inhalation exposure are local irritation at the site of entry, CNS effects (transient anaesthesia), kidney effects (chronic nephropathy) and liver effects (hepatocellular hypertrophy). The NOAEL for sub-chronic oral exposure is 300 mg/kg. The NOAEL for chronic inhalation exposure is 1,440 mg/m^3. This corresponds to retained MTBE-doses in the body of 102 and 113 mg/kg/day for male and female F-344 rats, respectively, and 182 and 184 mg/kg/day for the male and female mouse, respectively. (For calculation see page 29.)

Genotoxicity

MTBE has been tested extensively *in vitro* and *in vivo* for its genotoxic potential. It was not mutagenic in bacterial or yeast test systems, and no evidence of genotoxicity was seen for CA, gene mutation, and UDS determined in mammalian cells. A weak response in CHO cells (SCE) and a variable response in V79 cells (gene mutation) were observed . No genotoxicity was seen in the rodent cytogenetic assays, the *in vivo/in vitro* UDS assay and the sex-linked recessive lethal test in Drosophila. The weight of evidence suggests that MTBE is not genotoxic. This conclusion is supported by data on the genotoxicity of the MTBE metabolite TBA, which was negative in several *in vitro* tests and one *in vivo* assay. The other principle metabolite, formaldehyde, is mutagenic in a number of experimental systems, but toxicokinetic considerations, together with negative *in vivo* results for MTBE itself, suggest that is not a concern.

Conclusion

The available evidence does not raise concern with regard to genotoxicity of MTBE.

Neoplastic effects

The effect of high-doses of MTBE on tumour induction in experimental animals has been investigated in an oral gavage study in Sprague-Dawley rats and in inhalation studies with Fischer-344 rats and CD-1 mice. In the oral study, an increase in combined lymphoma/leukemia incidence in female rats was reported, but deficiencies in the design and reporting preclude meaningful interpretation of these results. The effect on Leydig cell tumour incidence after ingestion is regarded as specific to Sprague-Dawley rats and appears of doubtful relevance to human health. The inhalation study in rats demonstrated a tumourigenic response in the male kidney at 10,800 and 28,800 mg/m^3 (corresponding to 384 and 1023 mg/kg/day,respectively), but a non-genotoxic mechanism unique to the male rat is probably involved. The apparent increase in the incidence of Leydig cell tumours in male Fischer-344 rats treated via inhalation was considered a background event, within the historic

spontaneous range for this rat strain and, in addition, this tumour type is of doubtful relevance to humans. An inhalation study with mice showed an increase in the incidence of liver adenomas in female animals at 28,800 mg/m^3. This exposure concentration corresponds to a daily retained dose of 1824 mg/kg, a level in excess of the MTD and a non-genotoxic mechanism appears to be involved. The Task Force considers this increase in the incidence of benign liver tumours at this high-dose not relevant to humans.

Conclusion

MTBE induces tumours in rodents at doses exceeding the MTD. Since genotoxicity appears not to be involved, the mechanism of MTBE tumour induction is considered to be secondary to toxicity in the target tissues. Further mechanistic studies are currently being conducted to clarify this. The doses necessary to demonstrate neoplastic effects are equal to or greater than doses that induce chronic non-neoplastic effects in the target tissues, liver and kidney. Therefore, protection against non-neoplastic effects will also protect from any theoretical carcinogenic effect. It is concluded by the Task Force that MTBE does not require classification as a carcinogen according to the criteria presented in Annex VI of Directive 67/548/EEC on Dangerous Substances (EEC, 1993b).

Reproductive Effects

No effect on reproduction was observed at up to 28,800 mg/m^3 in two rat studies, the NOAEL for general toxicity was 1,440 mg/m^3 for the parental animals. For the rat and the rabbit, the NOAEL for developmental toxicity was greater than 9000 mg/m^3 or 28,800 mg/m^3, respectively. In mice, the NOAEL for developmental and maternal toxicity was 3600 mg/m^3. At higher concentrations (14,400 and 28,800 mg/m^3) foetotoxicity was observed and the incidence of one maleformation (cleft palate) was increased. These effects are not considered as direct effects on the fetus but secondary to concurrent maternal toxicity (reduced body weight and clinical signs of CNS effects such as hypoactivity, and ataxia).

Conclusion

The Task Force concludes that MTBE has no potential to cause adverse effects on human reproduction.

Human Experience

A large body of data is available from human experience with MTBE, including case reports of clincial use of MTBE for gallstone dissolution, studies reporting subjective complaints by garage workers and service station attendants, large population studies with sophisticated study design and controlled short-term exposure of volunteers. Whereas the early studies suggested a relationship between MTBE exposure concentration and health complaints, this has not been confirmed in subsequent studies. This absence of an association is in line with short-term experimental studies that showed no specific effects at concentrations similar to or greater than those observed in the population studies (< 3.6 - 181 mg/m^3).

