Risk Assessment Report for

Existing Substances

Methyl tertiary-Butyl Ether

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Risk Assessment Report for Existing Substances Methyl-tertiary-butylether

CONTENTS

LIST OF TABLES

EXECUTIVE SUMMARY

Methyl *tertiary-butyl* ether (MTBE) has been prioritised for risk assessment under the EU "Existing Substances" Regulation[a]. An ECETOC Task Force was established to support the official Finnish Rapporteur in identifying relevant critical health and environmental data, and to supplement an earlier human health risk characterisation (ECETOC, 1997). This report comprises a comprehensive risk assessment in accordance with EU guidance[b].

MTBE is produced in large tonnages and is widely used, mainly as a fuel oxygenate and octane booster in gasoline. MTBE is readily detected by analytical methods and by taste and odour due to its low organoleptic thresholds in air and water. The solubility in water is high and it is also very volatile.

The available hazard data reviewed in this report indicate that, although not readily biodegradable, MTBE is inherently biodegradable. It is not dangerous to aquatic and other environmental organisms. The toxicokinetic data in experimental animals do not give any reasons for concern with regard to bioaccumulation of MTBE or its metabolites, effects on the central nervous system, genotoxicity or potential effects on reproduction. For human health, skin and respiratory irritation are regarded as the primary concern. There is insufficient evidence for MTBE to be classified as a carcinogen.

Risk characterisation based on worst-case scenarios from EUSES[c] indicates that there is a need for more realistic emission factors to be calculated for some remaining MTBE production and gasoline blending sites. The Predicted Environmental Concentrations (PECs) for the terrestrial and aquatic environment should then be adjusted accordingly.

The conclusion for the environmental risk assessment is that more information is required. For the human population, exposed via all exposure scenarios, no further information/testing on the substance is needed and no further risk reduction measures (beyond those applied already) are considered necessary.

The overall conclusion for the risk assessment of MTBE is that there is a need for further information and/or testing.

[a] Council Regulation (EEC) 793/93 on the evaluation and control of the risks of existing substances (EC, 1993a)

[b] Technical Guidance Document in support of EU risk assessment of new existing substances (EC, 1996)

[c] European Union System for the Evaluation of Substanes (http://ecb.ei.jrc.it/existing-chemicals)

OVERALL RESULTS OF THE RISK ASSESSMENT

Name: methyl tertiary-butyl ether (MTBE)

IUPAC name: propane, 2-methoxy-2-methyl-

CAS No. 1634-04-4

EINECS No. 216-653-1

Overall results of the risk assessment:

i) There is a need for further information and/or testing;

ii) there is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already;

iii) there is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Summary of conclusions:

Environment

Result ii) applies to all environmental compartments and use patterns except for two situations listed below. This conclusion was reached because the environmental risk assessment produced acceptable (< 1.0) risk characterisation ratios (RCRs) and acceptable (> 1.0) margin of safety (MOS) values for all stages of the life-cycle.

Result i) applies to two situations, namely the local water and sediment compartments for Use Pattern 2, processing, in high-purity isobutylene synthesis. The recommended action was to consider carrying out sediment toxicity testing and to implement a statistically-designed sampling and analysis programme to measure concentrations of MTBE in wastewaters from sites using MTBE for isobutylene synthesis.

Overall, the results of the environmental risk assessment, using the factors described in this report, indicate that there is a low risk arising from MTBE as a fuel additive, process intermediate or solvent.

Human health (toxicity)

Result ii) applies to all human populations exposed to MTBE via all exposure scenarios.

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. The basis for the risk characterisation is a comparison of three different doses/concentrations for these effects with occupational and consumer exposure data. This produced MOS between 90 and 180 fold for workers involved in MTBE production, about 70-fold for workers handling gasoline containing MTBE, and between 250 and 510 fold for service station attendants and garage workers. A 17,000 fold margin of safety was calculated for consumer exposure during car refuelling. Compliance by workers with the short- and long-term occupational exposure limits for MTBE of 270 mg/m^3 (75 ppm) and 90 mg/m^3 (25 ppm) respectively is considered likely to protect for the above effects.

The risk characterisation produced here for occupational and consumer exposure to MTBE does not indicate concern for human health.

For humans exposed indirectly via the environment, all MOS are greater than one, which implies that there is no inherent risk.

Human health (physico-chemical properties)

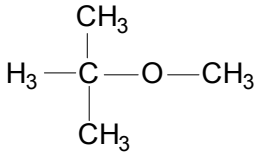
There are no risks to human health from the physico-chemical properties of MTBE.
In all, it is considered that for all human populations, exposed to MTBE via all exposure scenarios, there is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

1. GENERAL SUBSTANCE INFORMATION

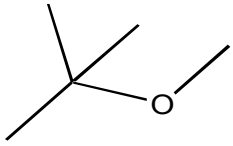
1.1 Identification of the substance

Name:	methyl tertiary-butyl ether (MTBE)
Synonyms:	tert-butyl methyl ether
	1,1-dimethylethyl methyl ether
	methyl-1,1-dimethylethylether
	1,1,1-trimethyl-dimethyl ether
IUPAC [a] (EINECS [b]) name:	propane, 2-methoxy-2-methyl-
CAS [c] Registry No.	1634-04-4
EINECS No.	216-653-1
Molecular formula:	$C_5H_{12}O$
Molecular mass:	88.15

Structural formula:



Spherical structure:



SMILES [d] notation: [O(C(C)(C)C)C] or [C(C)(C)(C)OC]

[a] International Union of Pure and Applied Chemistry

[b] European INventory of Existing ChemicalS

[c] Chemical Abstracts Service

[d] Simplified Molecular Input Line Entry System

1.2 Physico-chemical properties

Methyl *tertiary-butyl* ether (MTBE) is an oxygenated aliphatic organic compound. At normal temperature and pressure (20°C and 1,013 hPa) it is a clear, colourless, flammable liquid with a strong ethereal odour. MTBE is miscible with organic solvents and is highly soluble in water. A summary of the physico-chemical properties of MTBE from the HEDSET [a] data sheet (Arco Chemical Europe, 1997) is given in Table 1.1. Key properties for EUSES [b] calculations and risk assessment are reviewed in Sections 1.2.1 to 1.2.5.

Table 1.1: Physical and chemical properties of MTBE

Property	Value, unit	Reference
Melting point	-108.6°C [a]	Scholz *et al*, 1990; Arco Chemical, 1993
Boiling point	55.2°C [a]	Arco Chemical, 1993
	55.3°C	Scholz *et al*, 1990
Relative density D_4^{20} (density of water at 4°C is 1,000 kg/m3)	740.6	Kroschwitz and Howe-Grant, 1994
Vapour pressure at 25°C	334 hPa [a]	Ambrose et al, 1976
Surface tension at 20°C	20 mN/m	Scholz *et al*, 1990
Threshold odour concentration in air	0.19 mg/m^3 [b]	Vetrano, 1993
Threshold odour concentration in water	95 µg/l	Vetrano, 1993
Taste threshold in water	134 µg/l	Vetrano, 1993
Taste and odour in water	10 µg/l	Fawell and Young, 1997
Solubility in water at 25°C	42 g/l [a]	Stephenson, 1992
Miscible with ethanol and diethyl ether	Yes	
Partition coefficient, log K_{ow} (octanol/water) at 20 - 23°C	1.06 [a]	Hansch *et al*, 1968 [c]; Hüls, 1989 [d]
Henry's Law constant, at 25°C	59.5 Pa·m^3/mol [d]	Hine and Mookerjee, 1975 [d]
	70.1 Pa·m^3/mol [a,e]	Section 1.2.4
Flammability limits in air	1.5 - 8.5%	Arco Chemical, 1993
Flash point, closed cup	-28°C	Hüls, 1995
Autoflammability, ignition temperature	460°C	Hüls, 1995

[a] Figures used in EUSES calculations

[b] Reported as 0.053 ppm

[c] Calculation method

[d] Measurede

[e] Calculated

It should be noted that the HEDSET contains several values for some of the parameters, but only a single value can be inputed to EUSES. Thus, some selection criteria were required to decide which values to use. In general, where the difference between the values was minimal (1-2%), then the lowest value was selected. Where the difference between the values was greater than 1-2%, then an intermediate value was selected.

[a] Harmonised electronic data set

[b] European Union system for the evaluation of substances (EC, 1998)

1.2.1 Vapour pressure

Ambrose *et al* (1976) measured a vapour pressure of 334 hPa at 25°C, a value that was confirmed by Daubert and Danner (1985) who reported 330 hPa at 25°C. A company measurement is 268 hP at 20°C (Hüls, 1995) or 270 hPa (Arco Chemical, 1993). The value of Ambrose *et al* (1976) was used in the EUSES calculations. Other values reported in the HEDSET relate to higher temperatures.

1.2.2 Water solubility

Data on the solubility of MTBE in water are contradictory in respect of the relationship between solubility and temperature (Table 1.2). Results from solubility experiments by Stephenson (1992) show MTBE to exhibit a negative temperature relationship, with solubility increasing as the temperature decreases. This contrasts with the solubility data reported in the HEDSET, which show a positive temperature relationship.

Table 1.2: Comparison of solubility

Temperature(°C)	Stephenson, 1992(g/l)	HEDSET(g/l)	Reference
0	83		
9.7	51		
10		26	Hüls, 1995
19.8	42		
20		42	Stephenson, 1992
		48	Budavari *et al*, 1996
25		50	Hüls, 1993
29.6	31		
39.3	25		
48.6	19		

Plotting the data from Table 1.2 over temperatures (4-18°C) typical for many surface waters in Europe, the solubility of MTBE in water ranges from 40 to 65 g/l (Stephenson, 1992) or 20 to 45 g/l (HEDSET data); these are high solubility values for an organic compound. An intermediate value of 42 g/l was taken as the approximate water solubility at 20°C for the EUSES risk assessment.

Data from BenKinney *et al* (1994) produced during ecotoxicity testing (Section 3.2.1), suggest that although MTBE has a high solubility in water, it is relatively slow to dissolve. The slow dissolution rate has potential implications not only for the performance of physico-chemical and ecotoxicity tests on MTBE, but also for its likely fate and behaviour in the environment. However, this is the sole reference to such behaviour found in the scientific literature.

Most MTBE is used in gasoline (Section 2.3), and gasoline containing MTBE may come into contact with surface water, groundwater or rainwater. Huttunen (1997) determined the solubility of MTBE in water at 20°C using a 1/10 sample to water ratio, both from synthetic mixtures of aliphatic and aromatic hydrocarbons, and from gasoline. MTBE from gasoline containing approximately 1, 4 and 11% MTBE (w/w) dissolved in water to give concentrations of 0.3, 1.1 and 2.1 g MTBE/l. These concentrations are significantly lower than the solubility of neat MTBE in water.

1.2.3 Octanol/water partition coefficient

An octanol/water partition coefficient (log K_{ow}) of 1.06 was calculated using the linear free-energy relationship with aqueous solubility of organic liquids derived by Hansch *et al* (1968). A value of 1.06 was also measured at 23°C using OECD [a] Guideline 107 (Hüls, 1989). A measured value of 0.94 was reported by Funasaki *et al* (1985) and 1.3 by Veith *et al* (1983a). These four values were reported in the HEDSET. A value of 1.43 was calculated using the KowWin software from Syracuse Research Corporation (SRC, 1999) based on a structural fragment constant method. In view of the relatively small range (0.94 1.43) covered by the above values, the log K_{ow} of 1.06 was selected and used in the EUSES risk calculations.

1.2.4 Henry's law constant

Using the QSAR [b] software HenryWin by SRC (1999) which is based on the fragment constant approach, values of 2.02 x 10^{-3} atm·m^3/mol (205 Pa·m^3/mol) (bond estimation) and 1.44 x 10^{-3} atm·m^3/mol (146 Pa·m^3/mol) (group estimation) were calculated. Experimental values of 5.87 x 10^{-4} atm·m^3/mol (59.5 Pa·m^3/mol) (Hine and Mookerjee, 1975) and 4.33 x 10^{-4} and 5.28 x 10^{-4} atm·m^3/mol (43.9 and 53.5 Pa·m^3/mol) (Robbins *et al*,1993) were reported, all at 25°C. No specific value was entered into EUSES, so the slightly lower value of 70.1 Pa·m^3/mol (6.9 x 10^{-4} atm·m^3/mol), automatically derived by EUSES from the water solubility and vapour pressure, was used in the risk assessment.

1.2.5 Taste and odour

MTBE is a volatile liquid at normal room temperature and pressure and has a strong ethereal odour in air, with a threshold concentration of 0.19 mg/m^3 (0.053 ppm) (Prah *et al*, 1994).

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[a] Organisation for Economic Co-operation and Development

[b] Quantitative structure activity relationship

MTBE imparts a pronounced taste and odour to water at very low concentrations and this has the beneficial effect that exposure of consumers to toxic quantities via consumption of water is unlikely. Reported odour thresholds can vary considerably because of the wide range of sensitivity of response of the human population to tastes and odours and different approaches to setting guideline values for organoleptic parameters (Fawell and Young, 1997).

Vetrano (1993) reported an odour detection threshold in water of 95 μg MTBE/l and a taste threshold in water of 134 μg MTBE/l. The Oxygenated Fuels Association (OFA) in the USA commissioned an extensive test on the aesthetic properties of MTBE in water (Pirnie, 1998) using an odour panel of 57 participants. The results of the study supported the setting of a Secondary Maximum Contaminant Level (SMCL), an advisory guideline set for aesthetic, non-health effect parameters developed by the US-EPA, of 15 μg/l for taste and odour in drinking water. The actual US-EPA advisory level set for taste and odour considerations in drinking water is 20-40 μg/l, which is reported to provide a large margin of safety from toxic effects (US-EPA, 1997). Also in the USA, California has recently adopted an SMCL of 5 μg/l (California EPA, 2001). A study in the UK by Fawell and Young (1997) suggested a guidance value for taste and odour in drinking water of 10 or 34 μg MTBE/l, dependent on the considerations applied to the dataset used to establish the taste and odour threshold. (The health implications of the taste and odour properties of MTBE are discussed in more detail in Section 4.1.1.3 *Organoleptic (taste and odour) considerations)*.

1.3 Conversion factors

Conversion factors for concentrations of MTBE in air at standard conditions (20°C and 1,013 hPa) are:

1 ppm = 3.665 mg/m^3
1 mg/m^3 = 0.273 ppm

In this report, for the purpose of EUSES calculations, values were converted at 25°C and 1,013 hPa.

1 ppm = 3.604 mg/m^3
1 mg/m^3 = 0.278 ppm

1.4 Purity / impurities, additives

Degree of purity:	> 98% w/w
Identity and percentage of impurities:	tertiary-butyl alcohol (< 1% w/w)
	2,4,4-trimethylpent-1-ene (< 1% w/w)
	triisobutylene (< 0.5% w/w)
	methanol (< 0.5% w/w)
	C4-C6 hydrocarbons (< 1% w/w)
Identity and percentage of necessary additives:	not applicable

1.5 Classification

Provisional classification according to Annex I of Directive 67/548/EEC (EC, 2001):

Risk phrases:	R11, highly flammable; R38, irritant

2. GENERAL INFORMATION ON EXPOSURE

This section contains general information on exposure that is relevant to the risk assessment of MTBE for man and for the environment. There are a number of different exposure scenarios that must be taken into consideration for this substance, involving differences in the production and use of MTBE, the stages of its life-cycle and the human populations potentially exposed. This means that several sets of exposure data (both measured data and model calculations using EUSES) are presented in both the environmental and human sections (3.1 and 4.1.1) to allow for risk characterisation and assessment for each exposure scenario (Sections 3.3 and 4.1.3).

2.1 Production and consumption

2.1.1 Amount (tonnage)

Commercial production of MTBE began in Europe in 1973 and in the USA 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 1998 was 23.5 Mt and the actual production was 18 Mt (DeWitt, 1998). The estimated annual production of MTBE in the EU in 1996 was nearly 3 Mt, in addition about 200 kt was imported and 850 kt exported. Hence the annual consumption within the EU in 1996 was 2.35 Mt. By 1999, the estimated annual production had increased by about 10% to 3.3 Mt and the annual consumption to 2.65 Mt, an increase of nearly 13% (Table 2.1 to 2.4). (The main uses of MTBE are discussed in Section 2.2.).

The region of the EU with the highest production of MTBE is the Netherlands where in 1997 about 903 kt were produced, while 100 kt were imported and 471 kt exported. (A detailed breakdown for 1998-1999 is not available). Over the same period (1997), the highest use of MTBE as a fuel additive was in Italy where 509 kt were consumed, although this decreased slightly to 430 kt in 1998 and 1999. The highest production and consumption of MTBE in the EU occurred in 1999. However, since there were no figures available for regional production from the Netherlands for this year, the regional production volume figures were estimated assuming the same percentage regional production as for 1997. Thus the estimated regional production volume was 980 kt.

Table 2.1: Production and consumption (kt) of MTBE in the EU in 1996 *(DeWitt, 1998)*

	Total	Fuel additive	Isobutylene production	Solvent	Regional (Netherlands)	Ratio of region to total (%)
HPVC[a]	Yes	Yes	Yes	Yes	Yes	
Production	2,960				893	30.2
Amount imported	203				NS	NS
Amount exported	854				NS	NS
Amount consumed	2,309	2,274	29	6	99	4.2

Table 2.2: Production and consumption (kt) of MTBE in the EU in 1997 *(DeWitt, 1999)*

	Total	Fuel additive	Isobutylene production	Solvent	Regional (Netherlands)	Ratio of region to total (%)
HPVC	Yes	Yes	Yes	Yes		
Production	3,030				903.5	30
Amount imported	187				100.0	67
Amount exported	904				471.4	59
Amount consumed	2,313	2,278	29	6	99.2	4

Table 2.3: Production and consumption (kt) of MTBE in the EU in 1998 *(DeWitt, 1999)*

	Total	Fuel additive	Isobutylene production	Solvent	Regional (Netherlands)	Ratio of region to total (%)
HPVC	Yes	Yes	Yes	Yes		
Production	2,880				926	32
Amount imported	269				NS	NS
Amount exported	848				NS	NS
Amount consumed	2,301	2,266	29	6	169	7.3

Table 2.4: Production and consumption (kt) of MTBE in the EU in 1999 *(DeWitt, 2000)*

	Total	Fuel additive	Isobutylene production	Solvent	Regional (Netherlands)	Ratio of region to total (%)
HPVC	Yes	Yes	Yes	Yes		
Production	3,290				980 [b]	30
Amount imported	291				NS	NS
Amount exported	935				NS	NS
Amount consumed	2,646	2,611	29	6	150	5.7

[b] Calculated value based on regional percentage from previous years

[a] High Production Volume Chemical under the OECD difinition, i.e. manufactured or used in quantities exceeding 1 kt/y

There are 36 production plants for ether alkylates in the EU, the majority (29) of which produce MTBE. The distribution of the production capacity for MTBE (and other ether oxygenates) is shown in Table 2.5. The annual production capacity of the plants ranges from 15 kt to over 600 kt and the output of the plants is either used within the company producing the chemical (captive) or is sold on the open market (merchant).

Table 2.5: Inventory of European ether production plants at February 2000 *(Lambert, 2000)*

Company	Location	Country	Product [a]	Capacity (kt/y)	Feedstock [b]	Disposal [c]	Start-up [d]
Lyondell	Fos-sur-Mer	France	MTBE	610	TBA	M	1988
Lyondell	Botlek	Netherlands	MTBE	590	TBA	M	1988
Oxeno	Marl	Germany	MTBE	210	SC	M	1978
Total Fina Elf	Antwerp	Belgium	MTBE	180	SC, FCC	C	1986
Shell Chemie	Pernis	Netherlands	MTBE	160	SC	M	1986
Ecofuel	Ravenna	Italy	MTBE, ETBE	160	SC	C, M	1973
DSM	Beek, Geleen	Netherlands	MTBE	130	SC	C, M	1981
Exxon Chemical	Fawley	UK	MTBE	125	SC, FCC	C	1989
Repsol	Tarragona	Spain	MTBE	120	FCC, SC	C	1988
Fortum	Porvoo	Finland	MTBE	120	SC, FCC	C	1980
OMW	Karlsruhe	Germany	MTBE	120	FCC	C	1990
Fortum	Porvoo	Finland	TAME	114	FCC	C	1995
Lindsey Oil	Killingholme	UK	MTBE, TAME	100	FCC, SC	C, M	1990
PCK	Schwedt	Germany	MTBE	85	FCC	C	1995
Total Fina Elf	Feyzin	France	ETBE	75	SC, FCC	C	1987
Hellenic	Aspropyrgos, Athens	Greece	MTBE	70	FCC	C, M	1987
ÖMV	Schwechat	Austria	MTBE	70	SC	C	1983
Dea Mineraloel	Wesseling	Germany	MTBE	65	FCC	C	1993
Nerefco (BP/Texaco)	Rotterdam	Netherlands	MTBE	65	FCC	C	1994
Agip	Milazzo	Italy	MTBE	60	FCC	C	1992

Table 2.5: Inventory of European ether production plants at February 2000 (cont'd) *(Lambert, 2000)*

Company	Location	Country	Product [a]	Capacity (kt/y)	Feedstock [b]	Disposal [c]	Start-up [d]
Repsol	Puertollano	Spain	MTBE	60	SC, FCC	C	1990
Petronor	Somorrosteo, Bilbao	Spain	MTBE	60	FCC	C	1986
Nord ETBE	Dunkerque	France	ETBE	59	FCC	C	NS
Ouest ETBE	Gonfreville	France	ETBE	59	FCC	C	NS
Agip	Gela	Italy	MTBE	55	SC, FCC	C	1989
Agip	Gela	Italy	TAME	55	FCC	C	NS
Cepsa	San Roque, Algeciras	Spain	ETBE	54	FCC	C	1988
Agip	Priolo, Sicily	Italy	MTBE	54	FCC, SC	C, M	1986
Borealis	Stenungsund	Sweden	MTBE	50	SC	C	1993
Lindsey Oil	Killingholme	UK	TAME	50	FCC	C, M	NS
Agip	Sannazzaro	Italy	MTBE	47	FCC	C	1991
Fortum	Sines	Portugal	MTBE	45	SC	M	1992
Repsol	La Coruna	Spain	MTBE	40	FCC + COK	C	1988
Motor Oil Hellas	Corinth	Greece	MTBE	34	FCC	C, M	1986
RVI	Vohburg, Ingolstadt	Germany	MTBE	25	FCC	C	1986
Dea Mineraloel	Heide	Germany	MTBE	15	FCC, SC	C	1986
			Total EU:	3,991			

[a] MTBE, methyl *tertiary*-butyl ether; ETBE, ethyl *tertiary*-butyl ether; TAME, *tertiary*-amyl methyl ether

[b] FCC, fluid catalytic cracker; SC, steam cracker; TBA, *tertiary*-butyl alcohol; COK, cokery

[c] C, captive use: the product is used for blending into gasoline on site; M, merchant use: the product is sold on for blending into gasoline

[d] Year of first commercial operation; NS, not stated

2.1.2 Production process

MTBE is synthesised by reacting isobutylene with methanol over an acidic ion-exchange resin catalyst at 38 to 93°C under high pressure (100 - 200 psi[a] or 6,895 - 13,790 MPa). It can also be prepared from methanol or *tertiary*-butyl alcohol (TBA) (Budavari *et al*, 1996).

The feedstocks for MTBE manufacturing plants in the EU originate from different types of petroleum cracking units (Newenham, 1997):

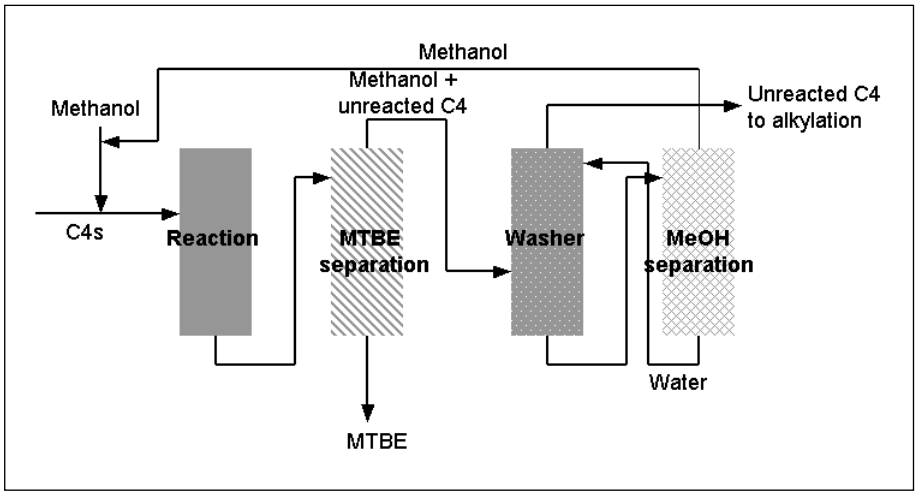
1. Field butanes. Mixed butanes are isomerised and dehydrogenated to yield isobutylene. The production plants are only viable on a large scale and usually have capacities in excess of 500 kt/y.
2. Propylene oxide. In this process propylene is reacted with isobutane to produce propylene oxide and TBA, which is dehydrated to isobutylene. These production plants can have capacities in excess of 500 kt/y.
3. Steam cracker C4 hydrocarbons. This synthetic route is much simpler than the previous two, since the isobutylene is extracted as a by-product from a steam cracker. Plant capacity is determined by feedstock constraints and production capacities of 50 to 210 kt/y are typical.
4. Fluid catalytic cracker C4 hydrocarbons. This synthetic method is similar to the steam cracker route, only the isobutylene is extracted from the FCC overheads. Production plant capacity is determined by feedstock availability, but many plants have a production capacity in the range of 20 to 70 kt/y.

Of the total amount produced in the EU in 1999, 32.5% originated from dehydrogenation of butanes, 13% was produced through dehydration of TBA, 23% originated from steam cracker and 31.5% from fluid catalytic cracker operations. Most of the recent growth has been based on butane dehydrogenation and fluid catalytic cracker units.

[a] Poundforce (lbf) per square inch;1 lbf/in^2 = 6,8948 kPa

A schematic diagram of the MTBE production process is shown in Figure 2.1.

Figure 2.1: MTBE process scheme



2.2 Use

The main uses of MTBE are as an additive in motor vehicle fuel, in the production of isobutylene and as a pharmaceutical solvent. The amounts consumed from 1996 to 1999 are reported in Tables 2.1 to 2.4.

2.2.1 Use as a fuel additive

By far the largest quantity of MTBE (2.6 Mt in 1999) is used as an oxygenated component of gasoline. The primary objective of adding MTBE to the fuel is to boost the octane number in unleaded petroleum (gasoline), especially in premium grades, where it is used at concentrations of up to 10% (w/w), but normally between 2 and 5% (w/w). A desirable additional effect of adding MTBE is a consequent reduction by dilution of the gasoline's benzene, aromatics and olefins contents. MTBE is also widely used at higher blending levels (up to 15% w/w) in reformulated gasoline to improve combustion efficiency. Reformulated gasoline blends meeting the latter specifications are widely used in North America and parts of Europe to improve air quality, by promoting complete combustion and thus reducing emissions of carbon monoxide and volatile organic chemicals (VOCs).

The former wide-spread use of alcohols such as ethanol as an oxygenate in gasoline has decreased over recent years, except in those countries where governments are subsidising the use of ethanol produced from agricultural products. MTBE and other ethers have the following advantages over alcohols (even when alcohols are used in combination with co-solvents):

- Generally improved road octane performance compared to the laboratory octane numbers;
- minor implications on the volatility characteristics, so no or minor "backing out" of butane[a] is necessary;
- no problems with phase separation;
- much less soluble in water.

2.2.2 Other use patterns

The second largest use of MTBE is as a chemical intermediate in the production of high-purity isobutylene; 29 kt were used for this purpose over the years 1996 to 1999.

A third use for high-purity MTBE is as a process reaction solvent in the pharmaceutical industry, but it is estimated that only about 0.2% of the total production in the EU (equivalent to 6 kt) is used for this purpose.

There are also a number of small scale uses of MTBE, such as in clinical practice to dissolve gallstones.

2.3 Life-cycle stages

Releases into the environment can take place at any stage of the life cycle of a chemical substance. The Technical Guidance Document (TGD, Chapter 3 Section 2.3.3) and EUSES distinguish five stages (EC, 1996, 1998) and their relevance with respect to MTBE is as follows:

2.3.1 Production

Production is the stage in which the substance is manufactured, i.e. formed by chemical reaction(s), isolated, purified, and drummed or bagged. For MTBE this will be the synthesis from petroleum derived precursor compounds (Use Patterns 1-3, Section 2.4). Production of MTBE is carried out on oil refinery sites and at other locations.

[a] When, during gasoline blending, the addition of an oxygenate component increases the recommended vapour pressure (RVP) of the blend, less butane must be used to maintain the specified RVP. Butane is a relatively inexpensive high-quality component of gasoline and consequently refiners are keen to maximise its usage.

2.3.2 Formulation

Formulation is the stage in which chemicals are combined in a process of blending and mixing to obtain a product or a preparation. In the case of MTBE used as an oxygenate in gasoline (Use Pattern 1) this will be when the MTBE is blended with gasoline. Such formulation is carried out at refineries and at some large gasoline storage areas.

2.3.3 Processing

There are various processing stages depending on how the substance, as a formulation or an article containing the substance assessed, is applied or used. The substance may be used as a processing aid or incorporated in a product. In the case of MTBE this includes the use of oxygenated gasoline including storage, transportation and delivery (Use Pattern 1), the use of MTBE as a starting material for the production of high-purity isobutylene (Use Pattern 2) and the use of MTBE as a speciality solvent in chemical synthesis (Use Pattern 3).

2.3.4 Private use

This stage considers the use and application of substances (as such or in formulations) on the scale of households. For MTBE, it could be argued that this would include the personal use of oxygenated gasoline (for example purchased from service stations), but this has been incorporated in the processing stage for MTBE. There is also no "private use" for the application of MTBE as a specialist solvent.

2.3.5 Disposal (recovery)

At the stage of disposal, the substance (or the products containing the substance) is disposed of with waste or wastewater. Waste water may be treated in a sewage treatment plant (STP). Waste materials may be incinerated or dumped. At the stage of disposal, recovery may take place. For some "Industrial Categories" (Section 2.4), recovery is considered. This stage of the life cycle is, however, not relevant for MTBE since it is reacted, burnt or emitted during use and disposal is not required.

2.4 Release estimation

The environmental releases of any substance are dependent upon its use patterns. EUSES distinguishes three types of categories, i.e. general (main), industrial and by function or use (Chapter 3, Section 2.3.3 of TGD).

2.4.1 Categories

A "Main Category" is a general description of the exposure relevance of the uses of a substance. Main Categories are also used to characterise release scenarios for the estimation of emissions during specific stages of the life cycle. The interpretation can differ for the particular stage of the life cycle under consideration (Table 2.6).

Table 2.6: Main categories for EUSES exposure scenarios *(According to TGD Chapter 5)*

Main category	Life cycle stage	Interpretation
Ia	Production	Non-isolated intermediates
Ib	Production	Isolated intermediates stored on-site, or substances (other than intermediates) produced in a continuous production process
Ic	Production	Isolated intermediates stored off-site, or substances (other than intermediates) produced in dedicated equipment
II	Formulation	Inclusion into or onto a matrix
	Processing	Inclusion into or onto a matrix
III	Production	Multi-purpose equipment
	Formulation	Multi-purpose equipment
	Processing	Non-dispersive use (industrial point sources)
IV	Processing	Wide dispersive use (many small point sources or diffuse releases; normally no emission reduction measures)

Different "Industrial Categories" specify the branches of industry (including personal and domestic use, and use in the public domain) where emissions occur during applications of the substance as such, or during the application and use of preparations and products containing the substance. Of the 15 possible Industrial Categories listed by EUSES (Chapter 5 of TGD), there are two relevant for MTBE, namely Mineral oil and fuel industry (Industrial Category 9) and Chemical industry: chemicals used in synthesis (Industrial Category 3). An emission-scenario document has been developed and described for Industrial Category 3 but this is not used for the release estimates of EUSES.

For some Industrial Categories additional information is required to obtain a more accurate emission estimation. Various Use Categories therefore depict the specific function or goal of the substance. In EUSES, relevant Use Categories can be chosen from a list of 55 (Chapter 5 of TGD), in the section "Emission input data". Three Use Categories were identified for MTBE, as an oxygenated component of gasoline (Use Category 28, fuel additives), as a starting material for production of high-purity isobutylene (Use Category 33, intermediates) and as a speciality solvent in pharmaceutical chemical synthesis (Use Category 43, process regulator).

2.4.2 Emission factors

EUSES uses by default standard emission factors that can be overridden by more specific information. The standard factors are based upon the so-called A- and B-tables, which can be found in Chapter 3, Appendix I of the TGD or Appendix IV of the EUSES documentation. The A-tables provide estimates of the total fraction from production released to the environment for all (relevant) stages of the life cycle for each of the 15 Industrial Categories. The B-tables provide the fraction of the main emission source that can be assumed to be released through a single point, and the number of days during which the substance is released, thus allowing the daily release rate at a main point source to be calculated.

The TGD states that where the default parameters are not thought to model adequately the true emission factors, and information is available to enable more accurate emission factors to be calculated, then the latter should be used for risk assessment. Consequently, where reliable data were available, specific emission factors have been calculated to override the default values.

2.4.3 Emission scenarios

Emission scenarios were modelled for each of the three use patterns of MTBE namely (i) as a fuel additive, (ii) in isobutylene production and (iii) as a speciality solvent. The relevant use categories and default emission factors that have been used in the EUSES calculations are shown in Tables 2.7 to 2.9.

It is important to envisage the extent of emissions that the default values indicate for MTBE, particularly for production and formulation where the annual amount is about 3 Mt. For example, an emission factor of 0.005 (the default value for emissions to air from production) equates to 150 kt/y and 0.003 (the default value for emissions to wastewater from production) equates to 9 kt/y.

The TGD provides default emission factors for the three emission scenarios that are modelled for MTBE, but in Scenario 1 (Table 2.7), some of the TGD default emission factors were replaced by the highest values for fractional emissions estimated using the available data from production and formulation plants as explained in Section 3.1 (Tables 3.6 and 3.16). This approach was used to ensure that the risk assessment considered a realistic, worst-case emission scenario. In Scenario 2 and 3, the emission factors for production and formulation were set at zero (Table 2.8 and 2.9).

Except for private use as a gasoline component, no surface water emission factors are given because for these use patterns there are no direct releases of MTBE to surface water in the emission scenarios.

Table 2.7: Emission Scenario 1 - MTBE used as a fuel additive

Use Pattern 1	**Mineral oil and fuel industry (Industrial Category 9)** **Fuel additive (Use Category 28)**			
Life cycle stage	**Main Category**	**Compartment**	**Emission factor[a]**	**Overriding value**
Production	Ib Intermediate on site	Air	0.005	0.000475
		Waste water	0.003	0.0000404
		Surface Water	0	0.0000404
		Soil	0.0001	
Formulation	Ib Intermediate on site	Air	0.005	0.000475
		Waste water	0.003	0.0000404
		Surface Water	0	0.0000404
		Soil	0.0001	
Processing	IV Wide dispersive	Air	0.01	
		Waste water	0.0005	
		Surface Water	0	
		Soil	0.001	
Private Use	IV Wide dispersive	Air	0.6	
		Waste water	0.0005	
		Surface Water	0.0001	
		Soil	0.0001	
Recovery	Not applicable			
Life cycle stage		**Number of days [b]**	**Fraction of main source [b]**	
Production		300	0.4	
Formulation		300	0.4	
Processing		350	0.02	
Private use	Only for wastewater	365	0.002	
Recovery	Not applicable			

[a] TGD default from A-tables

[b] TGD default from B-tables

Table 2.8: Emission Scenario 2 - MTBE used in the production of isobutylene

Use Pattern 2	**Chemical industry: chemicals used in synthesis (Industrial Category 3) Intermediates (Use Category 33)**			
Life cycle stage	**Main Category**	**Compartment**	**Emission factor[a]**	**Overriding value**
Production	Ic Intermediate off site	Air	0.005	0
		Waste water	0.003	0
		Surface Water	0	
		Soil	0.0001	0
Formulation	III Multi-purpose	Air	0.025	0
	equipment	Waste water	0.003	0
		Surface Water	0	
		Soil	0.0001	0
Processing	III Non-dispersive	Air	0.05	
		Waste water	0.007	
		Surface Water	0	
		Soil	0.0001	
Private Use	Not applicable			
Recovery	Not applicable			
Life cycle stage		**Number of days [b]**	**Fraction of main source [b]**	
Production		300	0	
Formulation		300	0	
Processing		300	0.25	
Private use	Not applicable			
Recovery	No applicable			

[a] TGD default from A-tables

[b] TGD default from B-tables

Table 2.9: Emission Scenario 3 - MTBE as a speciality solvent

Use Pattern 3	**Chemical synthesis (Industrial Category 3)** **Process Regulators (Use Category 43)**			
Life cycle stage	**Main Category**	**Compartment**	**Emission factor[a]**	**Overriding value**
Production	III Multi-purpose equipment	Air	0.005	0
		Waste water	0.003	0
		Surface Water	0	
		Soil	0.0001	0
Formulation	III Multi-purpose equipment	Air	0.025	0
		Waste water	0.003	0
		Surface Water	0	
		Soil	0.0001	0
Processing	III Multi-purpose equipment	Air	0.05	
		Waste water	0.007	
		Surface water	0	
		Soil	0.0001	
Private use	Not applicable			
Recovery	Not applicable			
Life cycle stage		**Number of days[b]**	**Fraction of main source[b]**	
Production		300	0	
Formulation		300	0	
Processing		230	0.3	
Private use	Not applicable			
Recovery	Not applicable			

[a] TGD default from A-tables

[b] TGD default from B-tables

2.5 Emission calculations

Automated EUSES calculations were performed using the three above emission scenarios (Section 2.4.3) for initial and refined risk assessment (Boxall and Watts, 1998; Watts *et al*, 1998; Watts and Mitchell, 2001). The (tabulated) results are presented in Sections 3.1 and 4.1.1.

In general, following TGD guidance, the total volume (tonnage) released is averaged over the year and used for the exposure calculation on the regional scale using the A-tables, resulting in a Predicted Exposure Concentration (PECregional). Local emissions from point sources were estimated using the B-tables for every environmental compartment and each relevant stage of the life cycle, and for every use pattern. This yields values for PEClocal. The emission rate is given averaged per day (24 hours). This implies that, even when an emission only takes place a few hours a day, the emission will be averaged over 24 hours. Emissions to air and water are presented as release rates during an emission episode.

For the regional and continental spatial scales, it is assumed that 70% of the emission to wastewater is treated in a sewage treatment plant (STP) and 30% reaches surface water directly. This split is accounted for in the total regional/continental emissions but not in the regional/continental releases per life-cycle stage. This cannot be done as the regional releases are also used to estimate local emissions where the entire amount of the chemical passes through an STP (for further explanation see TGD Chapter 3, Section 2.3.7).

For each stage, the losses in the previous stage are taken into account. Releases during production are not taken into account in the other stages, as these releases will generally already be accounted for in the reported production volume. If release is not applicable during a certain life-cycle stage, the release fraction must be set to zero in EUSES. Furthermore, for each stage of the life cycle, it is possible to define if intermittent release is appropriate. In that case, exposure may be of short duration only and a specific Predicted No-Effect Concentration (PNEC) is used in aquatic effects assessment.

After losses during the five stages of the life cycle are accounted for, the part of the tonnage remaining is assumed to end up entirely in waste streams. Quantitative methods for estimating emissions at the disposal stage are not currently available.

3. ENVIRONMENT

3.1 Exposure assessment

3.1.0 General discussion

There are no known natural sources of MTBE (IPCS, 1998).

MTBE is produced in large quantities in the EU; the majority of the MTBE manufactured is used as a fuel additive (Use Pattern 1). Consequently, it is important to use appropriate emission factors from the various life-cycle stages, as explained in the following sections.

MTBE is also used in significant quantities in the production of high-purity isobutylene. It is important to use appropriate emission factors from the relevant life-cycle stages for this Use Pattern 2.

The amount of MTBE used as a high-purity solvent much less, but is still sufficient to classify it as a HPVC (manufactured or used in quantities exceeding 1 kt/y). Since the amount used as a solvent is relatively small, this part of the risk assessment was carried out using only the TGD default parameters for fractional emissions to the environment.

The emission data gathered, both measured and from model calculations, indicate strongly that the compartment of primary release for MTBE is the air.

3.1.0.1 Release into the environment from production

Several manufacturing processes exist to produce MTBE (Section 2.1.2). Commercially available processes consist of a reaction-separation and a refining stage, and are comparable in terms of potential emissions. The production plants are so similar that MTBE emissions were expected to be at least within the same order of magnitude, if not equal to each other. Data on emissions to water, obtained from a 50% sampling of the production plants, were considered sufficient to give a statistically-valid reflection of the emissions.

Emissions to water

Manufacturing plants can be grouped into those using a "wet" or "dry" process. In neither process does the synthesised MTBE come into direct contact with water but the distinction is based on how the methanol is extracted from the unreacted C4-hydrocarbon-methanol stream (Figure 2.1). In the wet process, water is used to wash the hydrocarbon-methanol stream and extract the methanol, which is recycled. In the dry process, no water is used and there will be no wastewater. The excess methanol is separated and recycled to the feed-stream by using an azeotrope in the reaction-separation column.

In the wet process the hydrocarbon-methanol stream usually contains traces of MTBE that are washed with water. To avoid concentration, a small stream of process water is deviated and discharged to wastewater that this may thus contain low concentrations of MTBE (< 0.1 mg/l). The wastewater is treated on-site in an industrial wastewater treatment plant (WWTP) where small quantities of MTBE can be removed with high efficiency (generally > 80%). These concentration levels in the effluent are normally below the analytical limit of detection. Some MTBE producers operate the wet process in such a way that the MTBE is entirely separated before the excess hydrocarbon-methanol stream is washed with water. Thus, for a wet plant no MTBE is present in the process and wastewater.

Emissions to air

Airborne emissions from MTBE production plants differ to some extent, depending on the age and condition of the equipment (e.g. gaskets) and the sampling procedures used. Although these factors have some effect on the amount of MTBE emitted to air, the emissions from different sites are expected to be of the same order of magnitude. Releases to air from the production plants are generally low (i.e. a few ppm of the amount produced). Consequently, data on emissions to air from a 50% sample of the production and/or formulation plants were considered to be sufficient statistically to reflect the emissions from all sites. Furthermore the low odour threshold of MTBE in air (Table 1.1) acts as an excellent indicator of MTBE emissions to air.

Emissions in solid waste and to soil

Under normal operating conditions, emissions in solid waste and hence to soil are virtually non-existent for the MTBE manufacturing plants in the EU. However, emissions to soil may occur from dry and wet deposition of the MTBE emitted to air, and from MTBE adsorbed on sewage sludge spread on agricultural land. Both of these indirect inputs were accounted for in the EUSES model calculations.

3.1.0.2 Release into the environment from formulation

Formulation of MTBE involves blending (mixing) it with petroleum (gasoline) in the desired proportions. There are basically two formulation techniques used for blending gasoline (containing MTBE), namely in-line blending and batch blending. In in-line blending, the gasoline components (including MTBE) are pumped from their storage tanks to a common line and pumped further through the common line to the product storage tank. The components are blended both during pumping and passage through the common line and in the product tank.

In batch blending the gasoline components are pumped through separate lines to the storage tank. The blending of the components hence takes place only in the product storage tank.

Formulation is carried out at refineries or remotely at some large gasoline storage areas (terminals).

Refineries using and producing MTBE have similar systems for manufacturing MTBE and blending it into gasoline. The sites naturally differ from each other in some respects (as to emissions), such as the age and condition of the equipment, condition of gaskets and sampling procedures. Consequently, data on emissions to air from a 50% sample of the production and/or formulation plants can be seen as sufficient statistically to reflect the emissions from all sites. (Furthermore the low odour threshold of MTBE in air (Table 1.1) acts as an excellent indicator of MTBE emissions to air.)

When MTBE is blended into gasoline at locations outside the refineries, e.g. in commercial terminals, both techniques can be used. Batch blending is however used more frequently.

There are between four and eight commercial terminals within the EU that carry out blending of gasoline. The terminals are obliged to report the amount of their activities to the regulatory authorities to calculate VOC emissions, based on how much gasoline (with and without MTBE) and MTBE have 'gone through' the terminal. The MTBE emissions in these terminals should not differ significantly from the emissions from blending activities in the refineries (e.g. Fortum's refinery at Naantali, which uses batch blending), since the techniques used are similar.

It is estimated (see below) that most blending takes place at the same location as production and the manufacturers consider that the emissions from formulation will be of a similar level to those estimated for production. Consequently, the same emission factors to air and wastewater used for production were also input to EUSES for formulation. Where formulation takes place at a separate (remote) site from production, there can be no emissions to wastewater since formulation (blending with gasoline) occurs in closed vessels.

It is quite difficult to estimate the total amount of MTBE blended into gasoline outside of the refineries, as the seller does not always know where the product ends up, e.g. where the product is sold to traders and distributors. The annual quantities of MTBE blended at remote sites remote from refineries have been estimated by the producers (Table 3.1).

Table 3.1: Amounts (t/y) of MTBE blended outside refineries[a]

	1994	1995	1996	1997	1998	1999
Producer 1	-	-	5000	15,000	16,000	28,000
Producer 2	16,788	41,494	-	8,520	35,043	56,108
Producer 4	10,000	15,000	12,000	12,000	14,000	12,000
Producer 5	37,000	52,000	69,000	69,000	100,000	95,000
Producer 6	50,000	50,000	50,000	50,000	50,000	50,000
Total	113,788	158,494	136,000	154,520	215,043	241,108

[a] Estimates of sales to blenders including traders and distributors

Combining these figures with the knowledge that a large part of these are amounts that go to traders and distributors, it can be assumed that approximately 5% or less of the MTBE used in the EU is blended outside of refineries. One producer estimated that approximately 100 kt MTBE/y is blended outside refineries, which is somewhat less than 5% of the total amount used in the EU in 1999.

3.1.0.3 Release into the environment from use (processing) as fuel additive

MTBE used as fuel additive can enter the environment by two main routes: evaporation and spillage.

MTBE is a volatile compound primarily used in reformulated gasoline. It is distributed, stored and used mostly in this form, in which it can comprise up to 15% of the total volume. Measurements have shown that the percentage amount of MTBE in gasoline vapour (at normal ambient temperatures) is the same as that in the gasoline liquid. MTBE is emitted to the atmosphere at all stages of distribution and use of gasoline containing MTBE, the main emissions during normal use being fugitive emissions to air from (i) transfers and storage during bulk distribution to service stations; (ii) storage at service stations; (iii) car re-fuelling at service stations; (iv) car gas tanks, fuel lines and other fuel supply components; (v) emissions to air from car exhausts.

A fuel additive that is present to act as an octane enhancer will result in the majority of the MTBE being converted to water and carbon dioxide by combustion. Small quantities are also expected to be discharged unburnt from vehicle exhausts (0.001 and 0.02 g/km from cars with or without a catalyst, respectively; Section 3.1.2.2, Table 3.16). The resulting background concentrations in the atmosphere from fugitive emissions and incomplete combustion are expected to be low.

Direct emissions to water are less likely to occur although MTBE can enter surface waters directly, for example from the use of gasoline-containing MTBE as fuel in boats and other equipment used on or near water. Emissions to water are more likely to be indirect from washout from air (precipitation) and run-off from land.

The extent of use of MTBE varies considerably across the different EU member states and Table 3.2 provides a breakdown of the total gasoline use in 1999 and the estimated MTBE use for 1999 and the relative percentage of MTBE.

Table 3.2: Amounts of gasoline and MTBE used in EU member states *(DeWitt, 2000)*

Country	Total gasoline use in 1999 (kt)	MTBE use in 1999 (kt)	(%)
Austria	2,170	40	1.8
Belgium	2,560	64	2.5
Denmark	2,030	13	0.6
Finland	1,900	163	8.6
France	13,310	140	1.1
Germany	34,110	450	1.3
Greece	3,210	65	2.0
Ireland	1,340	NS	-
Italy	18,340	430	2.3
Netherlands	4,190	150	3.6
Norway	1,730	30	1.7
Portugal	2,100	NS	-
Spain	8,570	500	5.8
Sweden	4,140	54	1.3
UK	22,510	250	1.1

The largest tonnage (500 kt) of MTBE used in 1999 was in Spain, but the highest percentage (8.6) added to gasoline was in Finland. This is in contrast to production, since the largest production of MTBE in the EU is in the Netherlands, which was only the sixth highest user in 1999.

Leaking underground storage tanks

A more significant means of entry of MTBE into the environment is the accidental release of MTBE and, more commonly, of gasoline containing MTBE. Such accidental release can occur at all stages of the manufacturing/distribution systems but leaking underground storage tanks (LUSTs) at service stations have given rise to the most concern, as such releases may go undetected for some time and the gasoline components can pass to groundwater. Since MTBE has relatively high solubility in water, it will pass into solution in the groundwater and will be distributed further and faster than other, less water soluble gasoline components, such as benzene, toluene, ethylbenzene and xylenes.

In the USA, LUSTs and leaking pipelines have resulted in significant contamination of groundwater resources by MTBE, but concentrations measured in groundwater used for drinking water production have been less than the US-EPA advisory recommendation of 20 μg/l (Gullick and LeChevalier, 2000).

A major review of the threat to potable water supplies from MTBE used as a fuel additive was carried out recently for the UK Environment Agency (Turrell *et al*, 1996). This covered the usage, chemical properties and fate of MTBE in the environment and reviewed data from both the USA and Europe. The authors stated that "existing information indicates that spills from oxygenated fuels appear to be no more environmentally damaging than from non-oxygenated fuels". The report concluded, *inter alia*, that "there are insufficient data on which to make any accurate assessment of the current risk posed by MTBE to UK groundwater resources".

A more recent review of MTBE in almost 3,000 samples from public supply and monitoring boreholes for the UK Environment Agency (Dottridge *et al*, 2000) reported detectable concentrations (> 0.1 μg/l) at 13% of locations. However, most of the concentrations were low, typically < 1 μg/l, and a risk assessment concluded that "A forecast of future trends suggests that the number of boreholes (in England and Wales) with tasteable concentrations of MTBE is unlikely to rise if the concentration of MTBE in fuel remains the same". The overall conclusion of the project was that"the ether oxygenates do not currently pose a major threat to public water supplies in England and Wales".

It should be remembered that spillages and discharges to soil and air from leaking tanks represent abnormal operating conditions under the definition in the TGD, and as such should not form part of the risk assessment of existing chemicals. However, there is a need to minimise the risk of contamination of soil and groundwater from gasoline by ensuring that transport and storage results in minimal accidental and fugitive losses to the environment.

3.1.0.4 Release into the environment from use in isobutylene production and as solvent

Mostly, the use of MTBE for synthesis of high-purity isobutylene and as a specialist solvent in synthetic chemical reactions will result in fugitive losses to air from storage tanks, pipework and reaction vessels and in any wastewater derived from reaction vessel cleaning operations.

3.1.0.5 Emission summary

Table 3.3 presents a summary of emission calculations.

Table 3.3: Summary of emission calculations *(from Watts and Mitchell, 2001)*

Scenario	**1. MTBE used as a fuel additive**				**2. MTBE used in the production of isobutylene**	**3. MTBE used as a speciality solvent**
Life cycle stage	**Production**	**Formulation**	**Processing**	**Private Use**	**Processing**	**Processing**
Production volume						
EU (kt/y)	3,290[a]				29[a]	6[a]
Regional (kt/y)	980[a]				29[a]	6[a]
Continental (kt/y)	2,310				0	0
Emission factor						
Air	0.000475	0.000475	0.01	0.6	0.05	0.05
Waste water	0.0000404	0.0000404	0.0005	0.0005	0.007	0.007
Surface water	0.0000404	0.0000404	0	0.0001	0	0
Soil	0.0001	0.0001	0.001	0.0001	0.0001	0.0001
Emission days	300	300	350	365	300	230
Fraction of main source	0.4	0.4	0.02	0.002	0.25	0.3
Continental release						
Air (kg/d)	3,010	2,170	45,600	2,740,000	0	0
Waste water (kg/d)	256	184	2,280	2,280	0	0
Surface water (kg/d)	256	184	0	456	0	0
Soil (kg/d)	633	456	4,560	456	0	0
Regional release						
Air (kg/d)	1,280	1,280	26,800	1,610,000	3,970	822
Waste water kg/d)	108	108	1,340	1,340	556	115
Surface water (kg/d)	108	108	0	268	0	0
Soil kg/d)	268	268	2,680	268	7.95	1.64
Local emission during episode						
Air (kg/d)	621	621	560	0	1210	391
Waste water (kg/d)	52.8	52.8	28	2.68	169	54.8

[a] Set values (Section 2.4)

3.1.0.6 Environmental distribution and fate

Partitioning

EUSES uses three related partitioning models, to estimate the distribution of a substance at the continental, regional and local level. It was of particular interest to determine the theoretical equilibrium distribution of MTBE using the Level 1 fugacity model of Mackay *et al* (1992) (Table 3.4).

Table 3.4: Estimated distribution (%) between environmental compartments

Input parameter	**Value**
Chemical type	1
Molecular mass	88.15
Temperature	25°C
log K^{ow}	1.06
Water solubility	40 g/l
Vapour pressure	334 hPa
Melting point	-100°C
Compartment	**(%)**
Air	95
Water	4.6
Soil	0.004
Sediment	0.004
Suspended matter aquatic	NS
Biota (fish)	0

NS Not stated

Aerobic biodegradation

MTBE (initial concentration of 2 mg/l) was not degraded (0%) after 28 days in a closed bottle test (OECD Guideline 301D) using an inoculum of predominantly domestic sewage (Hüls, 1991a).

In a closed bottle test (OECD Guideline 301D) using a mixed population of micro-organisms, there was only 1.8% degradation of MTBE (initial concentration of 2 mg/l) after 28 days (Gnemi and Zanolo, 1996a).

Using a concentration of 120 - 200 mg MTBE/l and adapted sludge (inoculum from acclimated industrial activated sludge), 98.8% degradation was achieved after 8 hours (Salanitro *et al*, 1994).

Fortin and Deshusses (1999) reported almost full (97%) conversion of MTBE to CO_2 in biotrickling filters. No other degradation products in either the gas or liquid phase were detected.

In a comparative study of activated-sludge biodegradation rate constants of VOCs, including MTBE (in a test following US-EPA Method 304B and two batch tests), the average percentage removal at a feed concentration of 8.6 mg/l was 97.2%. Using a standard US-EPA 304B test protocol with an average feed concentration of 8.28 mg/l, the effluent concentration was 0.029 mg/l, equivalent to a 96.5% removal. The average first-order biodegradation rate constants calculated for MTBE (expressed as $l/g_{biomass}/h$) were 13.4 (SD 3.8) for the US-EPA 304B test, 16.3 (SD 6.7) for the batch test with oxygen addition and 11.8 (SD 0.9) for the serum bottle test (Cano *et al*, 1999).

Anaerobic biodegradation

Mormile *et al* (1994), using anaerobic sediment samples collected from sites that had been chronically contaminated with petroleum hydrocarbons, found a 50% reduction of the MTBE concentration in only one sample after 152 days of incubation.

Suflita and Mormile (1993) used anaerobic sediments collected from sites that were chronically contaminated with landfill leachate and observed no degradation (0%) after 182 days of incubation.

MTBE, amongst other gasoline oxygenates, was not degraded (0%) after 250 days in anaerobic organic-rich soil microcosms. Degradation of MTBE only occurred in soil with low organic content at pH 5.5. Addition of more readily biodegradable compounds inhibited MTBE biodegradation in this soil (Yeh and Novak, 1994).

Biodegradation in contaminated soil and groundwater (remediation)

There have been many studies of MTBE degradation using selected cultures specifically aimed at treating land contaminated by MTBE from LUSTs and other reformulated gasoline spillages. Some of these achieved high degradation rates of MTBE, but they are not relevant to typical degradation under normal environmental conditions and are therefore only considered briefly here.

Micro-organisms indigenous to the stream-bed sediments at two gasoline-contaminated groundwater sites have been shown, under mixed aerobic/anaerobic laboratory conditions, to be capable of significant mineralisation of MTBE and its prime degradation product TBA. Up to 73% of MTBE and 84% of TBA were completely degraded to CO_2 (Bradley *et al*, 1999). Such conditions may provide a significant microbial removal route for MTBE.

Although there have been many instances of contamination of soil and groundwater by LUSTs in the USA and many studies of the behaviour and fate of the released petroleum components, there is still some uncertainty regarding the natural attenuation of MTBE by biodegradation. A growing number of studies suggest that there may be significant attenuation of MTBE plumes by biological mineralisation *in situ*. Reisinger *et al* (2000) compared MTBE and benzene behaviour at a number of LUST sites in the USA and concluded that the attenuation rate constants and half-lives of these compounds in the spill plumes in groundwater were nearly identical. In another study examining contaminant concentrations in water from monitoring wells in California and Texas, comparison of the plume lengths of MTBE and benzene in the field showed no significant differences (Thomson, 2000). A study of a former USA Coast Guard Fuel Farm site in North Carolina showed that attenuation of MTBE in contaminated soils was extensive. The rate of removal *in situ* correlated well with the rates of removal achieved by microcosms of the aquifer material under laboratory conditions. There was no evidence of the build up of TBA *in situ* as the MTBE was attenuated (Wilson *et al*, 2000).

The data examined from these field studies of contaminated sites suggested that MTBE can degrade both aerobically and anaerobically, but that it is hindered from doing so in the presence of benzene. Thus, the area of MTBE degradation is restricted to a zone at the leading edge of a gasoline plume.

Photodegradation in air

In the atmosphere, MTBE is subject to indirect photolysis, i.e. reaction with photochemically produced hydroxyl radicals (·OH).

A rate constant of 2.84×10^{-12} cm^3/molecule/s at 25°C, equivalent to 100% degradation after 5.6 days, was calculated assuming a hydroxyl radical concentration of 5×10^5 ·OH/cm^3. With 10^6 ·OH/cm^3 at 22°C, the rate constant was 3.09×10^{-12} cm^3/molecule/s, resulting in 50% degradation after 2.6 days (Bennet and Kerr, 1990).

A rate constant of 2.5×10^{-12} cm^3/molecule/s at 25°C, equivalent to 50% photodegradation after 3.2 days, was reported with 10^6 ·OH/cm^3. Concentrations of MTBE between 0.34 and 3.7 ppm (1.23 and 13.3 mg/m^3) were tested. The major oxidation products were identified as *tertiary*-butyl formate and acetone (Cox and Goldstone, 1982). Smith *et al* (1991) confirmed that in the presence of hydroxyl radicals, photolysis of MTBE yielded *tertiary*-butyl formate and acetone; the authors also identified methyl acetate, TBA and formaldehyde as degradation products. The latter three compounds accounted for 95% of the photodegraded MTBE.