Human experimental data do not indicate irritation of the respiratory tract at concentrations of 180 mg/m^3 for two hours. Exposure to 270 mg/m^3 for three hours caused mild mucous membrane irritation in some volunteers.

Objective symptoms on the CNS were not observed in experimental volunteer studies up to 270 mg/m^3. Subjective symptoms at this concentration were reported by volunteers (mainly feeling of heaviness in the head). At 180 mg/m^3 no symptoms were reported.

Conclusion

No consistent relationship between MTBE exposure and subjective health complaints, symptoms or objective findings has been established in population studies. Experimental volunteer studies at concentrations possibly occuring as peak exposure levels at some workplaces demonstrated a NOAEL of 180 mg/m^3. Mild subjective symptoms and slight irritation of mucous membranes were reported at 270 mg/m^3.

Identification of relevant end points and NOAELs

Table 8-1 summarizes the conclusions with regard to MTBE-related effects observed after short-term and long-term exposure of human and experimental animals. Irritation observed after short-term exposure in humans, as well as liver and kidney toxicity observed after long-term exposure in experimental animals, are regarded as critical effects for the risk characterization of MTBE. Mild respiratory irritation occured at a concentration 270 mg/m^3 for three hours in human volunteers, while a concentration of 180 mg/m^3 for two hours did not cause such effects. The lowest NOAEL for liver and kidney effects after chronic inhalation exposure was 102 mg/kg/day (retained dose). In chapters 8.2 and 8.3, the NOAELs identified are compared with the doses/concentrations calculated for occupational and consumer exposures.

Table 8-1: Principal effects of MTBE and NOAELs

End point	Species	Route	Exposure Time	Principal Effects	NOAEL	Remarks	Reference
acute effects	human	inhalation	2 hours (during light physical exercise)	mucous membrane irritation	180 mg/m^3	subjective symptoms (like slight irritation and heaviness in the head) were reported at 270 mg/m^3 (3 hrs)	Johanson *et al*, 1995 Riihimäki *et al*, 1996
subchronic toxicity	rat	inhalation	90 days	liver and kidney toxicity (males)	2,880 mg/m^3	equivalent to 228 mg/kg bw/day (males)	Dodd and Kintigh, 1989
chronic toxicity and neoplastic effects	rat	inhalation	105 weeks	liver and kidney toxicity, kidney tumours (males)	1,440 mg/m^3	equivalent to 102 mg/kg bw/day (males)	Chun *et al*, 1992
	mouse	inhalation	18 months	liver tumours (females)	10,800 mg/m^3	equivalent to 669 mg/kg bw/day (females)	Burleigh-Flayer *et al*, 1992
neurotoxicity	rat	inhalation	6 hours	functional CNS effects	2,880 mg/m^3	LOAEL was 14,400 mg/m^3; effects were reversible	Gill, 1989
effects on fertility	rat	inhalation	two generations	no treatment related effects	> 28,800 mg/m^3	for parental toxicity a NOAEL of 1,440 mg/m^3 was determined	Myhr *et al*, 1991
developmental toxicity	mouse	inhalation	gestation days 6 to 15	no direct effect on the fetus	3,600 mg/m^3	higher concentrations caused maternal toxicity and secondary developmental toxicity	Tyl and Neeper-Bradley, 1989

8.2 OCCUPATIONAL EXPOSURE

The main route of exposure for workers who come into contact with MTBE or gasoline containing MTBE is inhalation. Although dermal exposure and subsequent uptake of MTBE is theoretically possible, current work practices as well as the personal protective measures recommended in material safety data sheets would render this unlikely. Dermal exposure is therefore considered as negligible for the assessment presented here.

8.2.1 MTBE production

Production is associated with relatively low exposures (<10 mg/m^3 over 8 h), while loading operations may result in higher exposures (20 mg/m^3, with peak exposure around 200 mg/m^3). Assuming an inhalation volume of 10 m^3 per 8-hour shift and a relative respiratory uptake of 40%, exposure to a concentration of 10 mg/m^3 would result in a retained amount of 40 mg MTBE per day corresponding to a dose of 0.57 mg/kg for a 70 kg adult. The lowest NOAEL from chronic animal inhalation studies is 102 mg/kg/day. Comparison of these two doses leads to an approximate 300 fold margin of safety for workers involved in production. For loading operations, a 180 fold margin of safety is apparent. Comparison of the maximum exposure values of about 200 mg/m^3 with the concentration, which caused slight mucous membrane irritation in some volunteers (270 mg/m^3), does not indicate concern.