Wallington *et al* (1989) reported a rate constant of 3.24 x 10^{-12} cm^3/molecule/s at 0°C, equivalent to 50% degradation after 2.5 days, with 5 x 10^5 ·OH/cm^3 at a tested concentration of 0.0044 mol MTBE/l (3,900 mg/m^3).

The above values for the rate constant are similar and show that the speed of MTBE photolysis is rapid, the slight differences in rate reflecting different experimental conditions used. Some other values available in the HEDSET (Arco Chemical Europe, 1997) are not reported here because key experimental details were not reported.

The half-life for degradation in air calculated by EUSES from the specific degradation rate constant with OH radicals of 2.84 x 10^{-12} cm^3/molecule/s at 25°C was 5.65 days.

Bioaccumulation

A bioconcentration factor (BCF) of 1.5 has been measured for MTBE (10 and 80 mg/l) in Japanese carp (*Cyprinus carpio*) using a flow-through system at 25°C. MTBE was completely eliminated after 3 days when the fish were transferred to clean water after the 28-day exposure period (Fujiwara *et al*, 1984).

A value of 1.59 was automatically predicted by EUSES based on the following linear QSAR valid if log $K_{ow} \geq 6$ (Veith *et al*, 1979 cited in EC, 1996) (TGD, p. 349/548).

$$\log BCFfish = 0.85 \times \log K_{ow} - 0.70 \qquad \text{(Eq. 1)}$$

Another value calculated by means of BcfWin software (SRC, 1999) using a QSAR based on log K_{ow} (Meylan *et al*, 1999), is 1.31.

For earthworms the BCF value was 1.63, calculated following (TGD p. 353)

$$BCFworm = Kworm\text{-}porewater \times rsoil / Ksoil\text{-}water \qquad \text{(Eq. 2)}$$

where Kworm-porewater = 0.25 x Kow x 0.16 = 0.459

ρsoil = bulk density of soil (1,700 kg/m^3)
Ksoil-water = 0.479

The BCF values selected for EUSES modelling were 1.59 for fish and 1.63 for worms.

3.1.0.7 Evaluation of distribution and fate

Based on Mackay Level 1 model calculations, the most (95%) of the MTBE released into the environment is likely to partition to the air compartment, while the remainder will be distributed to the water phase.

The *tertiary*-butyl group of the MTBE molecule is expected to be highly recalcitrant to biological degradation because of the carbon branching and would require specific enzymes to effect rapid degradation. In situations where micro-organisms are exposed to relatively high concentrations of MTBE for prolonged periods of time, they may be able to adapt and produce enzymes capable of rapidly degrading MTBE. This phenomenon could explain the differences in the biodegradation data on MTBE for activated sludge from industrial WWTPs, where it is rapidly degraded (and thus removed), and for domestic sewage treatment plants (STPs) and environmental samples (where it is slowly or not degraded).

The emissions of MTBE in wastewater from production plants are likely to be reduced by biodegradation at on-site WWTPs and hence the fractional emissions input to EUSES should reflect these reduced concentrations. As the majority of STPs will only receive small concentrations (if any) of MTBE from diffuse sources, the default value of zero (0%), both for aerobic and anaerobic biodegradation, was used in the EUSES modelling of STPs.

Published data for biodegradation of MTBE show conflicting results in terms of rate of degradation under aerobic and anaerobic conditions and do not apparently meet the requirements for classification as readily biodegradable (OECD Guideline 301D; TGD Section 2.3.6). MTBE has been reported to degrade rapidly in acclimated industrial activated sludge (US-EPA Method 304B and batch tests). It is also significantly attenuated *in situ* in contaminated groundwater aquifers and thus is inherently biodegradable. Consequently, for biodegradation in STPs, the "characterisation of biodegradability" in EUSES was specified, conservatively, as "inherently biodegradable, not fulfilling criteria".

The degradation of MTBE in air is expected to be rapid with a half-life of 3-6 days depending primarily on the hydroxyl radical concentration. The available rate constants are similar and a value of 2.84×10^{12} cm^3/molecule/s was used in the EUSES calculations. MTBE has a low potential for bioaccumulation in aquatic organisms. The calculated BCF value of 1.59 was used for further EUSES calculations.

3.1.1 Aquatic compartment (including sediments)

3.1.1.1 Measured exposure data

Surface water

Contamination of surface water by MTBE will arise from inputs through effluent discharges, soil runoff and deposition from the atmosphere. The available data are summarised in Table 3.5.

A study carried out in Germany, using solid phase micro-extraction and analysis by GCMS, reported concentrations of MTBE ranging from 7 to 160 ng/l (median 42.0) in river water (Achten and Püttmann, 2000). The Environment Centre of Helsinki measured MTBE in surface water in Finland at concentrations of 0.34 to 0.62 μg/l (mean 0.45) in a brook and 0.4 to 6.1 μg/l in sea water from marinas (Piilo and Salla, 2000). A small survey of MTBE concentrations in surface waters was carried in the UK in 1993. With one exception, all measured concentrations in the study (5 repeat samples from 2 lowland and 2 upland rivers) were less than the analytical limit of detection (0.1 μg/l); the only positive value was 0.2 μg/l (WRc, 1993).

In the USA, levels of MTBE in surface waters from atmospheric deposition were expected to be below 1 μg/l based on measured concentrations in air and in stormwater run-off (Zogorski *et al*, 1996).

Urban stormwater run-off has been sampled and analysed, by the US Geological Survey, for MTBE over the period 1991-1995. MTBE was detected in about 7% of the samples (Zogorski *et al*, 1996).

Table 3.5: Environmental (aquatic) concentrations of MTBE

Water/country	Location, number of sites	Year of sampling	Number of samples	Limit of detection (µg/l)	Number of positives[a]	Concentration (µg/l) Mean or median	Minimum	Maximum	Reference
Surface water									
Finland	Brook, 1	1999	4		NS	0.45	0.34	0.62	Piilo and Salla, 2000
Finland	Helsinki, marina (sea water), > 1	1999	17		NS	2.7	0.4	6.1	Piilo and Salla, 2000
Germany	River, 1	1999	7	0.007	7	0.042	-	0.160	Achten and Püttmann, 2000
UK	River, 4	1993	5	0.1	1	-	-	0.2	WRc, 1993
USA	California, > 450[b]	1998	455	3	21 c	-	-	> 13	California EPA, 1999a
Stormwater									
USA	GS survey		592	0.2	41	1.0	0.2 - 8.7		Zogorski *et al*, 1996

[a] Samples in which MTBE was detected

[b] Drinking water sources

[c] In at least 2 samples from a source

Oxeno (2000) analysed a series of water samples collected in and around the MTBE production facility at Marl (Germany). Most concentrations were below the limit of detection; the highest concentration found in surface waters was 0.5 μg/l (Table 3.6).

Table 3.6: MTBE in water in and around an MTBE production site *(Oxeno, 2000)*

Water type, location	Date	Concentration (μg/l)
Wastewater	16-Nov-99	<1
	07-Dec-99	< 0.5
WWTP effluent	17-Nov-99	< 1
Storage Lake in Haltern, Westfalia	26-Nov-99	< 0.1
River Lippe at Haltern	07-Dec-99	0.5
	09-Jan-00	0.2
City lake of Marl	26-Nov-99	0.2
	10-Jan-00	0.1

The available data suggest that background concentrations of MTBE in EU surface waters are below 1 μg/l.

Sediment

Few data are available for MTBE in sediments. Bianchi and Varney (1989) reported limited data on concentrations in estuarine waters and sediment samples taken from sites adjacent to motorways and centres of heavy urban road traffic density. The authors stated that the measured concentrations in samples were usually at trace levels and rarely exceeded 30 μg/kg.

Waste water from production and formulation

Samples of effluent from WWTP have been taken from 23 sites where MTBE is produced and blended with gasoline, and analysed for MTBE content (using methods based on headspace determination by GC-FID or GC-MS with detection limits of 1 and 0.1 μg/l respectively). The available data were solely for production plants and one site with a combined production and formulation (blending) facility where MTBE was mixed with gasoline. The total production capacity of the 23 sites was more than 3 Mt/y (equivalent to most of the total EU usage in 1999) and all major production processes and feedstock types were covered (Table 3.7). The supporting data were supplied confidentially to the Finnish Environment Institute and Fortum Oil and Gas.

Table 3.7: Measured concentrations of MTBE in wastewater from production plants in 1999

Plant number[a]	Manufacturing process	Feedstock[b]	Production (kt/y)	Number of samples	Sampling period	Concentration in WWTP effluent (µg/l)
1	Continuous	SC	135	NS	NS	< 100[c]
2	Continuous	SC, FCC	87.591	5	NS	80
3	Continuous	SC, FCC	176	4	NS	78
4	Continuous, wet, closed system at refinery	FCC	120	15	16 wk	720
5	Continuous, wet, closed system	TBA	590	NS	NS	< 10[c]
6	Continuous, wet, closed system	FCC	65	12	1 y	< 317
7	Continuous, wet, closed system	SC	45	NS	NS	0
8	Continuous, dry, closed system	SC, FCC	65	NS	NS	0
9	Continuous, wet, closed system	TBA	610	NS	NS	10[c]
10	Continuous, dry, closed system	SC	210	2[d]	2[d]	1[c]
11	Continuous, wet, closed system	SC, FCC	125	NS	NS	< 100[c]
12	Continuous	FCC	70.5	NS	NS	39
13	Continuous	SC, FCC	74.74	1 x/wk	NS	< 200[c,e]
14	Continuous	NS	22.35	NS	NS	5
15	Continuous	NS	38.74	NS	NS	75
16	Continuous	NS	30.4	NS	NS	5
17[f]	Continuous	SC	135	NS	NS	3.6
18	Continuous	SC	125	NS	NS	5
19	Continuous, wet, closed system at refinery	NS	39.174	NS	NS	0
20	Continuous	FCC	70	NS	NS	< 300[g]
21	Continuous	SC	80	NS	NS	8[c]
22	Continuous, wet, closed system	NS	35	NS	NS	< 2,000[c]
23	Continuous, wet, closed system	NS	45	NS	NS	< 2,000[c]

[a] For internal purposes

[b] FCC, fluid catalytic cracker; SC, steam cracker; TBA, tertiary-butyl alcohol

[c] Limit of detection

[d] One sample was a 24-hour composite

[e] Average value for weekly samples

[f] Data for year 2000

[g] Limit of detection for hydrocarbons

NS Not stated

In the dry processes, no wastewater is produced from the production plants. This is reflected in the measured concentrations of MTBE in the wastewaters from these sites being below the detection limits of the analytical methods used.

At the production/formulation sites where a wet process is used, there is no direct contact between water and MTBE, but traces of MTBE may be present in the methanol that contacts the water (Section 3.1.0.1). This is reflected in the measured concentrations in the wastewater from the site where MTBE is sometimes found at concentrations exceeding the limit of detection of the analytical method. For samples where no MTBE was detected, a mean concentration in the effluent was calculated by using the limit of detection as the MTBE concentration.

The data show that the average concentrations of MTBE in such WWTP effluents are 1 mg/l or less and have generally been below the limit of detection of the analytical method used.

The concentration of MTBE in the influent of a WWTP (one sample) in Finland was 0.91 μg MTBE/l (Piilo and Salla, 2000).

No data were available on concentrations of MTBE in WWTP sewage sludge samples, but where VOCs have been measured, no MTBE has been detected.

Gasoline tank bottom waters

One potential concern for the local soil and aquatic environment was disposal of the residues of water, which can accumulate in the bottom of some gasoline storage tanks. If MTBE is added to such a tank or is present in gasoline, which is stored in the tank, some of the water will dissolve in the MTBE and hence end up in the gasoline. Consequently some MTBE is also dissolved in the water. Addition of MTBE to gasoline that is stored in tanks that contain bottom waters is not recommended, since water content of gasoline is specified.

When the tank bottom waters are extracted from the tanks, the water is always treated in WWTPs before it is released to the environment and the majority of the MTBE present will thus be removed.

The following procedure is used in one commercial terminal (other terminals use similar systems). When bottom waters are extracted, they are collected in a small tank (20-30 m^3). If the water has a layer of petroleum hydrocarbons, this layer is separated and pumped back to the product tank. The water in the tank is then used when the crude in the tanks is treated. After this "crude treatment" the water contains less than 500 μg/l of hydrocarbons. The water is thereafter treated in a WWTP.

The following types of hazardous waste are defined in UK legislation and constitute "Special Wastes". Disposal of such waste has to be via a licensed waste disposal company (Table 3.8).

Table 3.8: Types of hazardous waste defined in UK legislation *(UK-DOE, 1996)*

Type	Code
Wastes from petroleum refining	05
Tank bottom sludges	050103
Oil wastes	13
Oil/water separator contents	1305
Oil/water separator sludges	130502
Interceptor sludges	130503
Waste from storage tank cleaning containing oil	160706

However, the high water solubility of MTBE means that it will be only a minor component of wastes that have been in contact with water and have been retained by an oil separation system. Consequently, facilities are required that collect and retain all components present in spilled gasoline.

"Grey waters" from service stations

A number of samples of "grey waters" (drainage waters downstream of the oil separators) were collected from service stations in Finland, and analysed for MTBE (and hydrocarbons) using a headspace GCMS method (detection limit 0.01 mg/l). The samples were collected in bottles with no headspace and transported and stored at temperatures < 6°C (Table 3.9) (Neste, 2000).

Table 3.9: MTBE in "grey waters" from service stations

Location	Sample	Concentration (mg/l)
Neste, Salpakangas	Forecourt	< 0.01
Neste, Laune	Forecourt	1.9
Neste, Hennala	Car wash	< 0.01
Neste, Hennala	Tank area	0.41

The concentrations of MTBE found in these waters were all < 2 mg/l.

STP organisms

No data were available for the concentrations of MTBE in STP samples, but where VOCs have been measured, no MTBE has been detected.

3.1.1.2 Model calculations

Entry of MTBE to the aquatic environment may occur through a number of routes:

1. Directly via:
   1.1. Discharges to surface waters from spillages and leaks from vehicles, transportation, pipelines and storage tanks, either as MTBE or in oxygenated gasoline (processing and private use).
2. Indirectly via:
   2.1. Discharges to wastewater from production/formulation plants and then through on-site or local WWTP effluents to rivers (production and formulation);
   2.2. emissions to air from production/formulation plants and subsequent wet or dry deposition to soil and water (production and formulation);
   2.3. emissions to air from vehicles and subsequent wet or dry deposition to soil and water (processing and private use);
   2.4. discharges to soil via spillages and leaks from vehicles, transportation, pipelines and storage tanks of MTBE or oxygenated gasoline and dissolution in porewate (processing and private use);
   2.5. disposal of solid waste containing either oxygenated gasoline or MTBE (for example sewage sludge from production/formulation site wastewater treatment plants) directly to soil or to landfills and subsequent leaching or evaporation (production and formulation).

It is likely that for MTBE, the major routes to the aquatic environment for consideration during the risk assessment process will be via 2.1, 2.2 and 2.3 above. The TGD states that spillages and leaks are not considered to be part of the normal life cycle, and should not form part of the risk assessment of existing chemicals.

Estimate of emission factors to water from MTBE production and blending

Using sampling and analysis data supplied by the major MTBE manufacturers (Table 3.7), emission calculations were made for route 2.1 above and these are shown in Table 3.10.

Table 3.10: Emissions and emission factors to water for MTBE production plants in 1999

Plant number[a]	Manufacturing process	Production (kt/y)	Concentration WWTP effluent[b]	Waste-water flow (km^3/y)	Emission (kg/y)	Emission factor for WWTP (g/t)
1	Continuous	135	100[c]	10,700	1,072	7.94
2	Continuous	87.591	80	2,960	237	2.71
3	Continuous	176	78	8,830	689	3.91
4	Continuous, wet, closed system at refinery	120	720	4,430	3,186	26.6
5	Closed, wet system	590	10[c]	250	2.5	0.00424
6	Continuous, wet, closed system	65	317	2,630	834	40.4[d]
7	Continuous, wet, closed system	45	0	2.63	0	0
8	Continuous, dry, closed system	65	0	NS	0	0
9	Continuous, wet, closed system	610	10[c]	200	2	0.00328
10	Continuous, dry, closed system	210	1[c]	26,300	26	0.119
11	Continuous, wet, closed system	125	100	5,480	548	4.38
12	Continuous	70.5	39	6,940	271	3.84
13	Continuous	74.74	200[c]	5,990	1,198	16
14	Continuous	22.35	5	3,500	18	0.783
15	Continuous	38.74	75	6,940	520	13.4
16	Continuous	30.4	5	1,990	10	0.337
17[e]	Continuous	135	3.6	31,500	114	0.841
18	Continuous	125	5	5,520	28	0.221
19	Continuous, wet, closed system at refinery	39.174	0	2,280	0	0
20	Continuous	70	300[c]	964	289	4.13
21	Continuous	80	8[c]	13,245	106	1.32
22	Continuous, wet, closed system	35	2,000[c]	35,040	70,008	200
23	Continuous, wet, closed system	45	2,000[c]	35,040	70,008	156

[a] For internal purposes

[b] From Table 3.7

[c] Limit of detection

[d] Selected for EUSES modelling

[e] Data for year 2000

NS Not stated

Emissions from some of these sites were over-estimated based on the detection limit that was assumed to be the concentration of MTBE. Mean concentrations in the effluent of other sites were calculated (using the limit of detection as the MTBE concentration for samples where no MTBE was detected). This again resulted in an overestimate of the emissions to water.

From the estimated emissions, emission factors were calculated for these production and formulation plants (Table 3.10). The two highest emission factors in 1999 were 200 and 156 g/t, which are one order of magnitude below the TGD default of 3,000 g/t (that would result in a significant overestimate of PECs). However, the highest estimated emission factors are still unrealistic, since they were both derived from emissions calculated on the basis of a high detection limit (2,000 μg/l), used as a surrogate value for the concentration in the WWTP effluent. The true concentration of MTBE in the effluent stream was probably much lower. Consequently, the next highest emission factor of 40.4 g/t was selected for EUSES risk assessment modelling as it represents a more realistic worst-case situation. This is also approximately the 90-percentile emission factor for the 23 production plants or the 75-percentile emission factor for those plants for which emission monitoring data were available. This emission factor was used for both the production and formulation life-cycle stages for MTBE used as a fuel additive, since most of the formulation occurs on production sites. In addition, as a worst-case scenario it was assumed that all the MTBE emitted to waste water would reach surface water. On this basis, the same emission factor was used for emission to surface water for the production and formulation life-cycle stages in EUSES.

PEC calculations

The PEClocal for the aquatic compartment from industrial point sources where MTBE is manufactured or formulated is calculated by EUSES as shown below. For MTBE, EUSES calculates the percentage removal by volatilisation to be about 43%, with the chemical considered to be non-biodegradable in an STP (TGD p. 291).

The concentration of MTBE in the industrial STP effluent is calculated using the following formula:

$$Clocal_{eff} = \frac{Elocal_{water} \times 10^6 \times Fstp_{water}}{EFFLUENT_{stp}} \qquad \text{(Eq. 3)}$$

where $Elocal_{water}$ = local emission rate to (waste) water during episode (kg/day)
$EFFLUENT_{stp}$ = effluent discharge rate of STP (default 2 000 000 l/day)
$Fstp_{water}$ = fraction of emission directed to water by STP (0.57 i.e. 57.0 %)
$Clocal_{eff}$ = concentration of the chemical in the STP-effluent (mg/l)

The local concentration of the substance in surface water is calculated as follows:

$$Clocal_{water} = \frac{Clocal_{eff}}{(1 + Kp_{susp} \times SUSP_{water}10^{-6}) \times DILUTION} \quad \text{(Eq. 4)}$$

where $Clocal_{eff}$ = concentration of the chemical in the STP-effluent (mg/l)
Kp_{susp} = solids-water partitioning coefficient of suspended matter (0.91 l/kg)
$SUSP_{water}$ = concentration of suspended matter in the river (default 15 mg/l)
DILUTION = dilution factor of discharged effluent in receiving water (default 10)
$Clocal_{water}$ = local concentration in surface water during emission episode (mg/l)

The concentration of the substance in the surface water, $PEClocal_{water}$, is calculated by adding the regional concentration of the substance to the local concentration, calculated as described above, arising from the point source discharge:

$$PEClocal_{water} = Clocal_{water} + PECregional_{water} \quad \text{(Eq. 5)}$$

For indirect human exposure and secondary poisoning, an annual average concentration in surface water is calculated:

$$Clocal_{water,ann} = \frac{Clocal_{water} \times T_{emission}}{365} \quad \text{(Eq. 6)}$$

where $T_{emission}$ = number of days per year that the emission takes place
$Clocal_{water,ann}$ = annual average local concentration in surface water (mg/l)

An annual average predicted environmental concentration, $PEClocal_{water,ann,}$ is calculated as follows:

$$PEClocal_{water,ann} = Clocal_{water,ann} + PECregional_{water} \quad \text{(Eq. 7)}$$

The PEC for sediment is calculated from the PEC_{water} as follows:

$$PEClocal_{sed} = \frac{K_{susp\text{-}water}}{\rho_{susp}} \times PEClocal_{water} \times 1000 \quad \text{(Eq. 8)}$$

where $PEClocal_{water}$ = concentration in surface water during emission episode (mg/l)

$K_{susp\text{-}water}$ = suspended matter/water partitioning coefficient (EUSES value 1.13 m^3/m^3)
ρ_{susp} = bulk density of suspended matter (TGD value 1,150 kg/m3)
$PEClocal_{sed}$ = predicted environmental concentration in sediment (mg/kg)

3.1.1.3 Summary of PECs

The PECs for the aquatic compartment calculated by EUSES according to the TGD guidelines, using the revised emission factor above are shown in Table 3.11.

Table 3.11: PECs for the aquatic compartment

Scenario	Use Pattern	Water (emission episode) (mg/l)	Water (average, dissolved) (mg/l)	Sediment (mg/kgww)
Local				
Production	1	1.18	0.969	1.16
Formulation	1	1.18	0.969	1.16
Processing	1	0.626	0.601	0.614
Private use	1	0.0631	0.0631	0.0618
Processing	2	3.77	3.1	3.7
Processing	3	1.22	0.772	1.2
Regional		0.0033	0.0033	0.00258
Continental		0.00019	0.00019	0.0001466

STP organisms

Some substances may have an adverse effect on microbial activity in a sewage treatment works, for example by inhibiting nitrification. Ideally, the risk characterisation for such effects should use the estimated concentration of the chemical in the aeration tank. Assuming that homogeneous mixing occurs in the aeration tank, then the dissolved concentration of a chemical in the tank is equal to the effluent concentration (TGD p. 293):

$$PEC_{stp} = Clocal_{eff} \quad \text{(Eq. 9)}$$

where $Clocal_{eff}$ = total concentration of chemical in STP effluent (mg/l)
PEC_{stp} = PEC for micro-organisms in the STP (mg/l)

Consequently the concentration of the substance in the effluent of the STP ($Clocal_{eff}$) is the concentration to which micro-organisms are exposed, and which is used as the PEC for micro-organisms.

The PECs calculated by EUSES according to the TGD guidelines for STP organisms using EUSES are shown in Table 3.12.

Table 3.12: PECs for STP organisms

Scenario	Use Pattern	Concentration (mg/l)
Production	1	11.8
Formulation	1	11.8
Processing	1	6.23
Private use	1	0.59
Processing	2	37.7
Processing	3	12.2

3.1.1.4 Comparison of modelling and monitoring data

Comparison of modelled and monitored concentration is only relevant if using European monitoring data that are truly background values in air, water, soil or sediment and not from areas contaminated by leaks e.g. from LUSTs. Such monitoring data are generally scarce, since many of the environmental monitoring exercises have focussed on locations that are known or suspected of being impacted by LUSTs or spillages. In the following discussion, the PECs calculated by the EUSES software are compared to the measured concentrations in appropriate environmental compartments.

Surface water

The highest PECs estimated for the local scenario, discharge from a production or formulation plant, for the receiving water are at the 0.5 to 1 mg/l level. This compares to the average concentration measured in a production site WWTP effluent of 317 μg/l that was used to estimate the worst-case emission factor. If the dilution factor of 10 (used in the EUSES modelling for STP effluent discharging to surface water) is used, then the effluent concentration translates into a surface water concentration of 32 μg/l, this is significantly below the estimated PEC. The most appropriate estimate of a background surface water concentration is provided by the regional PEC of 3.3 μg/l. This value is also significantly higher than the background concentrations measured for MTBE in river waters (generally below 1 μg/l). There are no monitoring data available for freshwater sediments, but with the physico-chemical properties of MTBE they would be expected to be of a similar magnitude to the water concentrations, i.e. less than 1 μg/kg.

STP organisms

The highest local PEC of 37.2 mg/l was estimated for processing (Use Pattern 2). This is significantly higher than the maximum concentrations measured in effluents of STPs on production plants of 317 μg/l. No measurements are available for MTBE concentrations in any other STPs.

3.1.2 Atmosphere

3.1.2.1 Measured exposure data for air

In the EU, no measured data are available for background concentrations of MTBE in ambient air. This section therefore relies heavily on data from the USA and other countries.

The levels of MTBE recorded in nine Canadian cities for the period of 1995 to 1996 were generally low, < 0.4 μg/m^3 (detection limit 0.1 μg/m^3). This study was carried out as part of the National Air Pollution Surveillance Programme, and the sites selected for monitoring were chosen based on usage of MTBE in gasoline or the vicinity of manufacturers of the substance (Environment Canada, 1996 cited in IPCS, 1998).

In Port Alegre (Brazil), MTBE concentrations in urban air for the period of March 1996 to April 1997 were 0.72 to 62 μg/m^3, with a mean value of 24 ± 16 μg/m^3 (Grosjean *et al*, 1998).

In Houston (Texas) and Boston (Pennsylvania) in 1990 and 1991, 24-hour ambient air sampling revealed concentrations of 0.7 to 10 μg/m^3 and 0.7 to 1.8 μg/m^3 respectively (Kelly *et al*, 1993).

In 1992-1993, Fairbanks (Alaska) saw a rise in complaints of ill health allegedly related to the introduction of MTBE into gasoline. (The health effects and data from similar studies are reported in Section 4.1.2.9). The concentrations of MTBE in ambient urban air (including road intersections) were measured along with sampling inside of buildings and residences. When the results were combined with those from other cities in the USA (Milwaukee, Albany, Stamford, Boston and Houston) the median concentration for ambient air ranged from 0.47 to 17 μg MTBE/m^3; the highest median value was found in Fairbanks. The levels were considerably higher near service stations, roadways and gasoline blending and distribution facilities (Zogorski *et al*, 1996).

Records in Milwaukee (Wisconsin) where MTBE has been used in reformulated gasoline (RFG) at approximately 11% by volume, have shown ambient air concentrations up to 15 μg/m^3. The concentration in 45% of the samples was below detection limit of 0.36 μg/m^3. The nearby cities of Madison and Green Bay where RFG has not been mandated, provided control samples with MTBE concentrations below the detection limit. Factors which might have elevated the airborne concentrations of MTBE included, for instance, the vicinity of busy intersections, or the effect on ambient air samples of starting cold engines (Allen and Grande, 1995 cited in IPCS, 1998).

Some MTBE measurements were recorded in California for the ambient air monitoring programme in 1996 for a number of cities. The overall range of concentrations taken over 24 hours was 1.4 to 44.7 μg/m^3, with the highest averages recorded for Los Angeles and Burbank (M. Poor, personal communication cited in IPCS, 1998).

The manufacturers in the EU carry out monitoring of emissions to air and to wastewater at their production plants. Levels of MTBE in air have been calculated for workplace exposure and the long-term exposure values are, on average, less than 1 mg/m^3 for production plants and a few (< 5) mg/m^3 for processing plants (Table 4.1).

There are limited data for MTBE concentrations in air around refineries. In one location in the USA, the 24-hour concentration of MTBE was 20 μg/m^3 in one sample (out of nine) taken downwind from the refinery perimeter (estimated release into the air for this refinery was reported to be 33 t/y). However, MTBE was not detected in any of 26 other upwind and downwind samples taken during the same year period, nor was it detected in 54 samples taken over 24 hours near two other refineries (limit of detection 6 μg/m^3) (API, 1989b cited in IPCS, 1998 and IARC, 1999).

With the data for MTBE around refineries being so limited, it has been considered useful to use data for other VOCs (such as benzene), which are more readily available, and which may provide a surrogate estimate for MTBE levels. More than 2,000 samples around the boundaries of 3 refineries were taken for benzene and then appropriate factors applied to the resulting data to calculate worst-case MTBE concentrations. It was considered that MTBE concentrations at the boundaries might reach 50 μg/m^3, although as residential dwellings are not generally located on the perimeters of these refineries the estimates were likely to be unrealistically high for human exposure (Concawe, 2000).

Given the high Henry's Law constant (Section 1.2.4), MTBE vapour present in the atmosphere will tend to partition to atmospheric water, including precipitation. However, this washing-out of gas-phase MTBE by rain will not greatly alter the gas-phase concentration in air. The concentration of MTBE in urban precipitation resulting from wash-out has been estimated to be up to 3 μg/l (Squillace *et al*, 1997). One small study in Germany reported concentrations of MTBE in 3 samples of rainwater at 9 and 70 ng/l (Achten and Püttmann, 2000).

The levels reached in receiving waters as a consequence of precipitation are unlikely to exceed the standard of 20 to 40 μg/l proposed to avoid tainting of drinking water in the USA (US-EPA, 1997). A Californian panel was unable to taste concentrations of 2-5 μg MTBE/l drinking water (Dale *et al* cited in US-EPA, 1997). Comparisons of modelled MTBE concentration data for stormwater in urban areas shows a very close fit between predicted and measured concentrations (Lopes and Bender, 1998). This suggests that urban land surfaces are the primary non-point source of most VOCs including MTBE. Lopes and Bender (1998) concluded that sources of drinking water in urban areas would be most effectively protected by controlling land-surface sources.