8.2.2 Handling of gasolines containing MTBE

Mean short-term exposure measurements for loading and delivery of gasoline containing 10-15% MTBE were between 13-91 mg/m^3 with a maximum of 226 mg/m^3. Since most fuels in Europe only contain MTBE at around 2% (as an octane enhancer), these findings were considered by the Task Force to be a 'worst case' situation . In general, these operations lasted around 30 minutes, with mean short-term exposure below 100 mg/m^3. Assuming a ventilation rate of 1.25 m^3/h and a relative respiratory uptake of 40%, exposure to such a concentration for 30 min would result in a retained amount of 25 mg MTBE per exposure period, corresponding to a dose of 0.36 mg/kg. Assuming a worst case of 4 exposures per day, this would lead to a daily dose of 1.44 mg/kg. The lowest NOAEL from chronic animal inhalation studies is 102 mg/kg/day. Comparison of these two doses leads to an approximate 70-fold margin of safety for workers involved in loading and delivery of gasoline containing high percentages of MTBE. Comparison of the maximum exposure values of about 226 mg/m^3 with the concentration, which caused slight mucous membrane irritation in some volunteers (270 mg/m^3), does not indicate concern.

8.2.3 Service-station attendants and garage workers

Monitoring data from the US show a highest mean exposure to MTBE of approximately 3.5 mg/m^3, with the highest individual value of 7.56 mg/m^3. Assuming a ventilation volume of 10 m^3 per 8-hour shift and a relative respiratory uptake of 40%, an exposure concentration of 3.5 mg/m^3 would result in a retained amount of 14 mg, corresponding to a dose of 0.2 mg/kg per day for a 70-kg adult. The lowest NOAEL from chronic animal inhalation studies is 102 mg/kg/day. Comparison of these two doses leads to an approximate 800 fold margin of safety for workers involved in loading and delivery of gasoline containing high percentages of MTBE. The highest observed exposure value would give an approximate 250 fold margin of safety. For service-station attendants and garage workers irritation due to MTBE is not a concern.

8.2.4 Recommended Occupational Exposure Limit

An occupational exposure limit of 90 mg/m^3 or 25 ppm (8-h TWA) is proposed for workers handling MTBE. This concentration corresponds to a daily retained MTBE dose of about 5.1 mg/kg (on the basis of a ventilation volume of 10 m^3/8-h shift and a relative respiratory uptake of 40%) and provides a margin of safety of 20 when compared with the lowest NOAEL determined in chronic animal inhalation experiments. Respiratory irritation is regarded as the critical effect for higher short-term exposures. In humans, at a concentration of 180 mg/m^3 for 2 hours no effects were observed while at 270 mg/m^3 for three hours only slight irritating effects on the mucous membranes were reported in some volunteers. Therefore, a limit of three times the TWA (270 mg/m^3 or 75 ppm) is considered to be an appropriate short-term, peak exposure limit (15-min STEL).

8.3 CONSUMER EXPOSURES

On the basis of the limited information available mean short-term exposures during car refuelling are calculated as 6.0 to 7.5 mg/m^3 for about 1 minute. The gasoline used in the Finnish study that provided these data had a MTBE content of 11% w/w, whereas in other European areas a lower MTBE content gasoline is used. Therefore maximum mean short-term exposures of about 10 mg/m^3 and a refuelling duration of about 5 minutes appear a realistic worst case scenario. Such exposure would lead to a retained amount of about 0.42 mg using an inhalation rate of 1.25 m^3/h and a retention of 40% for an adult human being (70 kg). This results in a dose of about 0.006 mg/kg. Comparison with the NOAEL of 102 mg/kg obtained in chronic inhalation studies indicate a margin of safety of approx. 17,000 for consumers in this situation.

9. ONGOING RESEARCH

Mechanistic investigations:

In animal studies, MTBE has been found to increase the incidence of certain tumours in male rats and in female mice following long term inhalation exposure. To explore mechanisms underlying these findings a research programme has been initiated at CIIT (the US Chemical Industries Institute for Toxicology, Research Triangle Park), which covers the following aspects:

- ***Kidney tumours in male rats:***

The species- and sex specificity of kidney tumours seen in male rats following inhalation exposure to MTBE and oral exposure to TBA resembles that of other chemicals which operate through an α_{2u}-globulin dependent mechanism. Studies will be conducted to determine if MTBE (up to 10,800 mg/m^3 by inhalation) and TBA (up to 5 mg/l in drinking water) bind to α_{2u}-globulin and cause it to accumulate in renal tubules with attendant nephropathy and increased cell proliferation in proximal renal tubules. Preliminary findings indicate an increased intensity of protein droplets in proximal tubules and a greater labelling index in treated animals (Borghoff *et al*, 1996; Prescott-Mathews *et al*, 1997).

- ***Liver tumours in female mice:***

The species- and sex specificity of liver tumours seen in female mice following inhalation exposure to high concentrations of MTBE resembles the tumours caused by unleaded gasoline. The studies at CIIT apply the initiation-promotion model of hepatocarcinogenesis using diethylnitrosamine. Preliminary results indicate increased hepatic cytochrome P450 activity, but no evidence that MTBE is a tumour promotor (Moser *et al*, 1996)

- ***Aspects of formaldehyde formation from MTBE:***

Formaldehyde formation from MTBE will be followed in liver and kidney cells *in vitro* with a macromolecular binding assay.