3.1.2.2 Model calculations

Entry of MTBE to the atmospheric environment may occur through two routes (cf. Section 3.1.1.2):

- Emissions to air from production/formulation plants and subsequent wet or dry deposition to soil and water (production and formulation);
- emissions to air from vehicles and subsequent wet or dry deposition to soil and water (processing and private use).

Emissions and emission factors to air from MTBE production and blending

Using data supplied by some of the major MTBE manufacturers, emission estimates were made for emissions to air from production and formulation plants. These data were prepared for the purpose of indicating manufacturers' compliance with regulations governing maximum allowed annual emissions of VOCs to air (Table 3.13).

Table 3.13: Airborne emissions and measured concentrations of MTBE in air at MTBE production plants in 1999

Plant number[a]	Manufacturing process	Feedstock[b]	Production (kt/y)	Emission to air (kg/y)	Number of samples	Sampling period	Concentration in air (µg/m^3)
1	Continuous	SC	135	21,258	NS	NS	NS
2	Continuous	SC, FCC	87.591	41,600[c]	12	2 months	300 - 31,000
3	Continuous	SC, FCC	176	45,000	NS	NS	NS
4	Continuous, wet, closed system at refinery	FCC	120	NS	NS	NS	< 400 - 36,000 < 400 - 7,000
5	Closed, wet system	TBA	590	NS	NS	NS	NS
6	Continuous, wet, closed system	FCC	65	0	NS	NS	0
7	Continuous, wet, closed system	SC	45	NS	NS	NS	NS
8	Continuous, dry, closed system	SC, FCC	65	NS	NS	NS	NS
9	Continuous, wet, closed system	TBA	610	31,810	NS	NS	NS
10	Continuous, dry, closed system	SC	210	35	NS	NS	NS
11	Continuous, wet, closed system	SC, FCC	125	NS	NS	NS	NS
12	Continuous	FCC	70.5	6,029	NS	NS	< 2,000[d]
13	Continuous	SC, FCC	74.74	2,946	NS	NS	< 100[d]
14	Continuous	NS	22.35	5,024	NS	NS	20
15	Continuous	NS	38.74	4,719	NS	NS	< 100[d]
16	Continuous	NS	30.4	5,998	NS	NS	< 100[d]
17[e]	Continuous	SC	135	5,409	NS	NS	< 165[f]
18	Continuous	SC	125	44,660	NS	NS	NS
19	Continuous, wet, closed system at refinery	NS	39.174	NS	NS	NS	NS
20	Continuous	FCC	70	NS	NS	NS	NS
21	Continuous	SC	80	NS	NS	NS	NS
22	Continuous, wet, closed system	NS	35	NS	NS	NS	NS
23	Continuous, wet, closed system	NS	45	NS	NS	NS	NS

[a] For internal purposes

[b] FCC, fluid catalytic cracker; SC, steam cracker; TBA, tertiary-butyl alcohol

[c] Calculated from VOC emissions

[d] Limit of detection

[e] Data for year 2000

[f] No detectable odour, so odour threshold used as surrogate concentration (not measured)

NS Not stated

Using the emission data of Table 3.13, emission factors were calculated for the air compartment and these are shown in Table 3.14.

Table 3.14: Emissions factors to air from production and formulation of MTBE in 1999

Plant number[a]	Manufacturing process	Production (kt/y)	Emission to air (kg/y)	Emission Factor for air (g/t)
1	Continuous	135	21,258	157
2	Continuous	87.591	41,600[b]	475[c]
3	Continuous	176	45,000	256
4	Continuous, wet, closed system at refinery	120	NS	NS
5	Closed, wet system	590	NS	NS
6	Continuous, wet, closed system	65	0	0
7	Continuous, wet, closed system	45	NS	NS
8	Continuous, dry, closed system	65	NS	NS
9	Continuous, wet, closed system	610	31,810	52.1
10	Continuous, dry, closed system	210	35	0.157
11	Continuous, wet, closed system	125	NS	NS
12	Continuous	70.5	6,029	85.5
13	Continuous	74.74	2,946	39.4
14	Continuous	22.35	5,024	225
15	Continuous	38.74	4,719	122
16	Continuous	30.4	5,998	197
17[d]	Continuous	135	5,409	40.1
18	Continuous	125	44,660	357
19	Continuous, wet, closed system at refinery	39.174	NS	NS
20	Continuous	70	NS	NS
21	Continuous	80	NS	NS
22	Continuous, wet, closed system	35	NS	NS
23	Continuous, wet, closed system	45	NS	NS

[a] For internal purposes

[b] Calculated from VOC emissions

[c] Selected for EUSES modelling

[d] Data for year 2000

NS Not stated

All of the estimated emission factors for emissions to air from production/formulation were lower than the default values in the TGD. The highest emission factor of 475 g/t was thus used in EUSES for the calculation of emission to air from production and formulation.

The data used were for 1999 except where indicated, but available data show that emissions of MTBE to air have declined year by year, as is illustrated by data for one of the largest production plants in the EU (Table 3.15).

Table 3.15: Year by year emissions and emission factors from a major production plant [a]

Year	Emission (kg/y)	Emission factor (g/t)
1992	39,000	66
1993	35,700	60
1994	11,000	19
1995	1,400	2.3
1996	1,200	2
1997	NS	NS
1998	2,500	4.24

[a] Plant number 5 in Table 3.14 (production 590 t/y)

The decline in emissions resulted from the installation of new equipment with better seals to prevent fugitive emissions of vapour, but may have reached a plateau since the emissions estimated for this plant for 1998 were higher than the 1996 emissions.

Estimate of emission factors to air from MTBE use

To estimate the MTBE emissions factors to air from vehicles equipped with internal combustion engines with and without catalyst (processing and private use), the exhaust emissions have been measured in Finland using standard emission test procedures (Table 3.16). The data stem from confidential measurements made at the emission laboratory of Fortum Oil and Gas.

Table 3.16: Measured emissions of MTBE to air from vehicle [a] exhausts

Exhaust	Gasoline blend[b]	Measured emission		Emission assuming unburnt		Emission factor[c]
		(g/km)	(mg/g fuel)	(g/km)	(mg/g fuel)	(mg/g MTBE)
With TWC[d]	Low	0.001	0.017	1.8	30	0.56
	High	0.003	0.05	7.2	120	0.42
Without TWC[d]	Low	0.02	0.33	1.8	30	11.11
	High	0.12	2	7.2	120	16.67
Average		0.07	1.17	4.5	75	13.89

[a] Standard fuel use of 60 g/km (35 miles/g or 12.4 km/l)

[b] Low blend, 2-3% MTBE (w/w); high blend, 11-12% MTBE (w/w)

[c] Fraction of MTBE present in the gasoline that is emitted to air from car exhaust

[d] Three-way catalyst

Table 3.16 shows that in these vehicles the average emission factor to air was 13.89 mg/g MTBE and the highest emission factor was 16.67 mg/g MTBE, i.e. much lower than the value of 0.6 g/g (600 mg/g) used as the default factor in EUSES for emission to air from Processing/Private Use. However, the emission factors calculated from the Finnish tests probably do not represent a 'realistic worst case' since the vehicles (and engines) were quite new, well-maintained and operated under optimum conditions. In addition they do not include emissions from other parts of the 'processing' stage, for example distribution and storage of gasoline.

It would have been possible to calculate emissions of MTBE to air from vehicle use in the EU using, *inter alia*, knowledge of the following:

- The current composition of the EU vehicle fleet in terms of age, engine size, kilometres travelled by each vehicle, typical travel cycles;
- exhaust emissions of MTBE for each part of the travel cycle for each engine size/age;
- the use of MTBE in gasoline within the EU member states;
- fugitive emissions of MTBE for example from vehicle fuel tanks, fuel lines, carburettors;
- fugitive emissions of MTBE from transport, storage and dispensing of gasoline.

While some of this information might be available, it was beyond the remit of the current risk assessment to collect and collate such data or to produce the data where it did not already exist. A study by the German UBA (Friedrich, 2000) reported that emissions of MTBE from evaporation, exhaust emissions, spilling and refuelling in Germany had declined from 5,990 tonnes in 1992 to 2,285 tonnes in 1999. Spilling and refuelling accounted for 440 tonnes in 1999, so the majority of emissions to air were from evaporation and exhaust emissions.

Consequently, for the purpose of this risk assessment, the high TGD default value of 600 mg/g was used for fractional emissions to air (and the other environmental compartments) to represent the worst-case scenario modelled in the EUSES calculations. It should be noted that this would result in a large overestimate of the amount of MTBE in the atmosphere.

Atmospheric PEC calculations

The concentration of the chemical in air is estimated at a distance of 100 metres from a point source, for example the production or formulation plant. Calculation of the PEC_{local} for air considers the emissions both from a point and from an STP and uses the higher value from the two concentrations as the PEC_{local} (TGD, p. 299):

$$Clocal_{air} = \text{maximum } (Elocal_{air}, Estp_{air}) \times Cstd_{air} \quad \text{(Eq. 10)}$$

where $Clocal_{air}$ = local concentration in air during emission episode (mg/m^3)
$Elocal^{air}$ = local direct emission rate to air during episode (kg/d)
$Estp_{air}$ = local indirect emissions to air from STP during episode (kg/d)
$Cstd_{air}$ = concentration in air at a source strength of 1 kg/d (mg/m^3)

The annual average concentration in air is calculated as:

$$Clocal_{air,ann} = Clocal_{air} \times T_{emission}/365 \quad \text{(Eq. 11)}$$

where $Clocal_{air,ann}$ = annual average concentration in air, 100 m from point source, mg/m^3
$T_{emission}$ = number of days per year that the emission takes place

The annual average predicted environmental concentration in air, $PEClocal_{air,ann}$ is calculated as:

$$PEClocal_{air,ann} = Clocal_{air,ann} + PECregional_{air} \quad \text{(Eq. 12)}$$

where $PEClocal_{air,ann}$ = annual average predicted environmental conc. in air, (mg/m^3)
$PECregional_{air}$ = regional concentration in air, (mg/m^3)

The indirect emission from an STP to air is given by the fraction of the emission to effluent that is directed to air:

$$Estp_{air} = Fstp_{air} \times Elocal_{water} \quad \text{(Eq. 13)}$$

where $Fstp_{air}$ = fraction of the emission to air from STP (0.43)
$Elocal_{water}$ = local emission rate to water during emission episode (kg/d)
$Estp_{air}$ = local emission to air from STP during emission episode (kg/d)

Calculation of the deposition flux from air to soil uses the sum of the emissions from the two sources (direct and STP) as follows:

$$DEPtotal = (Elocal_{air}+Estp_{air})x(Fass_{aer}xDEPstd_{aer}+[1-Fass_{aer}]xDEPstd_{gas}) \quad (Eq.14)$$

$$DEPtotal_{ann} = \frac{DEPtotal \times T_{emission}}{365} \quad (Eq.\ 15)$$

where

$Elocal_{air}$ = local direct emission rate to air during emission episode (kg/d)
$Estp_{air}$ = local indirect emission to air from STP during episode (kg/d)
$Fass_{aer}$ = fraction of chemical bound to aerosol
$DEPstd_{aer}$ = standard deposition flux of aerosol-bound compounds at a source strength of 1 kg/d, $mg/m^{-2}/d$
$DEPstd_{gas}$ = deposition flux of gaseous compounds as a function of Henry's Law coefficient, at a source strength of 1 kg/d
$T_{emission}$ = number of days per year that the emission takes place (d/y)
DEPtotal = total deposition flux during emission episode ($mg/m^{-2}/d$)
$DEPtotal_{ann}$ = annual average total deposition flux ($mg/m^{-2}/d$)

Local emission to air from an STP during an emission episode is calculated as follows:

$$Estp_{air} = Fstp_{air} \times Elocal_{water} \quad (Eq.\ 16)$$

where

$Fstp_{air}$ = fraction of the emission to air from an STP (0.43)
$Elocal_{water}$ = local emission rate to water during emission episode (kg/d)

3.1.2.3 Summary of PECs

The PECs (total) for air calculated by EUSES according to the TGD guidelines for the atmospheric compartment are shown in Table 3.17.

Table 3.17: Summary of PECs for the atmospheric compartment

Scenario	Use Pattern	Concentration(μg/m^3)
Local		
Production	1	172
Formulation	1	172
Processing	1	179
Private use	1	30.5
Processing	2	306
Processing	3	98.6
Regional		30.1
Continental		4.39

3.1.2.4 Comparison of modelling and monitoring data

The annual average local PEC of 172 μg/m^3, which relates to production is of a similar level to values reported for worker exposure that may be up to 1 mg/m^3. The highest reported concentrations in urban air are about 60 μg/m^3 and generally 10 μg/m^3 or less, which are within the same order of magnitude as the estimated regional PEC of 30.1 μg/m^3.

3.1.3 Terrestrial compartment

MTBE will be transported to the terrestrial environment through atmospheric deposition from air and in sewage sludge. The latter is used as a soil conditioner and fertiliser on agricultural land, or disposed of in landfills.

3.1.3.1 Measured exposure data

Little monitoring data are available for MTBE in soils in Europe. Almost all the data relate to sites contaminated by gasoline and hence there is no background data to compare with the PECs calculated from the EUSES model.

Groundwater

Most of the monitoring data for MTBE in the terrestrial environment are related to groundwater that has been contaminated by LUSTs. As a consequence of its high water solubility, MTBE migrates faster in aquifers than the other (hydrocarbon) components of gasoline and some high concentrations (up to 200 mg/l) have been reported in groundwater in the USA, reflecting extensive use of gasoline blended with high levels of MTBE. However, since these ground water concentrations do not relate to normal use, but rather to an abnormal situation (a leak), they are therefore not considered to be especially relevant to this environmental risk assessment.

Where routine monitoring data have been produced for groundwater in the EU (for example in the UK: Turrell *et al*, 1996; UK-DETR, 1999; Dottridge *et al*, 2000) they show that MTBE is not present in most samples and, where detected, the source is invariably a LUST or other spillage (Table 3.18).

In the survey of Turrell *et al* (1996), most (83%) of the detectable concentrations occurred during the winter season when use of oxygenate in gasoline is mandatory and MTBE emitted to air will partition more efficiently to rain water.

Table 3.18: Concentrations of MTBE in ground water

Water type/ country	Location	Number of sites and/or samples	Limit of detection (µg/l)	Number of positives[a]	Concentration (µg/l)			Reference
					Mean or median	Minimum	Maximum	
Finland	Service station	1, 2	0.1	2	53	37	69	Piilo and Salla, 2000
Finland	Water supply	1, 2	0.1	2	0.7	0.67	0.72	Piilo and Salla, 2000
UK	Thames EA survey	300	0.1	35	16.9	0.1	124	UK-DETR, 1999
UK	NE EA survey	100	0.1	3	NS	0.15	1.1	Turrell *et al*, 1996
UK	Midlands EA survey	17 (2 x)	0.1	3	NS	0.48	2.9	Turrell *et al*, 1996
UK	WSC survey	Ca. 25	0.05	4	NS	0.055	0.53	Turrell *et al*, 1996
UK	WSC survey	9, 13	0.2	3	NS	0.3	1.39	Turrell *et al*, 1996
UK	WSC and EA	1,178	0.1[b]	623	NS	< 0.1	> 5[c]	Dottridge *et al*, 2000
USA	California	2,297	0.1	8	NS	20	100,000	Happel *et al*, 1998
USA	USA GS survey	1,171	0.2	68	0.6	0.2	23,000	Squillace *et al*, 1997
USA	California	6,621	3	34[d]	NS	3	> 13	California EPA, 1999a

[a] Samples in which MTBE was detected

[b] 19% of samples had a detection limit of 50 and 15% had a detection limit of 0.2 µg/l

[c] All samples were from public water supply boreholes and only 3 had concentrations in excess of 5 µg/l

[d] In at least two samples from a source

The Danish EPA (Miljøstyrelsen, 1998) has published data on the concentrations of MTBE in groundwater samples taken in the vicinity of gasoline stations (Table 3.19).

Table 3.19: MTBE concentrations in groundwater from the vicinity of gasoline stations in Denmark

Local authority monitoring data		**Oil company monitoring data**	
	(µg/l)		(µg/l)
Fyn	3,000 - 6,000	Copenhagen 1	480
Ribe	1,000 - 3,700	Copenhagen 2	< 1
Arhus	22,000 - 550,000	Frederiksborg	30,000
Copenhagen	1 - 42	Fyn 1	1,700
Frederiksberg commune	0	Fyn 2	5
		Fyn 3	240
		Ringkobing 1	< 10
		Ringkobing 2	400
		Ringkobing 3	45
		Viborg	< 10

Clearly there are instances of significant pollution of groundwater by gasoline containing MTBE around many of these sites but some sites still show negligible pollution. The possibility of contamination of groundwater by gasoline components, including MTBE, is of particular concern in Denmark since virtually 100% of Danish drinking water is produced from groundwater and receives minimal treatment (i.e. not chlorinated) Morgenroth and Arvin, 1999).

These data show that MTBE can enter the soil and groundwater environment around service stations, probably as a result of minor spillages during refuelling operations of vehicles and, perhaps, refilling of underground storage tanks and leakages from pipes and LUSTS. Such emissions are preventable or containable (i.e. by the use of properly designed equipment, leak detection sensors, impermeable surfaces and run-off collection traps) and should not be taken as acceptable normal practice. In Germany a major programme of service station improvement has now been completed and all service stations have impermeable surfaces and double-skinned underground storage tanks with leak detection sensors (Morgenroth and Arvin, 1999).

Drinking water

In a small survey in Finland, MTBE was found in groundwater used for water supply (Table 3.18), but no MTBE was detected in drinking water (Piilo and Salla, 2000).

Little other information is available on the concentration of MTBE in surface water and groundwater specifically used as drinking water sources in EU member states. In California, where LUSTs have resulted in high concentrations of MTBE in some groundwaters, there has been a regulatory requirement since 1997 for the monitoring of MTBE in drinking water and their sources; some data were also collected prior to 1997. An extensive dataset has been built up and a summary of these data is shown in Table 3.20.

Table 3.20: MTBE detected in sources of California drinking water (1990-2001)[a]

Source[b]	Number of sources sampled	MTBE detected[c,d] in number of sources	Concentration > 5 µg/l	Concentration > 13 µg/l
Drinking water	8,565	72 (0.8%)	39 (0.5%)	19 (0.2%)
Ground water	7,986	46 (0.6%)	31 (0.4%)	17 (0.2%)
Surface water	579	26 (4.5%)	8 (1.4%)	2 (0.3%)

[a] Data from California Department of Health Services (California EPA, 2001).

[b] Monitoring is to be done by approximately 4,900 community and non-transient non-community systems, including some 11,000 ground water and 800 surface water sources. About 60% of ground water sources and surface water sources have been sampled

[c] MTBE was considered 'detected' if present in at least two samples from a source

[d] Detection limit 3 µg MTBE/l

The data show a higher percentage of detection of MTBE in surface water sources (4.6%) than in groundwater sources (0.5%). This could be due to LUSTs (impacting surface water sources through shallow groundwater inputs to the base flow), or through leaks and spillages (from vehicles or refuelling) to hard surfaces (via run-off to surface drains), or from atmospheric wash-out.

3.1.3.2 Modelling / estimated exposure data

PEC soil from production, formulation and processing

PEClocal calculations for soil are based on application of sewage sludge in agriculture and dry and wet deposition from the atmosphere.

The total deposition flux ($DEPtotal_{ann}$) is converted to concentration mg substance/kg soil/d (D_{air}) as follows (TGD p. 309):

$$D_{air} = \frac{DEPtotal_{ann}}{DEPTH_{soil} \times \rho_{soil}} \qquad \text{(Eq. 17)}$$

where $DEPTH_{soil}$ = mixing depth of soil (for the terrestrial ecosystem this is taken as the plough depth, i.e. 0.20 m)
ρ_{soil} = bulk density of soil (1700 kg/m^3)

Groundwater concentrations are estimated for indirect exposure of humans through drinking water. These estimates provide only a worst-case concentration in groundwater since they calculate the soil porewater concentration from the soil concentration. Such a calculation does not include degradation, sorption and dilution processes occurring in the aquifer.

$$PEClocal_{grw} = PEClocal_{agr.soil,porew} \quad \text{(Eq. 18)}$$

where $PEClocal_{agr.soil,porew}$ = predicted environmental concentration in porewater, mg/l
$PEClocal_{grw}$ = predicted environmental concentration in groundwater, mg/l

3.1.3.3 Summary of PECs

The average PECs for (agricultural) soil calculated by EUSES according to the TGD guidelines for the terrestrial compartment are shown in Table 3.21.

Table 3.21: PECs for the terrestrial compartment

Scenario	Use Pattern	PECsoil[a] (µg/kgww)
Local		
Production	1	24.3
Formulation	1	24.6
Processing	1	15.2
Private use	1	1.34
Processing	2	73.3
Processing	3	23.3
Regional		0.304
Continental		0.0435

[a] 30-d average

3.1.3.4 Comparison of modelling and monitoring data

The average local PEC estimated for production is 24.3 µg/kgww and the regional (background) PEC is 0.304 µg/kgww, but there are no measured concentrations to compare with these values.

3.1.4 Representativeness of monitoring data

The monitoring data reported in this report have been obtained from a wide variety of sources and there was little consistency in the methodology used to obtain the samples and/or carry out the analysis. Some of the key aspects relating to monitoring data quality are discussed in the following sections. ECETOC and the European Fuel Oxygenates Association (EFOA) have produced a specific overview of sampling and analysis protocols for the collection of emission data at production and formulation plants (Appendix B).

3.1.4.1 Sample collection and handling methods

In most instances of environmental monitoring, the non-homogeneous nature of the environmental matrix sampled, results in high variability in both the composition of the samples collected and the concentration of the determinand of interest. This is particularly true for samples of soil and sediment, which can show substantial spatial non-homogeneity. Losses of MTBE may occur during sample collection and handling by volatilisation, particularly where headspace is present in sampling equipment (or sample bottles) or during sample transfers. For MTBE, losses by sorption and chemical degradation should be insignificant, but biodegradation may occur. Samples should thus be kept at low temperatures, in the absence of light and with no headspace, and be analysed as soon as possible after collection.

3.1.4.2 Analytical methods

Analysis of MTBE in solid and aqueous samples is generally carried out by headspace extraction or purge and trap followed by gas chromatography (GC) with flame ionisation (FID), or mass spectrometric (MS) detection. Solvent extraction may also be used for solid and aqueous samples; air samples will usually be adsorbed on a sorbent for thermal desorption into the GC. MS is the preferred method of detection since it offers greater selectivity of detection than FID and, in Selected Ion Monitoring mode (SIM), the highest sensitivity of detection. Capillary chromatography is essential and preferably with a capillary column that is optimised for MTBE determination. This is important since MTBE can co-elute with other components present in gasoline on some columns. Co-elution and mis-identification is always a possibility for MTBE monitoring data that were obtained with GC-FID.

3.1.5 Non-compartment-specific exposure relevant to the food chain

No measured data on the uptake of MTBE by organisms through the food chain are available.

PECs were derived for top predators as fish- and worm-eating birds, based on the BCFfish of 1.59 and BCFworm of 1.63 (Section 3.1.0.5), using the following equations:

$$PEC_{oral,fish} = BCFfish \times 0.5(_{annual}PEC_{local,\ water,\ annual} + PEC_{regional,\ water}) \quad \text{(Eq. 19)}$$

$$PEC_{oral,worm} = BCFworm \times 0.5(PEC_{local,\ agric} + PEC_{regional,\ agric}) \quad \text{(Eq. 20)}$$

where $PEC_{local,\ agric}$ is the local PEC in agricultural soil, averaged over 180 days.

The $PEC_{local,\ agric}$ is a summation of the concentration due to deposition to agricultural soil from air and the concentration arising from spreading of sewage sludge.

The concentration in dry sewage sludge is calculated from the emission rate to water, the fraction of emission sorbed to sludge and the rate of sewage sludge production.

The contribution to the soil concentration from atmospheric deposition is estimated from the highest emission rate from the local source or STP.

The PECs for worm- and fish-eating predators calculated by EUSES for the various life-cycle stages of MTBE according to the TGD guidelines for predators through the food chain are shown in Table 3.22.

Table 3.22: PECs for predators through the food chain

Scenario	Use Pattern	PECoral, worm (µg/kgbw)	PECoral, fish (µg/kgbw)
Local			
Production	1	6.57	773
Formulation	1	6.57	773
Processing	1	5.37	480
Private use	1	0.646	52.7
Processing	2	16	2,460
Processing	3	5.02	616

3.2 Effects assessment: hazard identification and dose (concentration) - response (effect) assessment

3.2.0 General discussion

The effects assessment considers three trophic levels: primary producers (algae, plants), primary consumers (invertebrates) and secondary consumers (fish, mammals). The protection goals for the environment are the aquatic (including sediment and microbial activity in a STP) and terrestrial ecosystems, top predators (through the food chain) and the atmosphere. A PNEC has to be derived for each of these protection goals. In order to carry out the assessment a range of effects data must be entered for EUSES to extrapolate to PNECs (TGD, p. 321 and following).

In the TGD, certain assumptions are made in the environmental risk assessment concerning the extrapolation from single-species short-term toxicity data to ecosystem effects: (i) ecosystem sensitivity depends on the most sensitive species; (ii) protecting ecosystem structure protects community function. These two assumptions have important consequences. By establishing which species is the most sensitive to the toxic effects of a chemical in the laboratory, extrapolation can subsequently be based on the data for that species. Furthermore, the functioning of any ecosystem in which that species exists should be protected, provided the structure is not sufficiently distorted as to cause an imbalance.

3.2.0.1 Assessment factors

In principle, the PNEC is calculated by dividing the lowest short-term $L(E)C_{50}$[a] or long-term NOEC[a] value by an appropriate assessment (or safety) factor. The assessment factor should reflect the degree of confidence in extrapolation from laboratory toxicity-test data for a limited number of species to the real environment. A lower assessment factor may be used with larger and more relevant data sets than the base-set data (e.g. if data are available on the toxicity to organisms at a number of trophic levels, belonging to taxonomic groups and with lifestyles representing various feeding strategies).

In establishing the size of the assessment factor to be used a number of uncertainties must be addressed and these are summarised below:

- Intra- and inter-laboratory variation of toxicity data;
- intra- and inter-species variations (biological variance);
- short-term to long-term toxicity extrapolation;
- laboratory data to field impact extrapolation.

Assessment factors recommended in the TGD and used by EUSES in the derivation of a PNEC (for aquatic life) are given in Table 3.23.

Table 3.23: Assessment factors used to derive a PNEC *(TGD, p. 330)*

Information available	Assessment factor
At least one short-term L(E)C_{50} from each of 3 trophic levels of the base-set (fish, *Daphnia*, algae)	1,000
One long-term NOEC (either fish or *Daphnia*)	100
Two long-term NOECs from species representing two trophic levels (fish and/or *Daphnia* and/or algae)	50
Three long-term NOECs representing 3 trophic levels	10
Field/model ecosystem data	Case-by-case

A conservative assessment factor of 1,000 is applied to short-term toxicity data in the absence of longer-term data of sufficient quality. It assumes that each of the previously listed uncertainties makes a significant contribution to the overall uncertainty.

An assessment factor of 100 is applied to a single long-term NOEC (fish or *Daphnia*) if this has been generated for the trophic level showing the lowest L(E)C_{50} in the short-term tests. If this is not the case, then the NOEC cannot be regarded as being protective of other more sensitive species using the assessment factors available; the assessment is then based on the short-term data with an assessment factor of 1,000. An assessment factor of 100 is also applied to the lowest of two long-term NOECs from two trophic levels, when these values have not been derived for the trophic level with the minimum L(E)C_{50} value.

An assessment factor of 50 is applied to the lower of two long-term NOECs from two trophic levels when these values have been derived for the trophic level with the minimum L(E)C_{50} value. It is also applied to the lowest of 3 NOECs covering 3 trophic levels when such NOECs have not been generated for the trophic level with the minimum L(E)C_{50} value.

An assessment factor of 10 is normally applied only when long-term toxicity NOECs are available for at least three species across three trophic levels. It may sometimes be possible to determine with high probability that the most sensitive species has been examined and that further long-term NOECs would not be any lower; under these circumstances an assessment factor of 10 might be applied to the lowest NOEC from only two species. This might be particularly relevant where the substance shows no potential for bioaccumulation.

[a] Median lethal (or effective concentration)

The assessment factor to be used if field data or results from model ecosystems are available, is considered on a case-by-case basis.

There is still some discussion as to what are the most appropriate assessment factors to use and other recommendations have been made regarding the application of assessment factors in environmental risk assessment. ECETOC (1992) recommended an application factor of 200 to convert from (the lowest value of EC_{50}) a satisfactory set of acute toxicity data (at least 3 diverse species) to a true "field no effect concentration" (PNEC) and a factor of 5 from a set of chronic data. In a later report (ECETOC, 1993), the same overall recommendation was supported although there was a suggestion that a factor of 200 might be too conservative. At the time, however, there was insufficient evidence on which to recommend a lower value.

3.2.0.2 Equilibrium-partitioning approach in soil and sediment

In the absence of sufficient quantity or quality of ecotoxicological data for soil or sediment-dwelling organisms, the PNEC may provisionally be calculated using the equilibrium-partitioning method. This method uses the PNEC for aquatic life and the soil- or sediment-water partition coefficient (Di Toro *et al*, 1991; OECD, 1992b cited in TGD, p. 335-338). In the partitioning method it is assumed that:

- Sediment-or soil-dwelling organisms and water-column organisms are equally sensitive to the chemical;
- concentrations in soil/sediment, interstitial water and soil/benthic organisms are in thermodynamic equilibrium, thus the concentration in any of these phases can be predicted using the appropriate partition coefficients;
- sediment/soil-water partition coefficients can either be measured or derived on the basis of a generic partition method from separately measurable characteristics of the properties of the chemical and of the compartment.

However, this approach considers uptake via the water phase only; uptake via ingestion of soil or sediment is not taken into account, hence for some compounds the total uptake may be underestimated. Therefore, for lipophilic compounds ($\log K_{ow} > 5$) and species exposed primarily through food, the PEC is increased by a factor of 10 in the risk characterisation (TGD, p. 339).

A PNEC derived in this way may also be compared with one derived using the limited measured data if any exist. The lower value is usually adopted.

3.2.1 Aquatic compartment

The primary source for aquatic toxicity data was the HEDSET for MTBE (Arco Chemical Europe, 1997), which includes company data and information from the open literature. In addition, searches of Aquire (aquatic information retrieval database) and Aqualine databases were conducted.

The principal quality criteria for acceptance of data were that the test procedure was well described (with reference to an official guideline, if possible) and that the test concentrations were measured with an adequate analytical method. This latter requirement is particularly important for MTBE due to its volatility; data from tests with no analytical monitoring were not accepted. All the studies were in agreement in that MTBE was of low toxicity to the species studied.

The American Petroleum Institute (API) commissioned a number of tests in support of the development of ambient water quality criteria (US-EPA, 2000). The test reports were only available as abstracts. A brief overview was presented by Mancini *et al* (1999) [a].

3.2.1.1 Freshwater organisms

A summary of the freshwater toxicity data is provided in Table 3.24.

[a] The freshwater data were subsequently published by Wong *et al*, 2001

Table 3.24: Aquatic toxicity to freshwater organisms

Species	Method	GLP[a]	Concentration [b], test system[c]	Duration	Effect Parameter	Concentration (mg/l)	Reference
Algae					**Growth inhibition**		
Scenedesmus subspicatus (green alga)	88/302/EEC	Yes	A, C	72 h	NOEC	470	Hüls, 1991b
					EC_{10}	650	
					EC_{50}	>800	
Selenastrum capricornutum[d] (green alga)		Yes	A, SS	96 h	EC_{50}	184	BenKinney *et al*, 1994
Selenastrum capricornutum	ASTM E1218-90	Yes	A, S	96 h	EC_{50}	491	ENSR, 1999a; Mancini *et al*, 1999[e]
Invertebrates					**Immobility**		
Daphnia magna (water flea)		Yes	A	48 h	EC_{100}	> 100	Gnemi and Zanolo, 1996b
Daphnia magna		Yes	A, SS	48 h	EC_0	174	Hockett, 1997a
					EC_{50}	542	
Daphnia magna		Yes	A, C	48 h	EC_0	439	Hüls, 1991c
					EC_{50}	651.4	
					EC_{100}	>772.4	
Daphnia magna		Yes	A, SS	48 h	EC_{50}	681	BenKinney *et al*, 1994
Daphnia magna	EPA 850.1010	Yes	A, FT	48 h	EC_{50}	472	Mancini *et al*, 1999[e]
Ceriodaphnia dubia (water flea)		Yes	A, SS	48 h	EC_{50}	340	Hockett, 1997b
Physa gyrina (water snail)	ASTM E729-96	Yes	FT	96 h	EC_{50}	559	Mancini *et al*, 1999[e]
Hexagenia limbata (mayfly)	ASTM E729-96	Yes	FT	96 h	EC_{50}	581	Mancini *et al*, 1999[e]
Hyalella azteca (amphipod)	ASTM E729-96	Yes	FT	96 h	EC_{50}	473	Mancini *et al*, 1999[e]
Chironomus tentans (midge)	ASTM E729-96	Yes	FT	48 h	EC_{50}	1,742	Mancini et al, 1999[e]

Table 3.24: Aquatic toxicity to freshwater organisms (cont'd)

Species	Method	GLP[a]	Concentration [b], test system[c]	Duration	Effect Parameter	Concentration (mg/l)	Reference
Invertebrates					**Inhibition of reproduction**		
Ceriodaphnia dubia		Yes	A, SS	5 d	IC_{25}	203	Hockett, 1997c
					NOEC	202	
					LOEC	342	
Daphnia magna	EPA 850.1300	Yes	A, FT	21 d	IC_{20} [f]	42 [g]	Mancini *et al*, 1999; Wildlife International, 1999a [e]
Fish					**Lethality**		
Pimephales promelas (fathead minnow)		Yes	A, FT	96 h	LC_0	187	Hockett, 1997d
					LC_{50}	980	
Pimephales promelas		Yes	A, SS	96 h	LC_{50}	929	BenKinney *et al*, 1994
Pimephales promelas		No	A, FT	96 h	LC_{50}	672	Geiger *et al*, 1988
Pimephales promelas		No	A, FT	96 h	LC_{50}	706	Veith *et al*, 1983b
Leuciscus idus (golden orfe)		No	N, S	48 h	LC_0	1,000	Hüls, 1996
					LC_{100}	2,000	
Oncorhynchus mykiss (rainbow trout)		Yes	A, FT	96 h	LC_0	527	Hockett, 1997e
					LC_{50}	887	
Oncorhynchus mykiss		Yes	A, SS	96 h	LC_{50}	1,237	BenKinney *et al*, 1994
Brachydanio rerio (zebra fish)		Yes	A, SS	96 h	LC_{50}	> 100	Gnemi and Zanolo, 1996c
					LC_{100}	> 100	
Gasterosteus aculeatus [h] (three-spined stickleback)	EPA 850.1075	Yes	FT	96 h	LC_{50}	297	Mancini *et al*, 1999 [e]
Lepomis macrochirus (sunfish)	ASTM E729-96	Yes	FT	NS	LC_{50}	1,054	Mancini *et al*, 1999 [e]

Table 3.24: Aquatic toxicity to freshwater organisms (cont'd)

Species	Method	GLP[a]	Concentration [b], test system[c]	Duration	Effect Parameter	Concentration (mg/l)	Reference
Fish					**Growth inhibition**		
Pimephales promelas		Yes	A, SS	7 d	IC_{25} NOEC LOEC	288 234 388	Hockett, 1997f
Pimephales promelas, eggs and larvae/fry	ASTM E1241-92	Yes	A, FT	31 d	IC_{50}	279[i]	ENSR, 1999b; Mancini *et al*, 1999[e]
Amphibian							
Rana temporia (tadpole)		No	NS[j]	NS	LC_{50}	2,500	Paulov, 1987

[a] Good laboratory practice

[b] A, analysed or N, nominal; NS, not stated

[c] C, closed; S, static; SS, semi-static or static-renewal; FT, flow-through; NS, not stated

[d] Now known as *Pseudokirchneriella subcapitata*

[e] Subsequently published by Wong *et al*, 2001

[f] Inhibition concentration, estimated to cause 20% reduction in organism performance relative to control

[g] Acute to chronic ratio was 11.3

[h] Marine and freshwater

[i] Acute to chronic ratio was 3.4

[j] Abstract only

Freshwater algae

Toxicity data were found for two species of freshwater algae. BenKinney *et al* (1994) reported a 96-h EC_{50} value of 184 mg/l for the green alga *Selenastrum capricornutum* based on measured concentrations in a semi-static test conducted according to GLP. A higher EC_{50} value for *Selenastrum capricornutum* of 492 mg/l for a 96-h test was reported by Mancini *et al* (1999 [a]), using an EPA test protocol that exceeded all of the requirements of OECD Guideline 201 for a 72-h algal growth inhibition test (OECD, 1993). A 72-h NOEC of 470 mg/l (EC_{50} > 800 and EC_{10} approximately 650) was reported for *Scenedesmus subspicatus*. The NOEC was based on nominal concentrations, but measurement of dissolved concentrations of MTBE before and after the test gave results within 20% of the nominal values (Hüls, 1991b). These data indicate MTBE to be of low toxicity to freshwater algae.

Freshwater invertebrates

Six acute studies were located on the toxicity of MTBE in *Daphnia magna* and *Ceriodaphnia dubia*. All were conducted to GLP and were based on analysed concentrations. The studies indicate MTBE to be of low acute toxicity with EC_{50} values all well above 100 mg/l. The lowest 48-hr EC_{50} of 340 mg MTBE/l was reported for *Ceriodaphnia*, based on measured concentrations in a semi-static renewal test conducted to GLP (Hockett, 1997b).

Two acute toxicity tests have been reported for the water snail, *Physa gyrina*, and the mayfly, *Hexagenia limbata*. The 96 h LC_{50} values were 559 and 581 mg MTBE/l respectively (Mancini *et al* , 1999 [a]).

Two acute toxicity tests were carried out on sediment dwelling invertebrates, the amphipod *Hyalella azteca* and the midge *Chironomus tentans*, under flow through test conditions. *Hyalella* (LC_{50} 473 mg MTBE/l) was more sensitive to MTBE than Chironomus (LC_{50} 1,742 mg/l) (Mancini *et al*, 1999 [a]). However, since these tests did not include sediment, they can only be considered to be an extension of the dataset for the other freshwater invertebrates. The toxicity values obtained were consistent with the toxicity to MTBE shown by the other invertebrates.

Two chronic tests were available. A 5-d NOEC of 202 mg/l was reported for *Ceriodaphnia* after investigation of effects on reproduction and mortality over several broods (Hockett, 1997c). A NOEC of 51 mg/l and an IC_{20} value of 42 mg/l were reported for a 21 d test on *Daphnia* using an EPA test protocol. The acute to chronic ratio for this species was reported to be 11.3 (Mancini *et al*, 1999; Wildlife International, 1999a). The EPA test protocol was consistent with OECD Guideline 211 (OECD, 1998).

[a] Subsequently published by Wong *et al*, 2001.

Freshwater fish

Eleven studies were located which investigated the toxicity of MTBE to six species of freshwater fish. Most studies involved analytical monitoring and were conducted to GLP. All studies indicated MTBE to be of low acute toxicity, with reported LC_{50} values well above 250 mg/l, the lowest value being 297 mg/l from a study reported by Mancini *et al* (1999 [a]).

A sub-acute study in *Pimephales promelas* (fathead minnow) reported a 7-d NOEC of 234 mg/l based on measured concentrations in a static renewal test conducted to GLP (Hockett, 1997e). An IC_{20} value of 279 mg/l from a 31-day flow-through early-life stage test was reported for *Pimephales promelas* using an EPA test protocol (ENSR, 1999b [a]). The acute to chronic ratio for this species was reported to be only 3.4 (Mancini *et al*, 1999 [a]). The test protocol was largely, but not completely, consistent with OECD Guideline 212 (OECD, 1997).

Amphibians

Paulov (1987) reported the effect of MTBE on the survival and development of tadpoles of the frog *Rana temporia* and derived an LC_{50} value of > 2,500 mg/l, the highest (nominal) concentration tested. Lower concentrations in water (100 mg/l) led to a significant increase in the numbers of tadpoles and frogs compared to the controls and a sublethal MTBE concentration also accelerated the rate of metamorphosis, which occurred two days earlier than in unexposed animals.

3.2.1.2 Marine organisms

A summary of the freshwater toxicity data is provided in Table 3.25.

[a] Subsequently published by Wong *et al*, 2001.

Table 3.25: Aquatic toxicity to marine organisms

Species	Method	GLP[a]	Concentration [b], test system[c]	Duration	Effect Parameter	Concentration (mg/l)	Reference
Algae					**Growth inhibition**		
Skeletonema *costatum* (diatom)	EPA 850.5400 Algal toxicity Tiers 1 and 11	Yes	S	NS	EC_{50}	185 (114 [c])	Mancini *et al*, 1999
Invertebrates					**Lethality**		
Nitocra spinipes (copepod)		No	S	96 h	LC_{50}	> 1,000	Lindén *et al*, 1979; Bengtsson and Tarkpea, 1983
Mysidopsis bahia [d] (mysid shrimp)		Yes	A, C, SS	96 h	LC_{50}	136	BenKinney *et al*, 1994
Mysidopsis bahia		Yes	N	96 h	NOEC	15	Boeri *et al*, 1994a
					LC_{50}	44	
					LC_{100}	100	
Mysidopsis bahia	EPA 850.1035; ASTM E729-88a	Yes	FT	96 h	LC_{50}	187	Mancini *et al*, 1999
Palaemonetes pugio (grass shrimp)	EPA 850.1045	Yes	FT	96 h	LC_{50}	166	Mancini *et al*, 1999
Callinectes sapidus (blue crab)	EPA 540/9-82-024	Yes	FT	NS	LC_{50}	306	Mancini *et al*, 1999
Mytilus galloprovincialis (mussel)	Range-finding	Yes	N	NS	LC_{50}	1,309	Mancini *et al*, 1999
Rhepoxynius abronius (amphipod)	Range-finding	Yes	N	NS	LC_{50}	412	Mancini *et al*, 1999

Table 3.25: Aquatic toxicity to marine organisms (cont'd)

Species	Method	GLP[a]	Concentration [b], test system[c]	Duration	Effect Parameter	Concentration (mg/l)	Reference
Invertebrates					**Shell deposition**		
Crassostrea virginica (eastern oyster)		Yes	NS [e]	4 d	EC_{50}	150	Wildlife International, 1999b
					Growth inhibition		
Mysidopsis bahia	EPA 850.1350;	Yes	FT	28 d	IC_{20} [f]	36 [f]	Mancini *et al*, 1999; Wildlife
ASTM E190-90				NOEC[h]	26	International, 1999c	
Fish					**Lethality**		
Alburnus alburnus (bleak)		No	S	96 h	LC_{50}	> 1,000	Lindén *et al*, 1979; Bengtsson and Tarkpea, 1983
Menidia beryllina (inland silverside)		Yes	A, SS	96 h	LC_{50}	574	BenKinney *et al*, 1994
Cyprinodon variegatus		Yes	N, S	96 h	LC_{50}	> 2,500	Boeri *et al*, 1994b
(sheepshead minnow)					LC_{100}	> 2,500	*Cyprinodon*
variegatus	EPA 850.1075	Yes	FT	96 h	LC_{50}	663	Mancini *et al*, 1999

[a] A, analysed or N, nominal; NS, not stated

[b] C, closed; S, static; SS, semi-static or static-renewal; FT, flow-through; NS, not stated

[c] Cell density

[d] Renamed *Americamysis bahia*

[e] Not stated, abstract only

[f] Inhibition concentration, estimated to cause 20% reduction in organism performance relative to control

[g] Acute to chronic ratio was 5.3

[h] Juvenile growth

Marine algae

Toxicity data were available for one species, the diatom *Skeletonema costatum*, and showed an EC_{50} value of 185 mg MTBE/l (for biomass; 114 mg/l for cell density) (Mancini *et al*, 1999). The EC_{50} value suggests a low toxicity, similar to the most sensitive freshwater algae.

Marine invertebrates

Eight acute toxicity studies were located, three in the mysid shrimp *Mysidopsis bahia*. BenKinney *et al* (1994) reported a 96-h LC_{50} value of 136 mg MTBE/l for this species based on measured concentrations in a static test conducted under closed conditions and to GLP. Boeri *et al* (1994a) reported lower values, but the MTBE concentrations were not measured. The other five tests using EPA protocols in different species reported higher LC_{50} values, from 166 mg MTBE/l for *Palaemonetes pugio* (grass shrimp) (Mancini *et al*, 1999).

A 4-d EC_{50} value of 150 mg MTBE/l was reported for oyster shell deposition (Wildlife International, 1999b).

In a chronic test with *Mysidopsis bahia*, a 21-d IC_{20} value of 36 mg/l and NOEC of 26 mg/l (for juvenile growth) were reported using an EPA/ASTM test protocol for a 28 d flow-through life-cycle test. The acute to chronic toxicity ratio was 5.3 (Mancini *et al*, 1999; Wildlife International, 1999c). The protocol was similar to the OECD recommended protocol for chronic tests of daphnids (Guideline 211) (OECD, 1998).

Marine fish

Only four toxicity studies were located for marine fish, all of which indicated that MTBE is of low acute toxicity, with LC_{50} values well over 500 mg/l. BenKinney *et al* (1994) reported a 96-hour LC_{50} of 574 mg/l for *Menidia beryllina* (inland silverside) based on measured concentrations in a static renewal test conducted to GLP. This was the lowest LC_{50} reported for marine fish and is similar to values reported for freshwater fish. The value was chosen for this risk assessment. No longer-term data were available.

3.2.1.3 QSAR calculations

Where there are relatively few experimental data available, it can be useful to estimate toxicity, using well-developed and tested predictive methods based on quantitative structure activity relationships (QSARs) (ECETOC, 1998). In the context of risk assessment of new and existing chemicals, the QSARs listed in the TGD (p. 530) should be used (Table 3.26).

Table 3.26: Predicted aquatic toxicity using TGD recommended QSARs *(Verhaar et al (1995) and Van Leeuwen et al (1992) cited in TGD, p. 530)*

Organism	Duration	Endpoint	Concentration [b] (mg/l)
Green algae	71 - 96 h	EC_{50}	450
Daphnid	48 h	EC_{50}	415
Daphnid	16 d	NOEC	96
Fish	96 h	LC_{50}	450
Fish	28 - 32 d	NOEC	49

[b] Based on log K_{ow} 1.06 (Table 1.1)

Another widely used QSAR estimation approach is provided by the ECOSAR software. The results are shown in Table 3.27.

Table 3.27: Aquatic toxicity of MTBE calculated using ECOSAR, [a] *(US-EPA, 1999)*

Organism	Duration	Endpoint	Concentration [b] (mg/l)
Green algae	96 h	EC_{50}	300
Green algae	96 h	ChV [c]	17
Daphnid	48 h	LC_{50}	500
Daphnid	16 d	EC_{50}	17
Mysid shrimp	96 h	LC_{50}	280
Fish	96 h	LC_{50}	500
Fish (marine)	96 h	LC_{50}	73
Fish	14 d	LC_{50}	780
Fish	30 d	ChV	55

[a] Domain (class): neutral organics, baseline toxicity

[b] Based on log K_{ow} 1.06 (Table 1.1)

[c] Chronic value growth or survival

All of the predicted results show that the toxicity of MTBE to a wide range of aquatic species is relatively low, with the most sensitive organisms being daphnids and green algae in chronic tests, for which the ECOSAR QSARs predicted a value of 17 mg/l. This is similar to the measured chronic toxicity (NOEC, juvenile growth) value of 26 mg/l for *Mysidopsis bahia*.

3.2.1.4 Derivation of PNEC for aquatic life

A large amount of acute aquatic toxicity test data are available for organisms at different trophic levels and the results obtained from these are consistent with the conclusion that MTBE exerts a low toxicity to aquatic organisms.

The breadth of acute ecotoxicity data available for a variety of species at 3 trophic levels may justify the use of an assessment factor of 100 applied to the lowest EC_{50} value to generate a PNEC, rather than the standard factor of 1,000 (TGD, p. 330). Applying this assessment factor to the lowest EC_{50} value for a freshwater aquatic organism (184 mg/l for *Selenastrum capricornutum*), the $PNEC_{water}$ derived for the aquatic compartment would be 1.84 mg/l.

There was also chronic aquatic toxicity test data available for several species including a 72-h NOEC of 470 mg MTBE/l for *Scenedesmus subspicatus*, a 5-day NOEC for *Ceriodaphnia dubia* of 202 mg/l, an 21-d IC_{20} value of 42 mg/l for *Daphnia magna* and an 31-d IC_{20} value of 279 mg/l for *Pimephales promelas*. The *Ceriodaphnia* test encompassed several broods, but it is not the test species of choice for OECD daphnid chronic tests. The other two tests were carried out to ASTM and US-EPA protocols, rather than the OECD test protocols favoured by the TGD recommendations. An assessment of the full test reports showed that the *Daphnia* test conformed to OECD protocols, but the *Pimephales* test did not. Chronic test data were also available for the marine shrimp *Mysidopsis bahia*, where the lowest NOEC value of 26 mg/l was reported. There is no OECD test protocol for this type of test of juvenile growth, but an assessment of the test report showed that the protocol was similar to the OECD recommended protocol for chronic tests of daphnids.

Consequently, data from both the *Daphnia* chronic test, (NOEC for reproduction of 51 mg/l) and the *Mysidopsis* test (NOEC of 26 mg/l) data could be used to derive a PNEC. The US-EPA (2000) has set a preliminary ambient freshwater quality criterion of 51 mg/l for chronic effects based the *Daphnia* data. US-EPA (2000) further considered that valid chronic toxicity values were available for Daphnia, fathead minnow and algae although not all the tests conform completely to OECD test guidelines.

Conclusion

Since there is a wealth of acute toxicity data and some chronic data, derivation of a PNEC by application of an assessment factor of 10 to the lowest chronic (NOEC) value is justified for continuous release (chronic) situations. Thus, using the mysid chronic test data, a PNECaquatic of 2.6 mg/l is obtained, which is comparable to the PNEC derived from the acute data. Consequently, the EUSES risk assessment modelling was carried out using a continuous $PNEC_{water}$, value of 2.6 mg MTBE/l.

For intermittent release (acute) situations, the EC_{50} value for *Mysidopsis bahia* of 136 mg/l was used for EUSES with an assessment factor of 10, producing an intermittent $PNEC_{water}$ of 13.6 mg MTBE/l.

3.2.1.5 Derivation of PNEC for sediment life

In the absence of any ecotoxicological data for MTBE effects on sediment-dwelling organisms, the $PNEC_{sed}$ was provisionally calculated from PNECaquatic using the equilibrium partitioning method according to the following formula (TGD, p.335):

$$PNEC_{sediment} = K_{sed\text{-}water}/\rho_{sed} \times PNEC_{water} \times 1{,}000 \qquad \text{(Eq. 21)}$$

where $PNEC_{water}$ = predicted no effect concentration in water (mg/l)
ρ_{sed} = bulk density of wet sediment (1,300 kgww/m^3)
$K_{sed\text{-}water}$ = partition coefficient sediment water (m^3/m^3) = Foc_{sed} x Koc, where Foc is the fractional organic carbon content of the sediment
$PNEC_{sediment}$ = predicted no effect concentration in sediment (mg/kgww)

The $PNEC_{sediment}$ calculated by EUSES according to the TGD guidelines was 2.05 mg MTBE/kgww.

3.2.1.6 Derivation of PNEC for STP organisms

Although part of the aquatic protection goal of EUSES, the effects in STP organisms were separated from those of truly aquatic species. Two values were available in the HEDSET for effects on a specific species, *Pseudomonas putida*. These were an 18-h EC_{10} of 700 mg/l (in accordance with DIN 38412 Part 8) and a 5-h EC10 > 1,480 mg/l (using a Hüls in-house test procedure for the inhibition of oxygen consumption), both in accordance with GLP (Hüls, 1991d,e). As the long-term test protocol did not include monitoring of the MTBE concentration at the beginning and end of the test, some loss of MTBE may have occurred. However, the short-term test was carried out in a closed system where losses of MTBE could not have occurred and, as was expected, this produced an EC_{10} of more than twice that from the long-term test. The result from the long-term test therefore appears to be valid. In order to adopt a cautious approach, the longer-term value of 700 mg/l was input to EUSES for the risk assessment.

The $PNEC_{micro\text{-}organisms}$ derived for STP micro-organisms was 700 mg MTBE/l, while the assessment factor used was 1.

3.2.2 Atmosphere

There are no data on possible effects on environmental organisms exposed to airborne MTBE. A PNEC for the atmospheric compartment could not be derived in the same way as with the other compartments (TGD Section 3.7, p. 340 - 342).

The photochemical ozone creation potential (POCP) of MTBE was estimated to be 15.2, relatively low when compared to ethylene (100), which is used as a reference (Derwent *et al*, 1998).

3.2.3 Terrestrial compartment

No reliable experimental data were available for the terrestrial compartment. Although a 14-day LC_{50} (mortality) value could be estimated for the earthworm of 1,060 mg/kgww for baseline toxicity using a QSAR from the US-EPA ECOSAR software (this software used a QSAR for earthworms that had been derived from data produced by Neuhauser *et al*, 1985,1986), this derivation was not utilised. Thus no experimental data were used and EUSES calculated a PNEC value using the partitioning approach:

$$PNEC_{soil} = (Ksoil\text{-}water/\rho_{soil}) \times PNEC_{water} \qquad \text{(Eq. 22)}$$

where $PNEC_{water}$ = predicted no effect concentration in water (mg/l)
ρ_{soil} = bulk density of soil (1,700 g/l)

The $PNEC_{soil}$ derived by EUSES for terrestrial life was 0.732 mg MTBE/kgww.

3.2.4 Non-compartment-specific effects relevant to the food chain

As explained in Section 3.1.0.6, MTBE is not expected to bioaccumulate and hence no effects through the food chain are expected.

The $PNEC_{oral,\ worm/fish}$ derived by default for secondary poisoning of birds and mammals by EUSES was 100 mg MTBE/kgbw

3.3 Risk characterisation

3.3.0 Introduction

To evaluate the effects data (Section 3.2) with the exposure data (Section 3.1), EUSES calculates PEC/PNEC ratios (RCRs) and MOS for the different protection goals. In general, a RCR of less than one or a MOS of greater than one implies that the level of exposure is lower than the no effect level and hence there is no inherent risk resulting from that level of exposure for that substance. An acceptable result from a risk assessment of a substance (using EUSES) would ideally have all the RCRs less than one and all of the MOS greater than one. When there are large uncertainties associated with the risk assessment process, for example due to the absence of monitoring data or key ecotoxicity data, then a further margin of comfort may be desirable (TGD, p. 356-358).

3.3.1 Aquatic compartment

The RCRs for the aquatic compartment (including sediment) are shown in Table 3.28. These are estimated using the aquatic PECs during an emission episode. RCRs > 1 are underlined.

Table 3.28: RCRs for the aquatic compartment

Scenario	Use Pattern	Water	Sediment
Local			
Production	1	0.453	0.562
Formulation	1	0.453	0.562
Processing	1	0.241	0.299
Private use	1	0.0243	0.0301
Processing	2	1.45	1.8
Processing	3	0.47	0.583
Regional		0.00128	0.00126

3.3.1.1 Use as a component in gasoline (Use Pattern 1)

The RCR results for MTBE used as a fuel component obtained using realistic worst-case estimates of emissions to air and wastewater from production and formulation are all substantially less than one, except for the production local water where the value is about 0.5 and sediment where it is about 0.6. Since no sediment toxicity test data was available, the RCR for the local sediment scenario is based on $PNEC_{sediment}$ values estimated from the $PNEC_{water}$. Consequently it may be prudent to carry out sediment toxicity tests to derive an independent $PNEC_{sediment}$ value.

3.3.1.2 Use in the synthesis of high purity isobutylene (Use Pattern 2)

The largest RCR values (and the only ones > 1) for water and sediment are for MTBE used as an intermediate for isobutylene production for the processing part of the life-cycle. Both of these values are derived in EUSES from the local PECs during emission episodes (3.77 mg MTBE/l and 3.7 mg MTBE/kgww for surface water and sediment respectively) and the PNEC for surface water and sediment (2.6 mg/l and 2.05 mg/kgww respectively) and so represent a worst-case discharge. As stated in Section 3.1.1.3, the annual average surface water PEC is only 3.1 mg MTBE/l, which would produce an RCR of 1.19 for surface water rather than the 1.45 value.

These estimates are based on an emission factor calculated from a production plant showing high emissions of MTBE to wastewater and are therefore a realistic worst case. Monitoring of production plant wastewaters where MTBE is used to produce isobutylene, using specific methods of analysis and sampling programmes conducted over an appropriate time period, would produce more realistic estimates of MTBE concentrations from this scenario.

Since no sediment toxicity test data were available, the RCR greater than one for the local sediment scenario, is based on $PNEC_{sediment}$ values estimated from the $PNEC_{water}$. Consequently it may be prudent to carry out sediment toxicity tests to derive an independent $PNEC_{sediment}$ value.

3.3.1.3 Use as a high-purity solvent (Use Pattern 3)

These RCR tables show that for use of MTBE as a solvent, the risk characterisation performed using EUSES produces RCRs that are less than one.

3.1.4.4 Sewage treatment organisms

All the RCRs for STP organisms, calculated using EUSES, are substantially less than one (Table 3.29).

Table 3.29: RCRs for STP organisms

Scenario	Use Pattern	RCR
Production	1	0.0168
Formulation	1	0.0168
Processing	1	0.0089
Private use	1	0.000853
Processing	2	0.0538
Processing	3	0.0174

3.3.2 Atmosphere

In the absence of a $PNEC_{air}$, no risk characterisation for the atmospheric compartment could be made (Section 3.2.2).

3.3.3 Terrestrial compartment

The risk characterisation ratios for the terrestrial compartment, calculated using EUSES, are all substantially less than one (Table 3.30).

Table 3.30: RCRs for the terrestrial compartment

Scenario	Use Pattern	RCR
Local		
Production	1	0.0336
Formulation	1	0.0336
Processing	1	0.0208
Private use	1	0.00184
Processing	2	0.1
Processing	3	0.0319
Regional		0.000416

3.3.4 Non-compartment-specific effects relevant to the food chain

The risk characterisation ratios (RCRs) for non-compartment-specific effects relevant to the food chain, calculated using EUSES, are all substantially less than one (Table 3.31).

Table 3.31: RCRs for predators through the food chain

Scenario	Use Pattern	Worm-eating predators	Fish-eating predators
Production	1	0.0000657	0.00773
Formulation	1	0.0000657	0.00773
Processing	1	0.0000537	0.0048
Private use	1	0.00000646	0.000527
Processing	2	0.00016	0.0246
Processing	3	0.0000502	0.00616

3.3.5 Total MOS for man for indirect exposure (all routes)

Indirect exposure of humans via the environment may occur through consumption of food and drinking water, inhalation of air and ingestion of soil. Exposure via soil ingestion and dermal contact is not addressed because this represents significant exposure routes for specific situations of soil pollution, and is therefore not appropriate for a generic exposure scenario.