- ***Pharmacokinetic modelling in rats and humans:***

An initial PBPK model will be developed for MTBE and TBA in male rats and include separate compartments for liver, kidney and testes, then extended to include human metabolic and pharmacokinetic constants. Such modelling is necessary to quantitate the internal (effective) dose of MTBE in human organs following worker or consumer exposure. Differences in pharmacokinetic profile will have important consequences for risk characterisation.

■ ***Other tumours :***

A study will be conducted to determine the concentration of MTBE and its metabolites in testicular tissue from two strains of rat following oral administration. Thyroid tumours have been reported in mice after long term oral administration of TBA. The thyroid is a common target for chemically-induced toxicity, and tumours may arise as a result of enhanced hepatic microsomal metabolism and altered thyroid hormone homeostasis. The role of TBA and the formation of thyroid tumours as a function of the levels of TSH, T3 and T4 will be investigated.

Other studies

Metabolism studies

Comparitive metabolism studies in rats and humans will be sponsored for MTBE, ETBE and TAME by the Health Effects Institute (USA). Another study will examine the role of various P450 isozymes in the metabolism of these compounds.

Genotoxicity in vitro/ in vivo

The US Agency for Toxic Substances and Disease Registry (ATSDR) will sponsor studies on MTBE in a modified Ames test (using a microsuspension procedure), in a micronucleus assay and in a mutation induction assay at the lacZ gene in mouse kidneys.

Oxygenated gasoline

Belpoggi *et al* (1995) reported that a study is in progress to investigate the carcinogenic potential of gasoline containing 15% MTBE. Only limited experimental details are given. These indicate that oxygenated gasoline will be administered to male and female rats in olive oil, at a dose of 800 mg/kg/day, 4 days per week for 104 weeks. Control animals will be given olive oil only, with no inclusion of concurrent treatment groups exposed to MTBE alone or conventional unleaded gasoline. The treatment phase of the study appears completed, and it appears that the animals will be allowed to survive until they die from natural causes. No further details are available.

Exposure Studies and Environmental Monitoring

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health in the US will analyse blood samples for MTBE and other volatile compounds to determine frequencies of occurrence and background levels in the population. MTBE measurements will

continue to be included in the National Water-Quality Assessment projects in the US, together with measurement of other volatile organic compounds.

APPENDIX I: STUDIES OF SYMPTOMS CAUSED BY ODOUR

In a series of epidemiological studies, the relationship between objective exposure to odorant concentrations emitted by several industrial plants was investigated, as was the relationship between odour annoyance and subjective health complaints. Exposure was determined with a dispersion model of odorants, in which meteorological data and industrial emissions were used as input. Among others, it appears that the dispersion model performs moderately well in predicting annoyance (correlations between odorant concentrations and odour annoyance were about 0.35). The extent to which people regard malodour as a threat to their health is a relatively strong predictor of annoyance. Moreover, the subjective effects of long-term low exposure are similar to the effects of temporary high exposure (Cavalini, 1994).

Retrospective symptom prevalence data, collected from over 200 adult respondents living near three different hazardous waste sites, were analysed with respect to both self-reported "environmental worry" and frequency of perceived environmental (particularly petrochemical) odours. Significant positive relationships were observed between the prevalence of several symptoms (headache, nausea, eye and throat irritation) and both frequency of odour perception and degree of worry. Headaches, for example, showed a prevalence odds ratio of 5.0 comparing respondents who reported noticing environmental odours frequently versus those noticing no such odours and 10.8 comparing those who described themselves as "very worried" versus "not worried" about environmental conditions in their neighbourhood. Potential explanations for these observations are presented, including the possibility that odours serve as a sensory cue for the manifestation of stress-related illness (or heightened awareness of underlying symptoms) among individuals concerned about the quality of their neighbourhood environment (Shusterman *et al*, 1991).

A survey of young adult college students investigated the prevalence of self-reported illness from the smell of the five following common environmental chemicals (cacosmia): 1) pesticide, 2) automobile exhaust, 3) paint, 4) new carpet, and 5) perfume. Sixty-six percent of 643 students reported feeling ill from one or more of the five odours (Bell *et al*, 1993).

One study examined whether the emissions of a plant manufacturing fertiliser for mushroom cultivation constituted a health threat for the residents of the area. Apart from an extremely high degree of annoyance, the investigators found a growing number of complaints increasing from the control area to the medium range and to the close range around the plant; there were specific complaints (e.g. nausea, sickness, vomiting) and unspecified complaints (e.g. sleeplessness, headaches and stomach aches) in terms of effect gradients. After including moderating factors in multiple regression analysis, they found highly significant associations between the level of cortisol and odour exposure (Steinheider *et al*, 1993).

A group of 62 human subjects were exposed for 2.75 h to a mixture of 22 volatile organic compounds known to be indoor air pollutants. Three total concentrations of 0, 5, and 25 mg/m^3 of the same 22 compounds were used. Continuous evaluation of irritation in eyes, nose and throat showed significant correlation to exposure both at 5 and 25 mg/m^3. The effect was acute and showed no signs of adaptation. A digit span performance test showed decreased scores during exposure (Molhave *et al*, 1986).

APPENDIX II: REVIEWS OF TOXICOLOGICAL STUDIES

Table 5-1: Summary of acute toxicity studies

Table 5-2: Summary of data from repeat dosing studies (24 hours to approximately 90 days)

Table 5-3: Summary of studies conducted only to measure neurological effects of MTBE

Table 5-4: Summary of data from developmental / reproduction studies

Table 5-1: Summary of acute toxicity studies

Exposure Route	Species	LD_{50}/LC_{50}	Highlights	Reference/Source
Oral, gavage	Rat	3,800 mg/kg	2,000 and 10,200 mg/kg, deaths occurred at > 3,000 mg/kg	Hathaway *et al*, 1970a
Oral, gavage	Rat	3,866 mg/kg	Doses 1,900 - 6,810 mg/kg - Some degree of CNS depression at all doses; 1,900 mg/kg - diarrhoea; 2,450 mg/kg - ataxia, tremors, laboured breathing; 3,610 mg/kg - ataxia, loss of righting reflex, tremors; 4,080 mg/kg - as at lower dose + G.I. irritation and laboured breathing, 6/10 animals died; 6,810 mg/kg - all animals died.	Arco, 1980
Oral, gavage	Mouse	4,000 mg/kg	None reported	Little *et al*, 1979
Dermal	Rat	> 5 ml/kg (6,800 mg/kg)	Transient hyperactivity.	Shell, 1971
Dermal	Rabbit	> 10.000 mg/kg	None.	Arco, 1980
Dermal	Rabbit	> 10,200 mg/kg	6,800 and 10,200 mg/kg; initial pain followed by transient hyperactivity (3-5 min), local irritation, no deaths.	Hathaway *et al*, 1970a
Inhalation (5 m)	Mouse	400 mg/l EC 50; 200 mg/l	5 min exposures at concentrations of 125-800 mg/l. Transient anaesthesia in some mice at all dose levels. At 400 and 800 mg/l, 2 and 4 animals (respectively) died.	Hathaway *et al*, 1970a
Inhalation	Mouse	720 mg/l	LT50 was 5.6 minutes	Snamprogetti, 1980
Inhalation (10 m)	Mouse	648 mg/l (18 % in air)	Whole body exposure. Toxicity dose dependent.	Snamprogetti, 1980
Inhalation (15 m)	Mouse	141 mg/l	None reported	Marsh and Leake, 1950
Inhalation (4 h)	Rat	85 mg/l	44 and 395 mg/l, effects of treatment: hyperactivity, lachrimation, salivation, ataxia, tremors and unconsciousness.	Hathaway *et al*, 1970a

Table 5-1: Summary of acute toxicity studies (continued)

Exposure Route	Species	LD_{50}/LC_{50}	Highlights	Reference/Source
Inhalation (4 h) (MTBE = 99.1%),	Rat	120.3 mg/l	Doses - 68.1-230.5 mg/l. All doses - hypoactivity and ataxia in all animals within minutes. 68 mg/l - all rats prostrate or ataxic by end of exposure period. Upon cessation of exposure, survivors , rapidly recovered. CNS effects; tachypnea nasal discharge at > 68.1 mg/l.	Arco, 1980
Inhalation (4 h) (MTBE = 96.2%)	Rat	142.03 mg/l	70.7-201 mg/l - inco-ordination, hypoactivity, lachrymation and prostration at all doses. Rapid recovery in survivors.	
Intravenous	Rat	0.56 ml/kg	Lethal doses - death within 5 min, preceded by hypersalivation, urination, defecation and respiratory disorders. Survivors - CNS effects (various, as above), reversed within 15-20 min after cessation of exposure.	Snamprogetti, 1980
Subcutaneous (single injection)	Mouse	3.6 ml/kg	none	Snamprogetti, 1980
	Rat	6.7 ml/kg	none	Snamprogetti, 1980
Intraperitoneal (single injection)	Mouse	1.36 ml/kg	none	Snamprogetti, 1980
	Rat	1.67 ml/kg	none	Snamprogetti, 1980

Table 5-2: Summary of data from repeat-dosing studies (24 hours to approximately 90 days)