Human behaviour shows an appreciable amount of variation among the different EU countries; within countries, there may also be large deviations among individuals. As a consequence, indirect exposure will vary greatly among the population to be protected. The choice of an exposure scenario will have a major influence on the result of the assessment. This choice will always be a compromise, as a scientifically-sound solution is extremely difficult to obtain.

Indirect exposure is principally assessed on two spatial scales: locally near a point source of the substance, and regionally using averaged concentrations over a larger area. In the local assessment, all food products are derived from the vicinity of one point source, while in the regional assessment, all food products are taken from the regional model environment. Clearly, the local situation represents a worst case. People do not consume 100% of their food products from the immediate vicinity of a point source. Therefore, the local assessment represents a situation that does not exist in reality. However, one or two routes usually dominate the total exposure, and local exposure via these routes may not be unrealistic.

In contrast, the regional assessment represents an average exposure situation that cannot ensure protection of individuals who consume food products from the vicinity of point sources. A regional assessment indicates potential average exposure of the inhabitants of the region. In the light of the above limitations, it is clear that a generic indirect-exposure assessment, as required in this framework, can only be used to indicate potential problems. The assessment should be seen as a helpful tool for decision-making and not as a prediction of human exposure actually occurring at a specific place or time.

Assessment of indirect exposure via the environment comprises the following steps:

1. Assessing the concentrations in intake media (food, drinking water, air). The concentration in food is estimated from concentration in water, soil and air (from the distribution module) and bioconcentration or bioaccumulation behaviour. The exposure assessment in EUSES includes seven pathways: drinking water, fish (from surface water), root and leaf crops (from agricultural soil), meat and milk (from cattle grazing on grassland), and inhalation of air.
2. Assessing the intake rate of each medium (using a standard consumption pattern).
3. Combining the concentrations in the media with the intake of each medium, using a standard exposure scenario. The total daily intake of humans is estimated by multiplying these concentrations and the daily intake rate of each medium, and summing the contribution of each medium (Section 4.1.1.3). Each of these intake media is retrieved exclusively from within the contaminated system.

The MOS values produced for total exposure via the environment by indirect routes are shown and discussed in the Section 4.1.3.3.

4. HUMAN HEALTH

4.1 Human health (toxicity)

4.1.1 Exposure assessment

4.1.1.0 General discussion

The assessment in this section considers the following exposure scenarios: (i) occupational exposure (of workers), (ii) consumer exposure (of humans exposed through vehicle refuelling) and (iii) indirect exposure of humans via the environment. This last category includes exposure to MTBE from concentrations in the ambient air, and also exposure that may result from water containing MTBE. This may be in terms of drinking water, or contact with MTBE in bathing waters or recreational waters. Exposure through food is also considered.

Data on worker exposures to MTBE are available for different phases of MTBE production, handling, distribution and use as a fuel additive (Section 4.1.1.1). Consumer exposure has been measured at gasoline stations (Section 4.1.1.2). The data reviewed here relate 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 reviewed in the later section on human effects (Section 4.1.2). Data from a survey made by Concawe (2000) are also included. No measured data are available for exposure by man to MTBE as a solvent (minor use); therefore estimations of exposure for this use scenario were carried out using EUSES defaults alone.

Where reliable data for measured MTBE concentrations in the different waters are available, these have been summarised. Any data of sufficient quality pertaining to ambient air concentrations have been similarly treated, and the data themselves have been provided in the earlier Sections 3.1.1 and 3.1.2. However, it should be noted that the final estimations of indirect exposure for man via the environment have been carried out using ambient concentrations estimated by EUSES, rather than the reported measured data. This is a recognised and transparent method of producing total estimated doses for man via all routes, where the quality or reliability of the measured data for individual components may be less than ideal. It was considered that at this time, given the limitations and uncertainties regarding true representative environmental data, the available measured data might best be utilised for validation of the EUSES estimated values.

4.1.1.1 Occupational exposure

The main route of exposure for workers who come into contact with MTBE or gasoline containing MTBE is inhalation.

The majority of jobs where exposure to MTBE is a possibility are gasoline-related. Due to its benzene content, gasoline is classified as a carcinogen according to EU regulations. As a result of the "Carcinogens at Work" Directive (EC, 1990), amongst other measures, a risk assessment is carried out for any task involving exposure to gasoline, and it can be reasonably anticipated that because of regulatory pressure, employers do take the necessary measures to reduce exposure from all sources. These include the use of personal protective equipment when necessary.

Although dermal exposure and subsequent absorption through the skin is theoretically possible, current work practices and recommended personal protective measures (see company safety data sheets) would render this unlikely. There are no data available providing rates of absorption of MTBE through the skin in man (Section 4.1.2.1). (This becomes less of a concern when working practices can be shown to be capable of and likely to exclude most potential exposure via this route). Dermal exposure is therefore not considered further for the purposes of the present risk assessment. The need to include dermal exposure may have to be reviewed again in the light of new data of sufficient quality becoming available. For example, developments in PBPK modelling may allow better predictions of dermal absorption and subsequent body distribution (Leavens *et al*, 2000).

Following the TGD (p. 37), dermal exposure could be estimated through the use of general models such as EASE (Estimation and Assessment of Substance Exposure) to produce a crude estimated value based on exposure via other routes. However, based on the current limited data and models, and given the small percentage which exposure by this route is likely to constitute for the majority of the worker population, no EASE calculations have been carried out.

During production and formulation

Personal exposure measurements have been made for different jobs at an MTBE manufacturing plant including jetty operations (i.e. loading and unloading of ships) (Table 4.1).

Table 4.1: Personal exposure levels during MTBE production in Europe

Job type	Year of sampling	Sampling time (min)	Number of samples	Concentration (mg/m^3)		Reference
				Range [a]	8-h TWA [b]	
Production operator	1993	NS	7	0.04 - 3.71	0.86	Arco Chemical Europe, 1995
- Emptying	1993	NS	13	0.04 - 3.95	0.63	Arco Chemical Europe, 1995
- Start-up	1993	NS	6	0.14 - 7.92	2.12	Arco Chemical Europe, 1995
Plant operator	1981-1995	100 - 390	10	0.08 - 19	2.4	Concawe, 1997 [c]
Plant operator	1981-1995	100 - 390	5	< 3.6	< 3.6	Concawe, 1997 [c]
Sample room operator	1993	350	2	5.76 - 11.16	8.64	Arco Chemical Europe, 1995
Jetty operator	1993	325 - 540	6	0.72 - 20.5	11.88	Arco Chemical Europe, 1995
Jetty operator	1981 - 1995	100 - 390	2	45 - 46	46	Concawe, 1997 [c]

[a] Measured

[b] Average adjusted to 8-h working day

[c] As cited by IARC, 1999

In each case, the operator was equipped with a small charcoal sampling tube fixed close to his breathing zone. The sampling tube was connected to a pump to collect the air contaminated by MTBE and other VOCs. The samples were analysed subsequently according to NIOSH method 1615 (NIOSH, 1994).

Ship loading (at the jetty) is changing from open to closed loading, whereby the gasoline vapour is vented at a high level or at high velocity rather than at deck level.

Personal exposure data from short-term loading operations at production sites were obtained in the same way (Table 4.2). The measurements were adjusted to a 15-min interval and these values were considered short-term peak exposures.

Table 4.2: Personal exposure measurements during loading in Europe

Job type	Year of sampling	Sampling time (min)	Number of samples	Concentration (mg/m^3)		Reference
				Range [a]	Peak [b]	
Jetty operator	1993	12 - 55	7	47.9 - 205.6	106.6	Arco Chemical Europe, 1995
Other loading	1993	14 - 25	7	48 - 153.8	119.5	Arco Chemical Europe, 1995
Other loading	1993	3 - 56	5	62 - 140.1	100	Arco Chemical Europe, 1995
Other loading	1993	17 - 40	5	62.1 - 228.5	122	Arco Chemical Europe, 1995
Other loading	1993	2 - 13	3	1.4 - 31.8	13.0	Arco Chemical Europe, 1995
Plant operator	1981 - 1995	15 - 40	3	< 3.6	< 3.6	Concawe, 1997 [c]
Check tank levels	1981 - 1995	15 - 40	2	1.2 - 4.2	2.7	Concawe, 1997 [c]
Hose disconnection	1981 - 1995	15 - 40	2	8.3 - 38	23	Concawe, 1997 [c]

[a] Measured

[b] Mean value adjusted to 15-min interval

[c] As cited by IARC, 1999

Loading activities, which may be associated with relatively high exposure levels (for gasoline itself, not purely MTBE), are expected to change in the near future. Indeed due to regulatory measures requiring vapour recovery during roads tanker loading, loading activities are changing from top-loading to bottom-loading. The higher values reported in Table 4.2 may have arisen from top-loading procedures and therefore it is possible that these older data may no longer be representative. Though at present, this cannot be confirmed. Whilst some top loading is still carried out, it is scheduled to be completely phased out by 2004.

Concawe (1997) reported personal monitoring data available from the petroleum industry including exposures to the product MTBE and formulated MTBE, i.e. blended into gasoline. The average personal exposure level ranged from 3.6 to 45.5 mg/m^3 over time periods of 100 to 390 minutes (equivalent to 9.5 mg/m^3, 8 h TWA) for MTBE products. Exposure levels for MTBE from gasoline ranged from 0.3 to 85.6 mg/m^3 (maximum 162 mg/m^3) over time periods of 2 to 599 minutes. Area sampling of the workplace for MTBE in gasoline revealed levels of 0.2 to 18 mg/m^3 over a period of 23 to 480 minutes.

During distribution and handling of gasoline containing MTBE

Hakkola and Saarinen (1996) measured personal exposure levels of workers during handling of gasoline 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 during subsequent distribution to related service stations. The Northern depot used bottom loading of road cars, equipped with a vapour recovery system, as opposed to the top loading of road cars without vapour recovery in the Southern depot. The tanker drivers were handling one to four loads per day, more than half containing gasoline with MTBE, and were monitored during loading and unloading operations (Table 4.3). No individual data were provided for the service stations and no further distinctions in the results can be made.

Table 4.3: Exposure to MTBE during loading/unloading of gasoline containing 10 to 15% MTBE (Hakkola and Saarinen, 1996)

Operation, location	Sampling time (min)		Number of Samples	Concentration (mg/m^3)		Vapour recovery
	Mean	Range		Mean	Range	
Bottom loading of road cars, Northern depot	28	15 - 40	6	13	2.8 - 42.0	Yes
Delivery at service station, North	33	22 - 44	5	16	4.3 - 27.0	NS
Top loading of road cars, Southern depot	20	10 - 30	4	91	20 - 226	No
Delivery at service station, South	26	10 - 37	6	71	10 - 98	No

NS Not stated

In a similar study, Saarinen *et al* (1998) recorded personal exposure levels of tanker drivers in different parts of Finland. The tankers used gasoline blended with 11 to 12% MTBE (and other ethers such as *tert*-amyl methyl ether). Three depots were involved, all of which had vapour recovery systems, however none of the service stations did so. The aim of the study was to monitor exposure (via inhalation) and to ascertain the usefulness urinary metabolites as exposure biomarkers. Concentrations in air were determined using personal samplers, and metabolites were analysed in urine. The mean concentration of MTBE during a shift was 8.1 ± 8.4 mg/m^3, with exposure occurring 3 times per shift for periods of 21 ± 14 min on each occasion. These values would seem to be significantly lower than those presented by Hakkola and Saarinen (1996) for operator exposure. With regard to biomarkers, it was noted that urinary MTBE levels performed well as a biomarker for exposure; however, the authors concluded that the level of MTBE exposure was so low, that other metabolites such as TBA did not perform so well.

McKee *et al* (1997) 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, 8-h TWA).

At garages and service stations

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.

Many measurements were collected in the course of the population studies described in Section 4.1.1. These measurements were carried out by the US Centre for Disease Control and Prevention (CDC, 1993b,c). Representative data from these studies are summarised in Table 4.4. To simplify the presentation, Table 4.4 has been limited to data for car mechanics and service station attendants. Job categories that could not be clearly defined in relation to possible exposures to gasoline and MTBE have been excluded, e.g. foremen, chauffeurs, plant managers.

Table 4.4: Personal exposure at garages and service stations in the USA

Number of samples	Concentration (mg/m^3)		Reference
	8-h TWA	Range	
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	
7	0.44	< 0.1 - 1.62	CDC, 1993b
5	0.96	0.43 - 1.66	
3	0.35	< 0.14 - 0.54	
5	0.11	< 0.1 - 0.5	CDC, 1993c
4	0.1	< 0.1 - 0.1	
3	0.1	< 0.1 - 0.1	
4	0.45	< 0.1 - 1.19	CDC, 1993b
4	3.47	0.83 - 7.56	
Service station attendant			
10	2.98	0.5 - 6.82	API, 1991 [b]
10	1.08	0.36 - 3.38 [a]	

[a] One high value (43.34 mg/m^3) has been excluded

[b] Hazard Evaluation and Technical Assistance (HETA) data

Two of the studies reviewed measured exposures in regions of the USA 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 USA where MTBE was present in gasoline at levels > 11% (CDC, 1993b,c; API, 1991). Personal breathing zone samples were collected over a normal day period and analysed, and 8-h TWA concentrations calculated. Where MTBE levels in gasoline were low, exposure for car mechanics was in the range of < 0.1 to 1.62 mg/m^3; in regions with higher levels, exposures ranged from < 0.1 to 1.66 mg/m^3.

Hartle (1993) compared exposure among personnel at two different types of service station with high volume MTBE use (12.5 - 13%) in the USA: two stations with advanced vapour recovery systems, and two stations without this facility. The high volume MTBE % results ranged from 0.14 to 13.97 mg/m^3 (average 1.08 mg/m^3), while those of the vapour recovery station, the results ranged from 0.07 to 2.63 mg/m^3 (average 0.5 mg/m^3). These results showed that the presence of vapour recovery systems have a major impact on the potential levels of exposure of service station attendants to MTBE.

These studies were carried out to a reasonable technical standard, although jobs were often poorly defined. It is also unclear to the ECETOC 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.

Occupational exposure limit values

The USA and some EU countries have established occupational exposure limit (OEL) values for MTBE (Table 4.5). OEL values exist for other Scandinavian countries, but these are believed to be currently under revision.

Table 4.5: Occupational exposure limit values

Country	TWA [a] (ppm)	(mg/m^3) [c]	STEL [b] (ppm)	(mg/m^3) [c]	Reference
Finland	50	180			HTP-arvot, 2000
Germany	50	180	100 [d]	360 [d]	DFG, 2000
Italy	50	180			ACGIH, 2000
Netherlands	50	180	100	360	Ministerie van SZW, 2000
Spain	40	147			INSHT, 2001
Sweden	50	180	75	250	AFS, 1996
UK	25	92	75	275	UK-HSE, 2000
USA	40	144			ACGIH, 2000

[a] Time-weighted average concentration (8-h working period)

[b] Short-term exposure limit (15-min average, unless specified otherwise)

[c] Official values; some countries use different conversion factors and/or other ambient temperature

[d] 10-min peak

Evaluation

Production of MTBE is associated with low personal exposure levels (< 3 mg/m^3, 8-h TWA), except for sampling and loading which may result in relatively high exposures (8 - 46 mg/m^3, 8-h TWA). Peak exposures during loading ranged from 1 to 228 mg/m^3. When adjusted to 15 minutes, the maximum short-term peak exposure was around 120 mg/m^3.

Whilst some top loading of road tankers is still carried out, it is scheduled to be completely phased out by 2004. Similarly, ship loading is changing from open to closed loading, whereby the gasoline vapour is vented at a high level or at high velocity rather than at deck level.

Maximal worker exposures of up to 9.5 mg/m^3 (8-h TWA) occurred during production and formulation of MTBE in the European petroleum industry, although limited details are available for sampling and analysis. Peak exposures of up to 162 mg/m^3 have been measured. These values are comparable to those from the gasoline industry in the USA, where the mean and range of personal worker exposure results are similar.

High peak exposures (up to 71 mg/m^3) were reported for top loading of gasoline road cars without vapour recovery system during distribution and handling of gasoline containing MTBE (11 - 15%) in Finland. Extrapolation from these Finnish data to other types of gasoline (where MTBE is present at low concentrations, primarily as an octane booster) and other regions in Europe (with different loading/unloading systems) is not feasible. Considerably lower exposure levels (from 0.71 to 0.26 mg/m^3) were reported for employees exposed to MTBE from oxygenated gasoline during bulk storage, maintenance, and also during dispensing by service station attendants in Italy. Levels reported in the USA were higher with mean exposures to MTBE of 3.5 mg/m^3. The highest individual value obtained was 7.56 mg/m^3.

4.1.1.2 Consumer exposure

The most likely source of consumer exposure to MTBE vapour arising from gasoline by evaporation appears to be car refuelling at service stations. The levels of MTBE recorded appear to be station-specific and dependent on many factors, including amount of gasoline sold, wind speed, exhaust emissions from passing traffic and deliveries of gasoline to the station (Vainiotalo *et al*, 1998).

Specific data have been obtained for the following three consumer exposure situations: (i) at the service station perimeter, (ii) at the pump island, and (iii) in the customer breathing zones, using personal samplers.

At service station perimeter

Vainiotalo *et al* (1996 cited in IPCS, 1998) reported MTBE concentrations of 0.5 to 121 μg MTBE/m^3 at the perimeter of two service stations (urban and rural). The author noted that the measurements were dependent on factors such as wind speed, the amount of fuel sold and/or delivery frequency, and the amount of traffic on nearby roads.

Thirteen subjects living near to service stations in Frankfurt am Main (Germany) were monitored for 24 hours in the summer of 1996, using personal samplers to record levels of MTBE; 6 subjects who did not live in the vicinity served as controls. The geometric mean concentration was 3.8 μg MTBE/m^3, with a maximum of 9.9 μg/m^3. Both levels were lower than those for the controls (mean 8.4 μg/m^3, maximum 20.1 mg/m^3), the latter reportedly following 20 hours of driving in a car. Moreover, crucial factors such as the distance between the station and the houses were not recorded. Therefore these results are of questionable significance (Heudorf *et al*, 1998).

At pump islands

Vainiotalo *et al* (1996 cited in IPCS, 1998) measured the concentration of MTBE in air surrounding pump islands in Finland. The levels ranged from 247 to 1,347 μg/m^3. A further study (Vainiotalo *et al*, 1999) reported mean concentrations of 70 to 160 μg/m^3 for the area around pump islands in Helsinki. Both studies were carried out over an 8-hour sampling period. As with the perimeter values, the concentrations were heavily dependent on factors, such as wind speed, nearby traffic volume and temperature.

In Italy, measurements were made at the pump island by Giacomello (1996 cited by IPCS, 1998). The geometric mean (535 samples) ranged from 38 to 108 μg MTBE/m^3, with an overall range of 0 to 2,533 μg/m^3. The amount of MTBE present in the fuel in this Italian study was between 2.1 and 2.7%, i.e. considerably lower than the usual amount used in Finland.

Some data from the USA are available for pump island measurements; these tend to be in the range 200 to 900 μg MTBE/m^3 (IPCS, 1998). The sample sizes were small, and the situation regarding leakage of fuel at USA service stations and percentage of MTBE within that fuel does not allow reasonable comparison of estimated exposure.

In the customer breathing zone

Exposure in the breathing zone of customers was measured at two self service stations in the south west of Finland. To determine exposures during tank filling, air samples contaminated by gasoline vapours (containing up to 11% MTBE) were collected by holding a sampling tube connected to a pump and a charcoal tube in the customer's breathing zone for the duration of the filling. Samples were collected at each station during 4 days in summer and winter (Vainiotalo *et al*, 1996) (Table 4.6).

Table 4.6: Breathing zone measurements of MTBE during tank filling *(Vainiotalo et al, 1996)*

Duration of filling(s)		Number of samples	Concentration (mg/m^3)	
Mean	**Range**		**Mean**	**Range**
Summer				
71	25 - 210	77	7.5	< 0.2 - 131
70	25 - 242	76	4.3	< 0.2 - 203
Winter				
58	21 - 275	80	7.4	< 0.4 - 138
60	23 - 150	80	6.0	< 0.2 - 245

Resulting values ranged from 0.2 to 245.0 mg MTBE/m^3 with an overall geometric mean concentration of 6.0 mg/m^3 calculated for 1-minute refuelling. There was a wide range in the individual values obtained, but the overall mean short-term exposures were remarkably similar (6.0 - 7.5 mg/m^3 for approximately 1 minute). These data provide a reasonable insight into probable consumer exposure at the breathing zone during car refuelling.

The breathing zone of 40 randomly chosen customers at two gasoline service stations was monitored for hydrocarbons and MTBE. The stations were fitted with either a Stage I or Stage II vapour recovery system. (With Stage I equipment vapours are recovered during delivery; Stage II includes additional recovery measures during refuelling). The mean MTBE concentration during refuelling at the station with Stage I system was 15.3 mg MTBE/m^3 (range 1.8 - 74 mg/m^3), while at the Stage II station it was lower at 3.4 mg/m^3 (0.2 - 16 mg/m^3). This demonstrates the impact of improved vapour recovery systems on potential customer exposure (Hakkola and Saarinen, 2000).

Evaluation

Data for concentrations of MTBE in air recorded around the perimeter of service stations in Europe are both limited and highly variable. Despite this, they are of value, as they provide some information on the degree of dispersal of vapours from the pump island, and have been used to estimate the upper range of values for residential exposure in nearby dwellings. This can only be taken as a rough worst-case exposure, due to a number of environmental and other factors which impact on the concentrations reaching the perimeter of any station.

4.1.1.3 Indirect exposure via the environment

It is important to be aware that the majority of the information in this section for measured concentrations of MTBE in water and in air comes from Finland and the USA, where the percentage of MTBE used in fuel is significantly higher than that which is used in the rest of the EU (Tables 2.1 to 2.4). This means that the data may not be truly representative for the European situation and real exposure is likely to be lower than these data would suggest.

Exposure via ground and surface water

MTBE has been found, usually at low concentrations, in ground and surface waters (predominantly in those shallow ground waters underlying urban areas) (Tables 3.5 to 3.8). Typically it enters these waters as a result of leakage or spills of fuel. There may be both point and non-point sources; point sources include LUSTs, pipelines and accidental spills, while non-point sources include the partitioning of MTBE into precipitation and run-off. MTBE is relatively persistent in natural waters (Section 3.1.0.6) and so may remain in these waters long enough to provide a means by which the human population might become exposed. It should be borne in mind, however, that monitoring data and exposure data are not the same, i.e. concentrations of MTBE in water do not necessarily equate to exposure levels. True exposure levels for man are determined by a number of different factors, of which measured concentration in the source is one. The concentrations allow an estimate to be made, other factors having been taken into account.

Current available monitoring data (largely for the USA) for these two potential sources are provided in Section 3.1.1.

Exposure via drinking water

In general, monitoring data for MTBE in drinking water sampled from municipal distribution systems are limited. Some data have been published recently for concentrations of MTBE in drinking or potable water in the USA, Finland and Denmark, although these data reflect a mixture of 'clean' sites and sites that have suffered from known local contamination. Monitoring for MTBE is not a federal requirement in the USA and data are thus available for only a few individual States. Based on the data recorded by the US-EPA for New Jersey, Iowa, Colorado, Illinois and Texas, 51 public water systems had MTBE concentrations above the limit of detection, but usually below 20 µg/l (Zogorski *et al*, 1996; US-NTP, 1998). For uncontaminated water supplies the value is likely to be less than 0.1 µg/l across much of Europe.

The following information has been produced by the California Department of Health Services (California EPA, 2001) regarding reported MTBE detections in California drinking water (1990 to present). In 2,350 public water systems sampled 44 (1.9%) MTBE detections were recorded, of which 24 (1.0%) were > 5 μg/l and 13 (0.6%) were > 13 μg/l. Of 8,565 drinking water sources sampled, 72 (0.8%) MTBE detections were recorded, of which 39 (0.5%) were > 5 μg/l and 19 (0.2%) were > 13 μg/l. About 60% of ground water sources and surface water sources have been sampled overall. The 2,350 systems collectively serve nearly 30.5 million people, about 90% of the state's population of 34 million. MTBE is considered "detected" if present in at least two samples from a source. It is interesting to note that the detection of MTBE in Californian drinking water supplies (rather than sources) is only 1.9% of the total number sampled and less than 1% have concentrations above the odour threshold concentration (California EPA, 2001).

Where there has been monitoring following known contamination by MTBE in gasoline, much higher levels have been recorded; values have been reported in excess of 100,000 μg/l in the USA, and mean values of 330,000 μg/l were recorded at a contaminated site following a spill of gasoline in Finland (IPCS, 1998). Potable water and ground waters in the vicinity of service stations have been studied in Finland and in the USA for MTBE and there is a large amount of variation within the data produced. Some of the MTBE levels are very high (up to hundreds of mg/l). These are not representative of the background drinking water concentrations supplied to the human population, however, since the water would be unpalatable due to the low taste and odour threshold which this compound possesses (see below under *Organoleptic considerations and standards*). At concentrations above around 20 μg/l the organoleptic properties of MTBE generally render drinking water unacceptable for human supply and consumption.

Organoleptic (taste and odour) considerations and standards

As outlined above (*Exposure via drinking water* and Section 1.2.5), MTBE has a very low taste and odour threshold of 20 to 50 μg/l. Vetrano (1993) reported an odour detection threshold in water of 95 μg/l and a taste threshold of 134 μg/l. In the UK, Young *et al* (1996) used taste and odour panels to investigate thresholds for a range of substances including MTBE. The geometric mean for detection in this study was 34 μg/l but the lowest detected concentration was 15 μg/l, with no detection at a level of 6.25 μg/l. It should be noted that these threshold values do not reflect acceptability but merely the ability to detect the substance. By convention, the geometric mean of measured odours is used to provide guidance on targets for water acceptability and, all other factors being equal, it provides a sound basis for the comparison of studies. However, there is considerable variation within the human population for sensitivity in detecting this compound. A more conservative approach was taken by Du *et al* (1998) who stated that a concentration of 40 μg/l would be unlikely to be detected by the majority of the population. The "secondary" standards and advisory values based on taste and odour, which have been proposed and/or established for MTBE, take into account this inter-individual variation in detection.

It is universally agreed that any potential health effects in man resulting from exposure to MTBE in water would occur only at levels far in excess of those which produce a detectable odour. The US EPA suggested that the setting of a Secondary Maximum Contaminant Level (SMCL) of 15 μg/l to "avoid aesthetic non-health effects in drinking water supplies" could be supported based on the results of a panel study commissioned by the Oxygenated Fuels Association in the USA (Pirnie, 1998). The US-EPA has set a Health Advisory [a] value of 20 to 40 μg/l for MTBE based on aesthetic (taste and odour) parameters on the assumption that this will provide a significant margin of safety for the consumer.

The 1996 Young *et al* study led to the suggestion by Fawell and Young (1997) that a guidance value of either 10 or 34 μg/l would be appropriate for use in protection against taste and odour effects (depending on certain considerations applied to the dataset). The adoption of a guidance value as opposed to a standard is preferred on the grounds that there are too many uncertainties surrounding the detection versus the acceptability of odour, and also in relation to the potential impact of environmental ("real world") factors such as temperature (especially for a volatile substance such as MTBE) and other potentially masking substances (such as chlorine) which could be present in a residential setting. This approach is favoured by WHO, the EU and also by the Australian and Canadian governments, who prefer the flexibility of scientifically-considered guidance values or recommendations ("advisories") alongside health-based considerations. The only organisation known to set actual standards to govern taste and odour in water is the California EPA in the USA. This body has recently established a SMCL of 5 μg/l for MTBE in water (OEHHA, 1999).

All these values stem from the low taste and odour threshold for MTBE in water and from a desire to limit the unacceptability of drinking water (and thus consumer complaints). However, it is clear that because MTBE has such a low organoleptic threshold with a distinct taste and odour imparted to water at concentrations considerably below levels which may produce effects in man, the low taste and odour thresholds for MTBE provide an effective safeguard for the health of the consumer.

[a] The US-EPA Health Advisory values are set on the basis of non-carcinogenic effects only.

Exposure via ambient air

Human exposure to MTBE in ambient air is considered in this report to be exposure that occurs only in urban background air. MTBE in the air within or surrounding service stations and pump islands is considered in Section 4.1.1.2. There are sampling data on MTBE levels in ambient urban air from countries such as Canada and the USA; with the exception of data from Finland and one or two other reports, no such data exist for the majority of European countries. While these existing data are useful in establishing a link between use of MTBE and ambient urban air concentrations in these locations, the true picture for the majority of the EU is likely to be somewhat different with estimated ambient air concentrations being considerably lower. More data are needed to confirm this.

Based on the reported data from the USA and Canada, the general range of values for MTBE concentrations in ambient urban air in these locations is from 0.5 to 3 $\mu g/m^3$. More details of the studies from which this information is derived are provided in Section 3.1.2.

There have been some reports of ambient air concentrations taken at service stations (Section 4.1.1.2). It is worth noting, however, that whereas the pump island data from these reports are considered to be a "microenvironment", the data that may be derived from sampling at the perimeter of the stations have been used by some to represent the upper range of ambient air concentrations in urban neighbourhoods. Where such data exist they have been included in Section 4.1.1.2 of this report.