Species (Strain)	Duration	Doses	Highlights	NOEL / NOAEL	Reference
Route of exposure - Oral					
Rat (Sprague-Dawley)	14 d	0, 357, 714, 1071, 1428 mg/kg/d	All treatment levels - diarrhoea (from day 3). 1,071 mg/kg- increased relative kidney weights (males). Increased Hb and Hct (males). Decreased monocyte numbers (males). 1,428 mg/kg- transient anaesthesia (recovery within 2 h);reduced body weight gain; relative kidney weights; hyaline droplets; decreased BUN (females). Absolute and relative lung weights decreased. No effects on other main organs, immune of reproductive tissues	NOAEL 714 mg/kg/d (male kidney effects)	Robinson *et al*, 1990
Rat (Sprague-Dawley)	28 d	0, 90, 440, 1,750 mg/kg/d	90 mg/kg - increased relative kidney weight (females). 440 mg/kg- hyaline droplets; increased relative kidney weights (males); Mean RBC increased (males); occasional CNS effects (salivation, hypoactivity, ataxia). 1,750 mg/kg- slight increase in relative liver weight; increased relative kidney weight (males and females) and relative adrenal weight (males); MCT increased (females); increased serum cholesterol; localised G.I. irritation; no gross or histopathological effects on lung.	NOAEL 90 mg/kg/d (male kidney effects)	IIT Research, 1992

Table 5-2: Continued

Species (Strain)	Duration	Doses	Highlights	NOEL / NOAEL	Reference
Route of exposure - Inhalation					
Rat (Sprague-Dawley)	90 d	0, 100, 300, 900, 1200, mg/kg/d	No treatment-related deaths; dose-related effects on body weight / body weight gain, significant at top dose; no effect on immune or reproductive tissues; hyaline droplets in kidney at all doses, dose-related. Diarrhoea (from day 3), decreased BUN and increased blood cholesterol at all doses Occasional salivation, ataxia, hypoactivity in all treated groups immediately after dosing. 300 mg/kg - increased relative kidney weight (females), glucose and Co^{++} decreased 900 mg/kg - increased absolute kidney weights (males). Increased relative liver and kidney weights. 1,200 mg/kg - profound transient narcosis; increased monocytes, decreased MCV (males); hyaline droplets with severe tubular changes (males); increased RBC, Hb, Hct and decreased WBC (females); increased absolute and relative lung and kidney weight (males), relative liver weight (males), adrenal weights (females).	NOAEL - 300 mg/kg/d (male kidney effects)	Robinson *et al*, 1990
Mouse (CD-1)	1-2 d, 6h/d	0, 28,800 mg/m^3	No deaths, no other adverse effects	NOAEL 28,800 mg/m^3	Vergnes & Kintigh, 1993
Mouse (CD-1)	5 d, 6 h/d	0, 1,440 and 10,800 mg/m^3	No effects at < 10,800 mg/m^3 10,800 mg/m^3- ataxia, hypoactivity and loss of startle response	NOAEL 1,440 mg/m^3	Chun & Kintigh, 1993
Monkey (Rhesus)	5 days, 6 h/d	0, 12,400 - 341,000 mg/m^3	10,800 and 18,000 - no effects; 38,800 - ataxia at 65 min; 68,600 - ataxia, emesis, prostration, unconsciousness at 58 min; 112,000 - ataxia, prostration, tremors, bradypnoe at 50 min 173,000 - unconsciousness at 43 min 341,000 - unconsciousness at 24 min. Apnoea after 85 min. All effects reversed to normal upon removal of exposure.		Hathaway *et al*, 1970c

Table 5-2: Continued

Species	Duration	Doses	Highlights	NOEL /NOAEL	Reference
Route of exposure - Inhalation					
Rat (Sprague-Dawley)	9 d, 6 h /d, 5 d /w	0, 360, 1,080, 3,600, 10,800 mg/m^3.	360 and 1,080 mg/m^3- no effects. 3,600 and 10,800 mg/m^3 - chronic inflammation of nasal mucosa and trachea (27/40 rats). Serum phosphorus increased (females); BUN and urine analysis normal. Statistically significant increased relative liver weights (10,800mg/m^3, both sexes)	NOAEL 360 mg/m^3	Terrill and Daly, 1984
Mouse (CD-1)	13 d, 6 h /d	0, 7,200, 14,400, 28,800 mg/m^3.	No deaths. Clinical signs in all groups but primarily at 28,800 mg/m^3. Effects included ataxia, hypoactivity, periocular irritation. No gross or histopathological changes	NOAEL 14,400 mg/m^3.	Dodd & Kintigh, 1989
Rat (Fischer 344)	13 d, 6 h /d	0, 7,200, 14,400, 28,800 mg/m^3	No deaths. Clinical signs in all groups but primarily at 28,800 mg/m^3. Effects included ataxia, hypoactivity, periocular irritation. 8,000 ppm - Reversible behavioural changes (ataxia, decreased startle and pain reflexes and / or muscle tone) in both sexes Decreased body weight gain at 14,400 and 28,800 mg/m^3. Relative increase of weights of liver, kidney and adrenals at 14,400 and 28,800 mg/m^3. No gross or histopathological changes	NOAEL 7,200 mg/m^3	Dodd & Kintigh, 1989
Rat (Charles River)	14 d, 6 h/d 5 d/wk	0, 10,000, 30,000 mg/m^3.	Rat - No deaths, no clinical signs, no effects on body weight, no effects on haematology, clinical chemistry or urine analyses. No effects on main organs, including CNS.	NOAEL> 30,000 mg/m^3	Hathaway *et al*, 1970a
Rat (Fischer 344)	2 wk, 6 h/d , 5 d/wk	0, 1,440, 10,800, 28,800 mg/m^3.	No deaths. 10,800 mg/m^3- increased tubular epithelial cell proliferation after 5 days (males) 28,800 mg/m^3- decreased body weight gain.	NOAEL (body weight) 10,800 mg/m^3	Chun & Kintigh, 1993

Table 5-2: Continued

Species	Duration	Doses	Highlights	NOEL / NOAEL	Reference
Route of exposure - Inhalation					
Mouse (CD-1)	28 d, 6 h/d, 5 d/wk	0, 1,440, 10,800, 28,800 mg/m^3	1,440 mg/m^3 - No treatment related effects; 10,800 mg/m^3 - increased absolute and relative liver weight (females). 28,800 mg/m^3 - as above + CNS effects (ataxia, lack of startle response); decreased spleen weight (females); increased absolute and/or relative liver weight (both sexes); centrilobular hepatocellular hypertrophy (both sexes); reversible increase in hepatic cell proliferation (females, day 5 only). No kidney or thyroid effects. No deaths	NOEL (female), 1,440 mg/m^3 ; (male) 10,800 mg/m^3.	Chun & Kintigh, 1993
Rat (Fischer 344)	28 days, 6 h/d , 5 d/w	0, 1,440, 10,800 , 28,800 mg/m^3	1,440 mg/m^3- No treatment related effects; 10,800 mg/m^3 - increased absolute and/or relative liver weight (both sexes); increased absolute and relative kidney and adrenal weight (females only); increased protein accumulation in kidney detected histopathologically in males; reversible. 28,800 mg/m^3 - ataxia; decreased body weight gain in males (reversed during recovery); increased urine volume; increased absolute and/or relative liver weight (both sexes), kidney weight (males), adrenal weight (females); decreased absolute and/or spleen weight (both sexes); increased protein accumulation in kidney (males); reversible increase in renal cell proliferation in males (day 31 only). No evidence of α2u-globulin from antibody staining.	NOAEL (liver, kidney) 1,440 mg/m^3	Chun & Kintigh, 1993
Mouse (Swiss) Rat (Wistar)	30 d, 5-10 min/d, 5d/wk	0, 180,000, 288,000 mg/m^3 (0, 180 and 288 mg/l)	No treatment-related increase in deaths or clinical signs. Body weights, food intake, similar in all groups. No differences between control and treated groups in clinical chemistry, urine analysis, blood, organ weights and motor activity co-ordination. Same end points as above, except for behavioural changes.	NOEL >288,000 mg/m^3	Snamprogetti, 1980
Rat (Wistar)	10 min/d, 5d/w, 120 d	180,000 mg/m^3 (180 mg/l)	No treatment related changes.	NOEL >180,000 mg/m^3	

Table 5-2: Continued

Species	Duration	Doses	Highlights	NOEL / NOAEL	Reference
Route of exposure - Inhalation					
Rat (Sprague-Dawley)	13 wk, 5 d/wk, 6 h/d	0, 900, 1,800, 3,600 mg/m^3	No deaths. No treatment-related effects on haematology, clinical chemistry, urinalysis, organ weights (3,600 mg/m^3 - slight decrease in absolute and relative lung weight in females, but not treatment-related), gross or Histopathology. Dose-related anaesthesia.		Greenough *et al*, 1980
Rat (Sprague-Dawley)	13 wk, 5 d/wk, 6 h/d	0, 2,880, 14,400, 28,800 mg/m^3	2,880 mg/m^3- mild haematological changes, not treatment-related 14,400 mg/m^3 - hypoactivity. Decrease in hind leg grip (males). 28,800 mg/m^3 - No deaths. Ataxia. Not neurotic. Reduced food intake, body weight gain and final body weight. Elevated serum corticosterone. Significant increase in absolute and relative weights of kidney, liver and adrenals. Hyaline droplets in kidney (males). Haematocrit and neutrophils increased in females. Increased incidence of lymphoid hyperplasia in lymph nodes (males).		Dodd and Kintigh, 1989
Route of exposure - Other					
Intraperito-neal. Rat (Wistar)	15 d , daily	0, 185 mg/kg/d (0.25 ml/kg/d)	No deaths. Body weight gain depressed (75 % of controls). Urinary parameters and organ weights at termination similar to controls. no treatment-related histological changes.	NOAEL 185 mg/kg/day	Snamprogetti, 1980

Table 5-3: Summary of studies conducted only to measure neurological effects of MTBE