Some data for MTBE levels have been reported in relation to commuting situations, i.e. concentrations for the in-cabin levels during set periods that included refuelling stops. Lioy *et al* (1994) recorded micro-environmental in-cabin MTBE concentrations for a 60-minute journey which ranged from 3.6 to 576.6 $\mu g/m^3$ with a geometric mean of 21.6 $\mu g/m^3$. The highest values were found to be associated with an older vehicle that had significantly higher evaporative emission rates. This study was performed in New York, New Jersey and Connecticut where MTBE used in fuel is 10 to 15% by volume. Rhodes *et al* (cited in IARC, 1999) measured in-vehicle concentrations of MTBE (% use in fuel unknown) in roadways over 2 hours, and produced results of 3 to 90 $\mu g/m^3$. Vayghani looked at the concentrations of MTBE that could be recorded within vehicles while they were refuelling over the winter months when RFG was being used. A geometric mean value of 432 $\mu g/m^3$ was reported (cited in IARC, 1999). Additional data are provided in Table 3.13 for measured emissions of MTBE to air from vehicle exhausts.

EUSES indirect exposure estimation

The exposure of man via the environment is assessed by estimating the total daily intake of a substance in food, drinking water and air at both the local and the regional scale. A summary description of the approach used in EUSES for Indirect Exposure Estimation for humans exposed via the environment is given in Section 3.3.6 and a full description of the procedure is given in Section II 5.2 of the EUSES model documentation. The total intake via air is converted to an external oral dose by route to route extrapolation and taken together with estimated intakes via food and drinking water. Data used in the estimation of MTBE in food, water and air intakes as predicted using EUSES are presented in Table 4.7 for the use of MTBE as a fuel additive (Use Pattern 1), with use as a production intermediate for isobutylene, and as a speciality solvent as Use Patterns 2 and 3, respectively.

Table 4.7: Daily human intake (µg/kgbw/d) of MTBE through various media, calculated by EUSES for the regional and local scenarios

Medium, Fraction (%) of total daily intake	Regional	Production Use Pattern 1, fuel additive	Formulation Use Pattern 1 fuel additive	Processing Use Pattern 1, fuel additive	Processing Use Pattern 1, private use	Processing Use Pattern 2, isobytylene production	Processing Use Pattern 3, solvent
Drinking water	0.095	27.7	27.7	17.2	1.80	88.5	22.1
%	1.5	41	41	30	21	54	49
Fish	0.0087	2.53	2.53	1.57	0.17	8.09	2.01
%	0.1	3.8	3.8	2.7	1.9	5.0	4.4
Leaf crops	0.019	0.11	0.11	0.11	0.019	0.19	0.062
%	0.3	0.2	0.2	0.2	0.2	0.1	0.1
Root crops	0.0064	0.16	0.16	0.13	0.010	0.40	0.12
%	0.1	0.2	0.2	0.2	0.1	0.2	0.3
Meat	0.000013	0.00026	0.00026	0.00019	0.000025	0.00071	0.00019
%	0.0002	0.0004	0.0004	0.0003	0.0003	0.0004	0.0004
Milk	0.00025	0.0048	0.0048	0.0035	0.00046	0.013	0.0035
%	0.004	0.007	0.007	0.006	0.005	0.008	0.008
Air	6.45	36.8	36.8	38.4	6.54	65.6	21.1
%	98	55	55	67	77	40	47
Total daily intake	6.58	67.3	67.3	57.4	8.53	163	45.4

It can be seen from Table 4.7 that relatively high total daily intakes, mainly through consumption of drinking water and inhalation of contaminated air, are to be expected at the local scale, around MTBE production sites and plants using MTBE as an intermediate.

The following examples may explain some of the underlying model calculations performed by EUSES, (according to Appendix VII of the TGD or Section III.5.2 of the EUSES documentation).

For the regional daily dose of MTBE ingested through drinking water, it is assumed that the concentration in drinking water equals PEC_{water} of 3.3 μg/l (Table 3.11), since no MTBE removal is expected during water treatment (Henry's Law constant < 100 and no aerobic biodegradation < 10 d). Also the (regional) concentration in surface water is higher than in groundwater, the other major source of drinking water. Based on a standard human water consumption of 2 l/d and body weight of 70 kg, the dose is 2 x 3.3 / 70 = 0.095 μg MTBE/kgbw/d.

Similarly, the regional dose through fish is derived from the concentration of MTBE in the fish (BCF x PEC_{water}), i.e. 1.59 x 3.3 = 5.25 μg/kgww. Assuming a standard fish consumption of 115 g/d by a person of 70 kgbw, the dose is 0.115 x 5.25 / 70 = 0.0087 μg MTBE/kgbw/d.

The airborne dose is based on the regional PECair of 30.1 μg MTBE/m^3 (Table 3.17) and standard human ventilation rate of 20 m^3/d. Assuming, by default, that 75% of the inhaled MTBE is bioavailable, the retained dose is 451.5 μg/d, or 6.45 μg/kgbw/d for a person weighing 70 kg.

The doses via drinking water, fish and air account for approximately 1.5%, 0.1% and 98% of the total regional intake of MTBE via all routes.

Example calculations for the different local scenarios are as follows. Air surrounding production sites may contain 172 μg/m^3 (Table 3.17). Again assuming that 75% of the MTBE in inhaled (20 m^3) air is bioavailable, the daily dose is 0.75 x 20 x 172 / 70 = 36.8 μg/kgbw/d. Private use of MTBE as a fuel additive (PECair = 30.5 μg/m^3 in Table 3.17) yields a dose of 0.75 x 20 x 30.5 / 70 = 6.54 μg/kgbw/d, while air around isobutylene production plants (PECair = 306 μg/m^3) amounts to 0.75 x 20 x 306 / 70 = 65.6 μg/kgbw/d. The local air doses account for up to 40 to 77% of the total daily intake.

For the intake via local drinking water, the starting point is again the PEC_{water} values in Table 3.11 and an average human being of 70 kgbw consuming 2 l/d. Thus, around production sites (PEC_{water} = 969 μg/l) the intake is 2 x 969 / 70 = 27.7 μg MTBE/kgbw/d, again assuming no water purification and surface water the main source. Drinking water contaminated with MTBE privately used as a fuel additive (PEC_{water} = 63.1 μg/l) may yield a daily intake of 2 x 63.1 / 70 = 1.80 μg MTBE/kgbw/d. Similarly, the daily dose from drinking water around isobutylene production (PEC_{water} = 3,100 μg/l) is approximately 2 x 3,100 / 70 = 88.5 μg MTBE/kgbw. The doses represent around 40% of the daily intake for these scenarios.

Finally, human consumption of fish (115 g/d) locally caught would, using a BCF of 1.59, give rise to partial intake of 0.115 x 1.59 x 969 / 70 = 2.53 μg MTBE/kgbw. Similarly, the MTBE intake from fish contaminated with MTBE from fuel use or isobutylene production are calculated as 0.17 and 8.09 μg/kgbw/d, based on PEC_{water} values of 63.1 or 3,100 μg/l (Table 3.11). The doses are low and hardly contribute to the total daily intake.

4.1.2 Effects assessment: hazard identification and dose (concentration) - response (effect) assessment

Toxicokinetics data for both humans and experimental animals are presented in Section 4.1.2.1.

All toxicological effects data presented in Sections 4.1.2.2 to 4.1.2.8 are animal toxicity data alone. Human effects data are presented separately in Section 4.1.2.9.

4.1.2.1 Toxicokinetics, metabolism and distribution

MTBE has been studied extensively with regard to its toxicokinetic properties. The information presented here 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 (pre-1991) addressing some of these aspects are listed in Table 4.8 (studies indicated as A to J) and experimental findings are described either in Tables 4.9 to 4.10 or are given in the text. More recent work (1997 onwards) relating to the toxicokinetics of MTBE is described in more detail in the text.

Uptake in experimental animals

Miller *et al* (1997) administered single doses of MTBE to F344 rats by the intravenous (40 mg/kgbw/d), oral (40 and 400 mg/kgbw/d), dermal (6 h, 40 and 400 mg/kgbw/d) and inhalation (6 h, 400 and 8,000 ppm) routes, and repeated doses of 400 ppm by inhalation for 15 days. On plotting the concentration-time course, the plasma levels of MTBE were

higher following oral dosing than after intravenous dosing. This was considered by the authors likely to be due to rapid elimination of the substance from the lungs. Absorption across the gastro-intestinal tract appeared to be rapid and complete. Dermal absorption, on the other hand, was relatively low, with only 16% of the low dose and 34% of the high dose absorbed. Inhaled MTBE was much more readily absorbed followed by a rapid rise and a steady state of the plasma level within 2 hours.

In study A2, peak plasma concentrations of MTBE were reached within 1 hour after oral (gavage) administration, indicating rapid uptake from the gastro-intestinal 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 (study A3 - closed chamber, 6-h contact), the peak plasma concentrations were 20-fold lower than observed after oral administration and were reached about 2 hours after the start of the experiment. This demonstrates a slower uptake of MTBE through the skin compared with the uptake from the gastro-intestinal tract. The bioavailability of MTBE after dermal administration was 20% and 39% (40 and 400 mg/kgbw, both routes respectively) of the oral bioavailability.

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

During the single inhalation exposures in study B, plasma levels of MTBE increased rapidly between 10 min to 2 hours after the start of exposure and then more gradually up to a maximum concentration (Cmax), at approximately 4 to 6 hours 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 gastro-intestinal 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 gastro-intestinal tract within 3 hours after dosing the animals. Following dermal application of 40 mg MTBE/kgbw, about 60% of the radioactive material was still present in the application chambers, after administration of 400 mg/kgbw 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 publication by Borghoff *et al* (1996) 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.

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 and 49 min for female rats. In study F using 2 rats of either 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 4.8: Animal studies (pre-1991) on aspects of MTBE toxicokinetics

Study	Species, strain/ Number/group, sex	Route of administration	Time of exposure, duration	Target dose or concentration	Test substance MTBE, position of ^{14}C-radiolabel	Reference	Confidence in results
	Rat, F344						
A-1	40 M+F	Intravenous, bolus [a]		40 mg/kgbw	Unlabelled	Bio-Research Laboratories, 1990a	High
A-2	40 M+F	Oral, gavage [b]		40, 400 mg/kgbw	Unlabelled		High
A-3	60 M+F	Dermal, occluded [c]		40, 400 mg/kg	Unlabelled		High
B-1	60 M+F	Inhalation, nose only	6 h	1,440, 28,800 mg/m^3	Unlabelled	Bio-Research Laboratories, 1990b	High
B-2	40 M+F	Inhalation, nose only	6 h/d, 15 d	1,440 mg/m^3			High
C-1	6 M+F	Intravenous, bolus [a]		40 mg/kgbw	Central butyl carbon	Bio-Research Laboratories, 1990c; 1991	High
C-2	6 M+F	Oral, gavage [b]		40, 400 mg/kgbw	Central butyl carbon		High
C-3	6 M+F	Dermal, occluded [c]		40, 400 mg/kgbw	Central butyl carbon		High
D-1	6 M+F	Inhalation, nose only	6 h	1,440, 28,800 mg/m^3	Central butyl carbon	Bio-Research Laboratories, 1990d	High
D-2	6 M+F	Inhalation, nose only	6 h/d, 15 d	1,440 mg/m^3	Unlabelled first 14 d, further as D-1		High
	Rat, Sprague-Dawley						
E	3 M+F, 11 time points	Intraperitoneal		232 mg/kgbw	Methyl and central butyl carbon	Zacharias and Eschbach, 1984	High
	Rat, Charles River						
F	2 M+F	Intraperitoneal		7.3, 14.6 mg/kgbw	NS	Kennedy and Keplinger, 1972	Low

Table 4.8: Animal studies (pre-1991) on aspects of MTBE toxicokinetics (cont'd)

Study	Species, strain/ Number/group, sex	Route of administration	Time of exposure, duration	Target dose or concentration	Test substance MTBE, position of ^{14}C-radiolabel	Reference	Confidence in results
	Monkey, Rhesus						
G	2 F	Intraperitoneal		58.4 mg/kgbw	NS	Kennedy and Keplinger, 1972	Low
	Rat, Wistar						
H	5 M	Inhalation, whole body	6 h/d, 5 d/ wk, 15wk	180, 160, 1,080 mg/m^3	Unlabelled	Savolainen *et al*, 1985	High
J	5 M, 8 time points	Oral, gavage		0.379 mg/kgbw	Unlabelled	Li *et al*, 1991	Low

[a] MTBE dissolved in isotonic saline, injected into the caudal vein

[b] 10 ml MTBE/kgbw dissolved in isotonic saline

[c] 10 ml MTBE dissolved in isotonic saline, applied via occluded dermal chamber on the shaved dorsal flanks

NS Not stated

Table 4.9: Toxicokinetics of MTBE and TBA in F344 rats a after administration of MTBE by different routes *(Bio-Research Laboratories, 1990a,b)*

Study	Route	Received dose	MTBE				TBA				Total plasma clearance
		(mg/kg)	Cmax (µg/ml)	tmax (h)	AUC (µg·h/ml)	$t_{1/2}$ (h)	Cmax (µg/ml)	tmax (h)	AUC (µg·h/ml)	$t_{1/2}$ (h)	(ml/h)
	Male Animals										
A-1	Intravenous	36.7	NS	NS	10.7/NS	0.45	7.2	2	26.7/NS	0.9	413
A-2	Oral	33.5	17.2	0.25	17.0/NS	0.52	10.0	0.75 - 2	39.0/NS	1.0	392
A-2	Oral	420	124.0	0.15 - 0.75	230/NS	0.79	50.3	2 - 4	304/NS	1.6	358
A-3	Dermal	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	373	5.4	4	46.9/14.6	1.80	13.3	4	93.9/34.0	1.9	364
B-1	Inhalation	242 [c]	14.9	4 - 6	84.3/10.5	0.52	39.7	6.5	404/116	3.3	531
B-1	Inhalation	4,709 [c]	556.0	6	2,960/406	0.57	536	6.5	6,010/1,790	3.4	298
B-2	Inhalation	220 [c]	9.0 [b]	6 [b]	NS/6.7 [b]	0.51 [b]	37.1 [b]	6 - 6.5 [b]	NS/127 [b]	1.8 [b]	NS

Table 4.9: Toxicokinetics of MTBE and TBA in F344 rats [a] after administration of MTBE by different routes (cont'd)

Study	Route	Received dose (mg/kg)	MTBE				TBA				Total plasma clearance (ml/h)
			Cmax (µg/ml)	tmax (h)	AUC (µg·h/ml)	$t_{1/2}$ (h)	Cmax (µg/ml)	tmax (h)	AUC (µg·h/ml)	$t_{1/2}$ (h)	
	Female animals										
A-1	Intravenous	33.5	NS	NS	7.9/NS	0.46	6.4	1 - 2	32.2/NS	1.3	466
A-2	Oral	42.7	11.2	0.25	12.5/NS	0.62	8.9	2	36.7/NS	1.0	481
A-2	Oral	388	115.0	0.25 - 0.5	193/NS	0.93	48.8	4	289/NS	1.9	287
A-3	Dermal	39.4	0.1	4	1.1/NS	0.90	0.5	4 - 6	3.8/1.2	2.2	458
A-3	Dermal	441	7.8	2	34.4/6.9	1.40	16.3	6	101/37.1	1.9	273
B-1	Inhalation	329 [c]	15.1	4	77.9/11.8	0.63	39.4	6	374/94.2	3.0	573
B-1	Inhalation	6,991 [c]	563.0	4	2,870/369	0.53	245	6	2,550/574	2.8	315
B-2	Inhalation	318 [c]	9.1 [b]	6 [b]	NS/6.3 [b]	0.48 [b]	44.2 [b]	6 [b]	NS/125 [b]	1.5 [b]	NS

[a] Group mean values are shown

[b] 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

c_{max} Maximum plasma concentration reached during sampling schedule

t_{max} Time to reach Cmax after MTBE administration

$t_{1/2}$ Apparent half-life calculated from 1- or 2-compartment models

AUC Area under the plasma concentration-time curve; the first value gives AUC 0 - 4 h, the second AUC 6 - 24 h

NS Not stated

Table 4.10: Total percent of radioactivity recovered from F 344 rats [a] within 48 hours after administration of radiolabelled MTBE *(Bio-Research Laboratories, 1990c,d; 1991)*

Study	Route	Received dose [mg/kg]	Radioactive dose [µCi/kg]	Urine (%)	Faeces (%)	Expired air (%)	Tissue, carcass (%)	Application site (%)	Total (%)
	Male animals								
C-1	Intravenous	31.54	37.66	27.9	0.89	46.8	2.14	-	77.7
C-2	Oral	33.38	43.57	36.2		45.8	2.00	-	85.2
C-2	Oral	349.65	43.68	16.0	0.28	65.2	0.31	-	81.9
C-3	Dermal	34.64	44.30	6.49	0.14	6.05	0.67	57.10	70.5
C-3	Dermal	350.95	43.80	12.4	0.19	19.6	0.28	38.29	70.8
D-1	Inhalation	215.0 [b]	NS	64.7	0.76	21.2	13.40	-	100.0 [c]
D-1	Inhalation	4,220.0 [b]	NS	41.6	0.75	53.6	4.07	-	100.0 [c]
D-2	Inhalation	245.0 [b]	NS	71.6	0.62	16.9	10.90	-	100.0 [c]
	Female animals								
C-1	Intravenous	30.96	3.6.98	222.4	0.81	54.60	2.97	-	80.9
C-2	Oral	34.22	44.21	29.0	0.87	54.40	1.94	-	86.3
C-2	Oral	341.74	42.82	10.8	0.26	68.70	0.53	-	80.2
C-3	Dermal	35.79	45.85	6.12	0.13	9.67	1.02	59.91	76.8
C-3	Dermal	358.44	44.60	10.7	0.11	23.20	0.67	33.71	69.4
D-1	Inhalation	293.0 [b]	NS	65.4	0.74	21.90	11.90	-	100.0 [c]
D-1	Inhalation	5,840.0 [b]	NS	35.0	1.06	59.00	5.02	-	100.0 [c]
D-2	Inhalation	344.0 [b]	NS	67.4	0.77	21.40	10.40	-	100.0 [c]

[a] Group mean values are shown

[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 inhalation exposures. Consequently the recovery of radioactivity was calculated as percent of the total radioactivity recovered from each animal.

[d] Comparison of the numbers derived from inhalation exposures with another route of administration is only possible to a limited extent.

NS Not stated

Uptake in humans

Data from Nihlén *et al* (1995, 1998a) 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 was 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 in the 1995 studies, and from 42 to 49% in the 1998 study. 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 h) in the earlier studies and four half-lives in 1998 (1 min, 10 min, 1.5 and 19 h). MTBE blood clearance was calculated to be 0.5 l/h/kgbw (1995) and 0.34 to 0.52 l/h/kgbw (1998) at all MTBE concentrations. The half-life of TBA elimination was 8 (1995, blood) and 8.2 / 10 (1998, blood /urine) hours.

Pekari *et al* (1996) exposed 4 healthy volunteers to 0, 90 and 270 mg MTBE/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 (2,556 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 monophasic elimination in blood with a mean terminal half-life of 11.9 hours. These results corroborated the findings of Nihlén *et al* (1995, 1998a) and Johanson *et al* (1995).

Two men and 2 women were exposed to 6.1 mg MTBE/m^3 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.

Buckley *et al* (1997), in a more extensive analysis of data presented earlier by Prah *et al* (1994), investigated the uptake of MTBE, and subsequent elimination of MTBE and TBA in a male and a female volunteer (25 years old and 21 years old, respectively) following 60-min exposure to 5 mg MTBE/m^3 vapour. Breath and blood samples were collected simultaneously before, during and for 7 hours subsequent to exposure. Pharmacokinetic clearance of MTBE from blood, and its elimination in expired air, was modelled by fitting the "decay" portion of the blood and expired air curves to a 3 compartment mathematical model, which was then used to predict the uptake portion. Estimated residence times were 5 min, 60 min and 32 h, and 2 to 3 min, 15 to 50 min and 3 to 13 h for alveolar air and venous blood, respectively. While the breath and blood models fitted the decay portion of the measured elimination curves quite closely, a consistent overestimate of concentrations during uptake was noted by the authors, who suggested that 7 to 9% of the MTBE present in inhaled or expired air was taken up reversibly by the mucous membranes of the upper airways. The exposure concentration used in this investigation was selected to reflect probable USA consumer exposures to MTBE following evaporative emissions from oxygenated gasoline. The authors' suggestion, namely that MTBE is absorbed and eliminated by upper respiratory mucosa, will require further substantiation in view of the inter-individual variation in metabolic and pharmacokinetic data reported in this study.

Prah *et al* (2000) reported the results of human volunteers drinking 6.7 µl of MTBE dissolved in 250 ml of a lemon-lime drink. Blood samples were taken and analysed over a 24-h period, and these showed that MTBE was absorbed into the blood, though not as rapidly as following inhalation. Peak levels ranged from 5 to 15 ng/ml and declined with a half-life of 1 to 3 hours. Blood TBA levels rose more slowly and had a half-life of about 13 hours, with peak blood concentrations of 15 - 20 ng/ml.

Limited human data on the uptake, 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 detected only 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.

Evaluation

The uptake of MTBE in experimental animals 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 gastro-intestinal tract is complete and rapid. The dermal absorption rate in rats, following occluded exposure, is about one third of the oral rate, as determined by bioavailability. Actual values pertaining to the rate of absorption through the skin are not available. 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 2 to 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 via inhalation and the gastro-intestinal tract in these species are similar to those in rats.

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, 1998a), 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 to 48 hours. There are no data at present which provide comparisons between the rates of absorption of MTBE through the skin of rats and humans, it is likely, however, that the uptake kinetics follow a similar pattern to those in the rodent, being considerably slower than absorption via inhalation and following ingestion.

Distribution

MTBE concentrations were determined in blood, brain and fatty tissues in rats, following repeated inhalation exposure for up to 15 weeks (study H). The MTBE concentrations in the tissues concentrations were linearly related to exposure concentration at all time points (Table 4.11).

Table 4.11: MTBE and TBA burden (nmol/g) [a] of rats exposed to MTBE vapour for up to 15 weeks *(Savolainen et al, 1985)* [b]

Concentration	Duration (wk)							
(mg/m^3)	2		6		10		15	
	MTBE	TBA	MTBE	TBA	MTBE	TBA	MTBE	TBA
Blood								
180	9 ± 118	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
1,080	67 ± 18	28 ± 8	66 ± 18	151 ± 24	55 ± 14	89 ± 32	72 ± 19	127 ± 39
Brain								
180	11 ± 3	Trace[c]	Trace[d]	Trace	Trace	Trace	Trace	Trace
360	27 ± 5	Trace	28 ± 5	77 ± 3	23 ± 5	66 ± 5	Trace	53 ± 16
1,080	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
1,080	642 ± 119	Trace	610 ± 28	Trace	645 ± 39	Trace	698 ± 67	Trace

[a] Mean of five rats ± standard deviation (SD)

[b] Study H in Table 4.8

[c] Detection limit 2 nmol TBA/g

[d] Detection limit 1.5 nmol MTBE/g

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 around 10-fold higher concentrations in fatty tissues as 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 must 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.

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. With the exception of 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 intraperitoneal administration. 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 killing intervals, respectively. The majority of the radioactivity present in tissues was found in the liver. In another intraperitoneal 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 8.89%), skin (1.77 3.58%) and fat (0.59 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* (1996) determined tissue/blood partition coefficients for male F344 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 for the slowly perfused tissue compartment. The partition coefficients measured with regard to distribution are in good agreement with the observations made in the toxicokinetic studies: 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 intravenous, oral and inhalation administration as described in studies A, B, C and D. Borghoff *et al* (1996) 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.

It has been shown in a number of animal studies that concentrations of MTBE may be higher in fatty tissues than in other tissues (Miller *et al*, 1997). Despite this, the evidence suggests that elimination of the substance is virtually complete within 48 hours and that MTBE is not preferentially retained within the fat. There are also no significant gender-based differences in the distribution of MTBE.

In the human study of Leuschner *et al* (1991) described previously, 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 fallen to the pretreatment level.

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 was observed when steady state was reached as compared with 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 caused no 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 observed in experimental animals.

Metabolism of MTBE and TBA

Data on the metabolism of MTBE and TBA are mainly available from studies conducted with MTBE. The metabolic fate of the other principal 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 formaldehyde detoxification rates. Accordingly, formaldehyde is discussed here in the detail necessary to address this issue (see Page 123, *MTBE and formaldehyde: quantitative aspects*).

Microsomal metabolism of MTBE in liver and other tissue fractions was blocked by carbon monoxide and undetectable in the absence of NADPH and negligible *O*-demethylase activity (1 pmol/min/mg protein or less) was detected in human or rodent liver cytosol. The results confirm a role for microsomal cytochrome P450 haemoproteins in the metabolism of MTBE (Hong *et al*, 1997a; 1999a,b, 2001; Turini *et al*, 1998). These studies are detailed below.

Hong *et al* (1997a; 1999a,b) investigated the conversion of MTBE to TBA by microsomal enzymes present in human and rodent liver fraction. Samples obtained from eight North Americans (morphologically-normal tissue taken from liver cancer patients) showed a mean specific activity of 125 pmol TBA/min/mg microsomal protein, while microsomal fractions from two Chinese patients expressed activities of 167 and 221 pmol/min/mg. In comparison, hepatic microsomes from 10 wk old male SD rats or 7 weeks old female A/J mice showed rates that were 284 and 288 pmol/min/mg, respectively. It would appear that there are significant inter-individual differences regarding the metabolism of MTBE in human populations, although it is impossible to draw firm conclusions with such a small sample, and comparisons with the metabolic rates in animals may not be valid without further data.

In another study involving 15 human liver samples from people who had died in accidents, the rates of MTBE metabolism were much higher than previously recorded (204 - 2,890 pmol TBA/min/mg protein was the range) and this activity appeared to correlate with CYP2A6 content within the microsomes. The second highest activity was shown by CYP 2E1 (Hong *et al*, 1999c).

The role of individual human cytochrome P450 isoforms was investigated using CYP2A6 and CYP2E1 (and human P450 reductase) obtained from a baculovirus expression system. Both haemoproteins converted MTBE to TBA, although CYP2A6 had the greater activity (6.1 nmol/min/nmol CYP2A6 versus 0.7 nmol/min/nmol CYP2E1). Previous studies by this group (Brady *et al*, 1990) had shown that monoclonal antibodies against CYP2E1 inhibited the demethylation of MTBE by rat liver microsome by around 35% (Hong *et al*, 1999a,b, 2001). Although the authors of this work noted that genetic polymorphism in

the expression of human cytochrome P450 isoforms has been reported (including CYP2A6 and CYP2E1), and speculated that this could lead to inter-individual differences in human response to MTBE, no evidence of any causal association was demonstrated. A committee of the US Health Effects Institute, who sponsored the study, evaluated these results. Their conclusions were as follows:

- CYP enzymes are important in the metabolism of ethers added to gasoline in rats and humans;
- human hepatic CYP2A6 and CYP2E1 appear to be involved in metabolism of ethers added to gasoline, but the relative contribution of these isozymes at ambient exposure levels has not been ascertained;
- specific activities for CYP enzymes in metabolising ethers added to gasoline are roughly comparable in rat and human liver tissue. Further, the enzymatic activity for ether metabolism in rat nasal mucosa is substantially higher than in rat liver. Whether comparable enzymes and activities for metabolism of ethers added to gasoline exist in the nasal mucosa of humans remains unknown (HEI, 2001).

It is important to note that the *in vitro* nature of the work using high doses of ethers has made it difficult to draw reliable conclusions with regard to the relative contributions made by the different isoforms. This is because the metabolic pathway may change as the dose increases.

Turini *et al* (1998) examined the role of cytochrome P450 in the demethylation of MTBE using hepatic microsomal fractions from rats pretreated with specific and four purified cytochrome P450 haemoproteins. Pretreatment of male SD rats (6 - 8 wk old) with pyrazole or phenobarbitone increased the Vmax for *O*-demethylation of MTBE by 2 or 4-fold, respectively, with an associated decrease in Km. In contrast, B-naphthoflavone pretreatment decreased both kinetic parameters, while dexamethasone was without significant effect. In studies using purified, reconstituted preparations of cytochrome P450, CYP2B1 had the highest activity (64 nmol/min/nmol P450), while CYP2CII, CYP1AI and CYP2EI were markedly less active (< 0.1, 0.3 or 3.9 nmol/min/nmol P450, respectively). These results are consistent with a role for CYP2B1, strongly induced by phenobarbitone, in the metabolism of MTBE. CYP2E1 may also be involved, although this contribution appeared relatively small.

Chemical and antibody inhibition studies have also been reported which help clarify the differential contribution of cytochrome P450 isoforms to convert MTBE to TBA. Metyrapone, a potent (but, according to the authors, possibly less-than-specific) inhibitor of CYP2B gave a 75% decrease in *O*-demethylation by a microsomal fraction from phenobarbitone pretreated SD rats, but was without appreciable effect after pyrazole pretreatment. 4-Methylpyrazole, an inhibitor of CYP2E1, produced a 27 to 42% decrease in microsomal activity from animals treated with phenobarbitone or pyrazole,

respectively. Addition of testosterone (an inhibitor of CYP2A1) to microsomal incubations from female control rats resulted in a 48% decrease in MTBE metabolism *in vitro*. Antibody studies demonstrated that anti-CYP2E1 leads to a 28% reduction in demethylation of MTBE in microsomes from pyrazole pretreated rats (i.e. CYP2E1-induced) (Turini *et al*, 1998). These results confirm the relative importance of CYP2B1 and CYP2EI in the demethylation of MTBE, and also suggest that CYP2A1 (involved in androgen metabolism) may be a constitutive isozyme expressed in the female rat.