Exposure route	Species	LD_{50} / LC_{50}	Highlights	Reference / source
Oral	Rat	-	40 mg/kg - no effects; 400 mg/kg - drowsiness	Bio-Research, 1990a
Oral	Rat	-	440 mg/kg - ataxia	IIT Research, 1992
Inhalation (6h)	Rat	-	Doses - 2,880, 14,400, 28,800 mg/m^3 2,880 mg/m^3- no effects. 14,400 mg/m^3- ataxia, duck walk; decrease in rectal temp; reduced hind leg grip (females); significant decrease in motor activity. All effects apparent after 1 h, absent by 6-24 h.	Gill, 1989

Table 5-4: Summary of data from developmental / reproduction studies

Species (Strain)	Dosing regime	Doses	Highlights	NOEL / NOAEL	Reference
Route of exposure - Inhalation					
Rat (Sprague-Dawley)	gd 6-15. 6 h/d	0, 900, 3,600, 9,000 mg/m^3	Developmental study. No effects on pregnancy rates, mean body weights and weight gain, in-life observations, corpora lutea or uterine implantation, liver weights, necropsy observations or foetal data (mean weights, crown-rump distances, sex distribution or ossification). Incidence of soft tissue malformations / foetus and /litter were comparable between control and treated groups. 900 mg/m^3 - lachrymation.	NOEL 9,000 mg/m^3	Schroeder and Daly, 1984a. Conaway *et al.* 1985
Mouse (CD-1)	gd 6-15. 6 h/d	0, 900, 3,600, 9,000 mg/m^3	Developmental study. No effects on mortality, pregnancy rates, maternal body weight, food and water intake, most clinical signs, implantation, liver weight, necropsy observations, foetal data (foetal weight, crown-rump distances, sex distribution and ossification). Slight increase in lachrymation (females).	NOAEL 9,000 mg/m^3	Schroeder and Daly, 1984b. Conaway *et al,* 1985
Mouse (CD-1)	gd 6-15. 6 h/d	0, 3,600, 14,400, 28,800 mg/m^3	Developmental study. No effects on mortality. 14,400/28,800 mg/m^3 - ataxia, hypoactivity, prostration, laboured breathing, increased eye lachrymation and periocular encrustation. foetal body weights significantly reduced. Reduced maternal weight and weight gain (significant at 28,800 mg/m^3); Increase in individual skeletal malformations consistent with developmental toxicity. 28,800 mg/m^3 - decreased food intake; laboured breathing. Reduced implantations and increased non-viable implantations (resorptions, dead foetuses) / litter and reduced sex ratio; Significant increase in cleft palate, pooled external and visceral malformations and total malformations.	NOAEL 3,600 mg/m^3	Tyl and Neeper-Bradley, 1989

gd = gestation day

Table 5-4 continued

Species (Strain)	Dosing Regime	Dose	Highlights	NOEL / NOAEL	Reference
Rabbit (Albino)	gd 6-18. 6 h/d	0, 3,600, 14,400, 28,800 mg/m^3	Developmental study. No effects on pregnancy rates, most clinical signs, maternal periodic weights, gross observations at necropsy, maternal body weights, maternal gestational weight change or absolute liver weight, mean foetal body weights / litter, malformations, gestational parameters (number of corpora lutea, total, nonviable or viable implantations / litter, sex ratio , pre- or post-implantation loss). 14,400 mg/m^3 - significantly reduced body weight gain and food intake. 28,800 mg/m^3 - significant increase in relative liver weights; laboured breathing; ataxia; hypoactivity and ataxia (d 6).	NOAEL, maternal toxicity: 3,600 mg/m^3 NOAEL, develop-mental toxicity: at least 28,800 mg/m^3	Tyl, 1989
Rat (Sprague-Dawley)	wk 16-28, 6 h/d	0, 900, 3,600, 9,000 mg/m^3.	One-generation, two-litter reproduction study No significant effects on dam of foetal parameters. Nasal discharge at 9,000 mg/m^3. Minor effects on pups were: Mid and high-doses between days 14-21 - weights slightly reduced. Pup gross observations at necropsy - most frequent observation was hollow kidney. Similar in treated and control groups. Frequently seen in this strain of animal.	NOAEL: 9,000 mg/m^3	Biles *et al,* 1987
Rat (Sprague-Dawley)	wk 14-19, 5-7 d/ wk, 6 h/d	0, 1440, 10,800, 28,800 mg/m^3	Two-generation, two-litter reproduction study 10,800 mg/m^3 (males) - hypoactivity; lack of startle response; increased liver weight in F1 but no associated lesions, reduced F1 and F2 pup weights, reduced weight gains, reduced body weight gain (males); periocular encrustation and ocular discharge. Overall, no effects on respiratory, renal, immune or reproductive systems, heart, GI tract or associated tissues.	NOAEL, 1440 mg/m^3 LOEL for pup development - 10,800 mg/m^3. NOAEL for pup develop-ment - 1440 mg/m^3	Myhr *et al,* 1991

gd = gestation day

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