Microsomal fractions from rat nasal mucosa also contained a NADPH, carbon monoxide-sensitive enzyme system, catalysing the conversion of MTBE to TBA. High levels of activity were present in olfactory epithelium (specific activity 46-fold greater than rat liver) and moderate activity in respiratory epithelium (9-fold greater than liver), but no activity was detectable in lung microsomes (Hong *et al*, 1997b).

A comparison of the above data on the metabolism of MTBE by microsomal fractions from rodents and man is given in Table 4.12. The greater specific activity in rat olfactory epithelium versus rat liver appears consistent with a lower Km and higher Vmax in nasal versus hepatic tissue. MTBE is metabolised by cytochrome P450-dependent mono-oxygenases via *O*-demethylation yielding formaldehyde and TBA as the main metabolites.

Table 4.12: MTBE O-demethylase activity in microsomal fractions from different tissues

Tissue	Number of samples	Activity [a] (pmol/min/mg protein)	K_m (mmol/)	V_{max} nmol/min/mg	Reference
Mouse liver	4	288 ± 29	NS	NS	Hong *et al*, 1997a
Rat liver	5	284 ± 14	NS	NS	Hong *et al*, 1997a
	6	200 ± 10	NS	NS	Hong *et al*, 1997b
	NS	NS	6.3	0.93	Turini *et al*, 1998
Human liver	8	125 ± 11	NS	NS	Hong *et al*, 1997a
	2	194 ± 27	NS	NS	Hong *et al*, 1997a
Rat olfactory mucosa	6	9,280 ± 1,330	0.11	10.3	Hong *et al*, 1997b
Rat respiratory mucosa	6	1,840 ± 260	NS	NS	Hong *et al*, 1997b
Rat lung	5	ND	NS	NS	Hong *et al*, 1997b
Rat kidney	5	ND	NS	NS	Hong et al, 1997b
Rat olfactory bulb	3	ND	NS	NS	Hong *et al*, 1997b

[a] Mean ± standard deviation (SD)

ND Not detectable

NS Not stated

Hong et al (1999c) and Poet and Borghoff (1998) observed significant inter-individual variation in human CYP isoform activity when metabolising MTBE to TBA, and in some subpopulations, such as the Japanese, it has been shown that there are a large number of the population who lack the gene for one isoform (CYP 2A6) (Shimada et al, 1996; Oscarson et al, 1999).

Other studies with information on the metabolism of MTBE and TBA are described as follows.

In the inhalation study H, an approximately linear relationship between MTBE dose and TBA concentration in blood was observed (Table 4.11). This relationship did not change after prolonged exposures, although 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 does not accumulate in fat and rapidly perfused tissues. MTBE exposure caused a transient increase in UDP-glucuronosyl-transferase 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 (study B), TBA was detected 10 minutes 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/kgbw, 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 contrast, only the half life of TBA was decreased in females. Since an additional decrease in MTBE-AUC and an increase in TBA-AUC were 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 be due, partly, 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.

In study J, the blood of rats was analysed 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, 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/kgbw MTBE are removed very rapidly, which apparently is not the case.

Borghoff *et al* (1996) described a PBPK model that predicted successfully the MTBE dosimetry after intravenous, oral and inhalation administration of MTBE as described in studies A, B, C and D. They optimised Vmax and Km for MTBE metabolism from gas uptake data and found that two saturable pathways were necessary to model adequately the measured data. The first pathway had high capacity and low affinity with a Vmax of 0.104 mmol/h/kgbw) 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/kgbw) and a Km of 0.001 mmol/l.

MTBE and formaldehyde: quantitative aspects

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; it is generated intracellularly 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, 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, E 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 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/kgbw). The corresponding values for formaldehyde detoxification were 0.127 mmol/l and 7.69 mmol/h/kgbw). 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/kgbw) can be calculated. Such recalculated values from Brady *et al*, (1990) have been used recently 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* (1996) used two saturable pathways to describe MTBE metabolism to TBA and formaldehyde. The Vmax values were 0.104 and 0.008 mmol/h/kgbw), 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/kgbw) reveals that the likelihood of an increased formaldehyde concentration following MTBE exposure is much smaller than for methanol.

In the human study of Leuschner *et al* (1991) described previously, traces of methanol were found in 3 out of 27 patients, but no formaldehyde or formic acid.

Evaluation

Metabolism of MTBE has been investigated in a number of well-designed *in vitro* and *in vivo* studies. These demonstrate that MTBE is metabolised to formaldehyde and TBA via *O*-demethylation of MTBE. Taken together with information reviewed previously (ECETOC, 1997), induction, inhibition and reconstitution experiments have shown the involvement of at least three cytochrome P450 haemoproteins (CYP2A1, CYP2B1, CYP2E1) in the *O*-demethylation of MTBE by hepatic microsomes. In the studies so far reported, there is limited evidence to suggest that the basal metabolism of MTBE in liver may be faster in animals than in humans. However, further investigation is required to establish whether there are any significant qualitative/quantitative species differences in basal metabolism.

Animal studies suggest that MTBE has a potential to increase CYP2B1 levels after intraperitoneal administration, and induce MTBE metabolism *in vivo* after repeat inhalation exposure (ECETOC, 1997). Broadly similar effects (on P450 content and activities *in vitro*) have also been described for ethyl *tertiary*-butyl ether (Turini *et al*, 1998).

Since at least one of the P450 isoforms (CYP2A1) linked to MTBE metabolism is also involved in the *in vivo* disposition of androgens, it is tempting to speculate that hepatic enzymology provides a basis for the induction of apparently sex hormone-dependent tumours reported in the rodent bioassays (male rat testis, female mouse liver). As noted previously, however, pharmacokinetic studies demonstrate a clear shift in elimination route (from urinary excretion to lung exhalation) with increasing internal dose, probably reflecting enzyme saturation (ECETOC, 1997). Hence, cytochrome P450 status in animals receiving repeated high doses of MTBE is unlikely to be predictive of the cytochrome P450 status in animals or humans when exposed to lower concentrations.

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.

Human data 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.

Elimination

In the study of Miller *et al* (1997) (Section 4.1.2.1), total radiolabel was determined in the urine, faeces, exhaled air and also in the tissues, and there appeared to be no difference in distribution between the sexes. With all routes of administration, MTBE was rapidly removed from the plasma by exhalation and by metabolism to TBA. Following administration, greater than 90% of the dose was represented by the radiolabel measured in the excreta and tissues, of this, the majority was recovered in the exhaled air within 48 hours and to an extent in the urine, while less than 2% was found in the faeces. After intravenous dosing, 60% of the dose was eliminated in exhaled air, this fell to 9 to 19% after dermal dosing. With inhalation it was observed that there was a dose-dependent shift in the metabolic pathway and subsequent route of elimination; a significantly larger fraction of the high dose was eliminated through the exhaled air than occurred with the lower dose, which showed a more rapid renal elimination. This apparent saturation of a metabolic pathway was also observed following oral administration of high doses.

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* (1996). 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 and repeated inhalation exposures.

Urinary radioactivity accounted for an average of about 3% of the administered intraperitoneal dose in study E. 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 - 5%) and faeces (0.5 - 1.5%) 48 hours following administration.

In study G, the bulk of the expired radioactivity was recovered in monkeys within 8 hours of intraperitoneal 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/kgbw (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 reported above indicate that elimination kinetics of MTBE and TBA do not differ from the data obtained in animal experiments.

Dekant *et al* (2001) provided a detailed characterisation of metabolites of MTBE, ethyl *tertiary*-butyl ether and *tertiary*-amyl methyl ether. This study attempted to quantify the time- and concentration-dependent excretion of metabolites of these ethers from both rats and humans following administration via ingestion and via inhalation, for comparison of routes and species. The results confirmed the metabolic pathway for MTBE and *tertiary*-butyl ether in rats and humans as being the same, and described a pathway for *tertiary*-amyl methyl ether that was different to that of the other two.

The metabolism of *tertiary*-amyl methyl ether was also quantitatively different between the two species. The authors noted that the data for both exposure routes (ingestion and inhalation) were similar for all of the ethers and that the pathway was the same: no hepatic "first-pass effect" was recorded. This supports the use of inhalation data in route-to-route extrapolation for health risk assessments.

A study was carried out to investigate the effect of acute and repeated inhalation exposure to the light fraction of unleaded gasoline (LFG) on the toxicokinetics of inhaled MTBE, an area that has received little attention thus far. Male rats were exposed by inhalation to the following protocol: 40 ppm ^{14}C-labelled MTBE for 4 hours, 200 ppm LFG containing 20% MTBE by weight (40 ppm) once for 4 hours, or repeated exposure (4 h/d) to 200 ppm LFG for 7 days (on the 8th day the rats were exposed to LFG containing MTBE as with the other groups). The results of this study suggested that MTBE inhalation in the presence of LFG appeared to enhance the clearance of MTBE equivalents from the body, through increased exhalation of MTBE and/or its volatile metabolite and by enhanced excretion of MTBE equivalents in the urine. There also appeared to be enhanced retention of MTBE equivalents in the testis following repeated inhalation with LFG (Benson *et al*, 2001). This finding is of interest given that repeated inhalation and ingestion of high levels of MTBE have been shown to induce Leydig cell tumours in rats (Section 4.1.2.6). A committee of the US Health Effects Institute, who funded this study, assessed the Benson data and concluded that:

- MTBE uptake is not linear between 4 and 400 ppm, suggesting that extrapolation from high to low doses for human risk assessment should also be non-linear;
- single and repeat co-exposure with gasoline reduces MTBE uptake and increases its elimination from blood and urine, thereby possibly reducing the toxic effects associated with inhalation of MTBE in a gasoline mixture (as occurs during refuelling) (HEI, 2001).

In the human study of Leuschner *et al* (1991) described above, the TBA concentration measured in urinary samples from 27 patients was about 40 μg/ml at 5 hours after beginning of the treatment. TBA was still detectable 12 to 18 hours later at about the same level.

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 largely completed 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.

Conclusion on toxicokinetics

Animal studies provide extensive information on the toxicokinetics of MTBE and there is adequate supplementary information available from humans. MTBE is absorbed by all routes of exposure, though with quantitative differences. Absorbed material is distributed uniformly in all tissues, according to the relevant tissue/blood partition coefficient, which for most tissues is around one. The fat/blood partition coefficient is in the region of 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. TBA and formaldehyde, 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.

MTBE is rapidly absorbed from the gastro-intestinal and respiratory tract. Absorption from the gastro-intestinal 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 10-fold 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 around 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 (CYP2E1/CYP2B1) resulting in the formation of 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 exposure

In humans, the average net uptake rate was 38%, i.e. the retained percentage of an inhaled concentration of 18 to 180 mg/m^3. In another study, lung retention was 40% for exposures up to 270 mg/m^3. Since this range of exposure concentrations is relevant for the human exposure situation, a percentage of 40% has been used by the ECETOC Task Force 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 (e.g. diethyl ether and benzene) with a similar blood/air and tissue/blood partition coefficients (EC, 1993b; 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) (EC, 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 ECETOC 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 from 3.6 to 360 mg/m^3 a retained percentage of 60% is expected.

Physiologically-based pharmacokinetic models

PBPK models may be used to refine the estimates of the retention values used here. PBPK models have been developed to describe the behaviour of MTBE in rats (Borghoff *et al*, 1996), and blood levels of the compound can be predicted in animals following exposure by different routes, such as inhalation, and using a variety of exposure concentrations. Apart from some differences in distribution in male rats (thought to be linked with $\alpha_{2\mu}$-globulin nephropathy), the behaviour of MTBE is considered to be similar in both man and animals. With insertion of human physiological and anatomical parameters, the model can be used to predict values for man, providing a picture of the blood levels and distribution of MTBE in the body under different consumer exposure scenarios. PBPK models have also been used to look at specific target tissues such as the brain, and to attempt to predict exposure following bathing and showering (see also Rao and Ginsberg, 1997).

Recently published abstracts in the area of PBPK modelling and MTBE have described the ability to predict blood and tissue levels of MTBE in humans following inhalation exposure (Licata *et al*, 2000), and also investigations into predictions of dermal absorption and subsequent distribution in man (Leavens *et al*, 2000). This latter model may prove to be particularly useful in allowing the prediction of the relative contributions of various routes of exposure to the total body burden of MTBE and TBA in man exposed via the environment.

4.1.2.2 Acute toxicity

The acute toxicity of MTBE has been investigated in several animal species using different routes of administration. The following LD_{50} and LC_{50} values were considered representative (Table 4.13). Details of the available studies are contained in Appendix C.

Table 4.13: Acute toxicity

Route	Species	Result	Reference
Oral		LD_{50} (mg/kgbw)	
	Rat	3,800	Hathaway *et al*, 1970a
		3,866	Arco Chemical, 1980
	Mouse	4,000	Little *et al*, 1979
Dermal	Rat	> 6,800	Shell, 1971
	Rabbit	> 10,000	Arco Chemical, 1980
	Rabbit	> 10,200	Hathaway *et al*, 1970a
Inhalation [a]		LC_{50} (mg/m^3)	
	Rat	85,000	Hathaway *et al*, 1970a
	Rat	120,300	Arco Chemical, 1980
	Rat	142,000	Arco Chemical, 1980
Intravenous		LD_{50} (mg/kgbw)	
	Rat	410	Snamprogetti, 1980
Subcutaneous	Rat	4,960	Snamprogetti, 1980
	Mouse	2,670	Snamprogetti, 1980
	Mouse	1,010	Snamprogetti, 1980

[a] 4 h

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 hyper-excitability).

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.

4.1.2.3 Irritation, corrosivity and sensitisation

Skin irritation

Undiluted MTBE (0.5 ml) was applied to abraded and non-abraded skin of 6 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 Chemical,1980) using a 24-h 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 six rabbits from 1 hour to day 8 of the 14-d observation period. On day 14, desquamation and flaking was observed at the treatment sites. The 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 caused congestion of the conjunctivae, thickening and hyper-secretion, all of which were reversible. Two samples were tested for eye irritation by Arco Chemical (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 72 hours and 7 days. One sample was practically non-irritant to washed and unwashed eyes. Erythema was the only reaction, reaching a maximum score of 1.0 in 3/6 animals, 24 hours after instillation and declining to zero by 72 hours (mean of the 24, 48 and 72-h scores was 0.2). The other sample was slightly irritant 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 72-h scores were 1.4 (erythema), 0.6 (chemosis) and 0.3 (corneal opacity).

Mastrie (1969) reported transient eye irritation after 0.1 ml of MTBE was instilled into the eyes of 5 rabbits. Effects appeared within 1 minute 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 EC 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 to 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. There was no evidence of lung injury as determined by measurements of total protein and lactate dehydrogenase activity in broncho-alveolar lavage fluid. Recovery to normal respiration patterns was almost complete, even at the highest dose level, within 15 minutes after removal of the animals from the exposure chamber. The authors calculated a 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 to MTBE vapours have been reported (Arco Chemical, 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 (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 revealed no treatment related local effects (Dodd and Kintigh, 1989; Biles *et al*, 1987).

Sensitisation

MTBE was tested in a Magnusson and Kligman maximisation 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 Chemical, 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 (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

MTBE is slightly irritant to rabbit eyes and an irritant to rabbit skin. It caused moderate to severe skin erythema and moderate oedema in a study conducted to OECD guidelines. These effects resolved after 2 weeks.

MTBE vapour causes slight to transient irritation to the respiratory system of laboratory animals. A sensory irritation threshold of 300 mg/m^3 and a 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 a provisional threshold limit or occupational exposure limit.

Neither of the two available animal studies indicated that MTBE is a potential skin sensitiser. The conduct of the two tests was adequate though not entirely consistent with current guidelines.

4.1.2.4 Repeated-dose toxicity

Discussed in this section are several subacute, subchronic studies in the rat and mouse, and one study in monkeys, following repeated dosing for up to 90-days duration via the oral (gavage) and inhalation routes. Study details are given in Appendix D. These studies were conducted to determine whether protracted exposure to MTBE might cause toxicity. The studies are presented here in order of increasing exposure period.

Oral

Groups of 10 male and 10 female Sprague-Dawley rats were administered MTBE in corn oil daily by gavage at doses of 0, 357, 714, 1,071 and 1,428 mg/kgbw/d 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 these were considered incidental to treatment, except for the local irritation of the upper gastro-intestinal tract (sub-mucosal oedema, sub-acute inflammation, epithelial hyperplasia) at ≥ 357 mg/kgbw/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 bodyweight gain, increased blood haematocrit and haemoglobin levels (males), decreased monocyte numbers (males), increased relative kidney weights in males at ≥ 1,071 mg/kgbw/d and decreased absolute and relative lung weights (females). Significant changes in several clinical chemistry values were also observed, blood urea nitrogen was reduced in males and females and creatinine levels were reduced at the highest dose in females. In males, aspartate amino transferase and lactate dehydrogenase were reduced at 1,071 and 1,428 mg/kgbw and cholesterol was increased at 1,428 mg/kgbw. 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/kgbw/d). The most significant effects were anaesthesia and renal changes. The NOAEL derived from this study was 714 mg/kgbw/d.

Sprague-Dawley rats (10/sex/group) were dosed by oral gavage (5 d/wk) with MTBE in water at 0, 90, 440 and 1,750 mg/kgbw/d for 4 weeks (IIT, 1992). There were no treatment-related deaths. Mean bodyweight 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 was 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/kgbw/d and the NOAEL 90 mg/kgbw/d.

In a 90-day study, Sprague-Dawley rats (10/sex/group) were administered MTBE by gavage in corn oil at doses of 0, 100, 300, 900 and 1,200 mg/kgbw/d (Robinson *et al*, 1990). Profound narcosis occurred at 1,200 mg/kgbw/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/kgbw (males) and 1,200 mg/kgbw (males and females) dose groups (e.g. small increases in red blood cell count, haemoglobin and haematocrit, and decreases in white blood cell count in females and increased monocyte numbers in males). Clinical changes were similar to those seen in the 14-day study described previously. None suggests that MTBE is a significant systemic or target-organ toxin but blood urea nitrogen levels were statistically reduced in all treatment groups when compared with controls. Additionally, in males, there was a trend towards elevated cholesterol levels. Absolute and relative kidney and relative liver weights were significantly increased in males at ≥ 900 mg/kgbw/d. Relative kidney weights were also significantly higher in females at ≥ 300 mg/kgbw/d but this effect was not dose-related. Kidney changes in males were reflected, histologically, by tubular degeneration, accumulation of hyaline droplets and increased $\alpha_{2\mu}$-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/kgbw/d. MTBE exhibited low toxicity and significant effects were limited to doses ≥ 900 mg/kgbw/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/kgbw/d. For other effects the NOAEL was 900 mg/kgbw/d.

Zhou and Ye (1999) carried out a 90-day oral gavage study in Sprague-Dawley rats dosed (5 d/wk) with 0, 200, 600 and 1,200 mg MTBE/kgbw/d. Liver enzyme levels, a number of other haematological parameters and organ weights (liver, lungs, kidney and testes) were determined at termination of the study. Light and electron microscopy was used to examine tissue sections. Some decreases were observed in levels of aspartate amino transferases in all treated animals, however these were found to be still within the normal range of values for this species and strain. All treated animals demonstrated elevated liver weights with respect to controls, while the top two dose groups also showed a statistically significant increase in kidney weight. Unexpectedly, no histological changes were observed in treated animals using light microscopy, while electron microscopy revealed only reversible alterations in cellular infrastructure within hepatocytes. While the biological significance of the increase in liver weight and of the minor cellular changes is not clear, a LOAEL based on these effects can be determined as 200 mg/kgbw/d.

A sub-acute, 28-day oral study was carried out in Sprague-Dawley rats by Williams *et al* (2000) to investigate the potential effects of MTBE on the endocrine system (details of this study are provided in Section 4.1.2.7).

Inhalation

Inhalation studies were conducted in the rat and the mouse and observations at exposures in excess of 3,600 mg MTBE/m^3 were similar to those reported in the studies using oral gavage. No significant toxicity was noted at lower exposures. No adverse effect was seen in monkeys exposed to concentrations up to 10,600 mg/m^3.

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, 1,080, 3,600 and 10,800 mg/m^3 applied 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 1,080 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. No effects on behaviour were noted during exposure.

Prescott-Matthews *et al* (1997) carried out a 10-day inhalation study in rats to investigate the induction of $\alpha_{2\mu}$-globulin nephropathy by MTBE. This study is described in detail below (Section 4.1.2.6).

A 2-week vapour inhalation study was carried out by Hathaway *et al* (1970a). Groups of 5 male and 5 female albino rats were exposed (6 h/d, 5 d/wk) to 0, 10,000 or 30,000 mg/m^3 MTBE vapour in air. 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.

Following exposure (6 h/d, 5 d/wk) of rhesus monkeys (*Macaca mulatta*) (2/sex/group) to 7,000 or 10,600 mg MTBE/m^3 for 2 weeks, there were no deaths. The only effects seen were weight loss in 6 of the 8 animals at the end of the exposure period and some sluggishness in the high-dose group on each test day after 3 hours of exposure. Haemaotogy, clinical blood/urine chemistry and gross or microscopic pathology parameters all appeared normal (Hathaway, 1970d).

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 (10 min/d, 5 d/wk) to MTBE concentrations of 0, 180,000 and 288,000 mg/m^3 for 30 days. 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 (5 or 10 min/d, 5 d/wk) to 180,000 or 288,000 mg/m^3 of MTBE vapour for 30 days. Motor activity and co-ordination were monitored in this study. No treatment-related effects were observed. In the third study, groups of Wistar rats (25 animals/sex/group) were exposed (10 min/d, 5 d/wk) to approximately 180,000 mg/m^3 of MTBE vapour for 120 days. 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 CD-1 mice and F344 rats. Toxicity 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. NOAELs 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 F344 rats were exposed (6 h/d, 5 d/wk) to vapour concentrations of 0, 2,880, 14,400 and 28,800 mg/m^3 for 13 weeks (Dodd and Kintigh, 1989; Lington *et al*, 1997). 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 4 weeks of the study. Occasionally minor changes were recorded at 14,000 and 28,800 mg/m^3 in the functional observation battery (e.g. elevated body temperature) and a slight reduction in weight gain during the first 3 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. These increases were 8% (liver) and 4% (kidney) at 2,880 mg/m^3, and 39% (liver), 49% (kidney) and 55% (adrenals) at 28,800 mg/m^3. 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 (6 h/d, 5 d/wk) to MTBE vapours at 0, 900, 1,800 and 3,600 mg/m^3 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 F344 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 (6 h/d, 5 d/wk) either to filtered air (control) or to MTBE vapour. Target concentrations were 0, 1,440, 10,800 or 28,800 mg/m^3. Additional animals (5/sex/group) were included in the control and high-dose groups to be killed after an additional 16-day recovery period. In addition to the standard endpoints, cell proliferation in rat kidney and mouse liver was investigated using a bromodeoxyuridine (BrdU) immuno-histochemical 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 (such as ataxia, blepharospasm), 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/m3 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 $\alpha_{2\mu}$-globulin. They observed no $\alpha_{2\mu}$-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 $\alpha_{2\mu}$-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 $\alpha_{2\mu}$-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 to the extent, that would be typical of a classical $\alpha_{2\mu}$ globulin inducer such as d-limonene.

Evaluation

MTBE has been studied in the rat, mouse and monkey in well-conducted studies that have involved multiple exposures by ingestion (oral gavage) or inhalation. Findings have been similar in all studies. In general, the findings show that MTBE possesses 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. Other treatment-related findings at high-doses were irritation of the respiratory and gastro-intestinal 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 $\alpha_{2\mu}$-globulin.

While 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' $\alpha_{2\mu}$-globulin mechanism.

NOAELs of 90 mg/kgbw (LOAEL 440 mg/kgbw) and 300 mg/kgbw were determined from a 28-day oral study in rats and a 90-day study, respectively. 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/kgbw 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 spacing between the exposure levels, but in spite of this, effects of treatment were similar and the derived NOAELs were 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 (or 800 ppm) 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 (1988) has compiled reference body weights and inhalation rates. 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%, a daily retained dose of 228 and 244 mg/kgbw/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 B6C3F_1 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/kgbw/d for males and females respectively.

4.1.2.5 Genotoxicity

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).

In vitro

The mutagenic potential of MTBE has been tested in several prokaryotic cell systems (Table 4.14).

Table 4.14: Genotoxicity of MTBE in vitro

Test system	End point	Concentration	Results [a]		Reference
			-S9 [b]	+S9 [c]	
Salmonella typhimurium TA 1535, 1537, 1538, 98, 100	Reverse mutation	0.007 - 7.40 mg/plate (2 samples)	-ve	-ve	Jagannath, 1978; Arco Chemical, 1980
Salmonella typhimurium TA 1535, 1537, 1538, 98, 100	Reverse mutation	0.341 - 5.41 mg/plate	-ve	-ve	Seeberg and Cinelli, 1989
Salmonella typhimurium TA 1535, 1537, 1538, 98, 100	Reverse mutation	0.004 - 2.66 mg/plate	-ve	-ve	Hüls, 1991f
Salmonella typhimurium TA 102	Reverse mutation	0.39 - 2.40 mg/plate	±	±	Williams-Hill *et al*, 1999
Saccharomyces cerevisiae	Gene conversion	0.007 - 7.40 mg/plate	-ve	-ve	Arco Chemical, 1980
Mouse lymphoma cells L51798Y TK +/-	Forward mutation	0.229 - 7.40 mg/ml 0.118 - 4.44 mg/ml (2 samples)	-ve ND	ND ±	Arco Chemical, 1979, 1980
Chinese hamster ovary cells	SCE; chromosome aberration	0.003 - 0.740 mg/ml 0.007 - 3.70 mg/ml	-ve -ve	ND ±	Arco Chemical, 1980; Galloway, 1980
Chinese hamster V79 cells	Forward mutation	0.003 - 5.40 mg/ml 0.148 - 1.33 mg/ml	-ve ND	ND ±	Seeberg, 1989a
Rat hepatocytes	Unscheduled DNA synthesis	0.148 - 5.40 mg/plate	-ve	ND	Seeberg, 1989b

[a] -ve, no genotoxic activity; ±, equivocal

[b] In the absence of S9 metabolic activation

[c] In the presence of S9 metabolic activation

[d] Sister chromatid exchanges

ND Test not performed

The results showed no induction of base pair substitution or frame shift mutations in bacterial cells, or mitotic gene conversion in a yeast, under the conditions of these studies.

MTBE is a volatile compound and some concern has been expressed that there may potentially be some loss through evaporation from test plates in microbial assays such as the Ames test. This, it has been hypothesised, could result in reduced concentrations of test substance. Kado *et al* (1998) used a micro-suspension assay involving a closed tube to eliminate this possibility, and the results of this mutagenicity assay were still negative.

One report has been published of a positive Ames test result using *Salmonella typhimurium* strain TA102. The authors claimed MTBE to be weakly mutagenic without S9, moderately mutagenic in the presence of rat S9, and weakly mutagenic in the presence of human S9 (Williams-Hill *et al*, 1999). There are a number of major flaws in this study. Most significantly, the test material used in the study was synthesised in-house, with no reference made as to the methods used or to the purity of the substance. Also, the 'positive' result was not based on a doubling of the colony revertant rate as would normally be required, neither was there an indication of a real dose-response. The Task Force therefore considered the results of this study should not influence the evaluation of MTBE as a mutagen.

MTBE has been tested for genotoxic activity using five end points in four eukaryotic cell systems (Table 4.14).

MTBE did not induce chromosome aberrations or sister chromatid exchanges (SCE) in Chinese hamster ovary cells, gene mutation in V79 cells or unscheduled DNA synthesis in rat hepatocytes, respectively, although equivocal results were obtained with metabolic activation.

Evidence of a weak positive result was obtained when mouse lymphoma L5178Y TK+/- cells were exposed to 0.115 to 4.44 mg MTBE/ml, 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.

MTBE has also been reported to cause a variable increase in SCE in Chinese hamster ovary 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 of 15 to 16 SCE/cell (with 12.5/cell for the solvent control) in the presence of S9 following incubation with 0.148 to 0.740 mg/ml. The magnitude of the change was independent of the concentration, i.e. there was 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.

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 in rat hepatocytes (*in vivo/in vitro* assay), and also in the *Drosophila* sex-linked recessive lethal test. The results are summarised in Table 4.15 and discussed below in more detail. MTBE is readily diffusing into all body compartments and these studies are considered to give a realistic assessment of the genotoxic potential since they integrate absorption, metabolism and excretion with *in vivo* endpoints of DNA damage.

Table 4.15: Genotoxicity of MTBE in vivo

Species, strain, sex	Endpoint	Route	Dose and regime	Result	Reference
Rat, Sprague-Dawley, M	Chromosome aberrations (tibial bone marrow)	Oral (gavage)	30, 96 and 296 mg/kgbw (as a single dose or 1x /d, 5 d)	No clastogenic activity	Stetka, 1979; Arco Chemical, 1980;
Rat, F344, M+F	Chromosome aberrations (femoral bone marrow)	Inhalation	0, 2,880, 14,400, 28,800 mg/m^3 (6 h/d, 5 d)	No clastogenic activity	Vergnes and Morabit, 1989
Mouse, CD-1, M+F	Chromosome aberration (femoral bone marrow)	Inhalation	0, 1,440, 10,800, 28,800 mg/m^3 (6 h/d, 2 d)	No clastogenic activity	Vergnes and Kintigh, 1993
Mouse, CD-1, M+F	Unscheduled DNA synthesis in hepatocytes (*in vivo/in vitro*)	Inhalation	0, 1,440, 10,800, 28,800 mg/m^3 (6 h/d, 2 d)	No increase in unscheduled DNA synthesis	Vergnes and Chun, 1994
Drosophila melanogaster	Sex-linked dominant lethal	Ingestion	0.3% in sucrose	No increase in recessive lethal events	Sernau, 1989

The clastogenic potential of MTBE was evaluated *in vivo* using a rat bone marrow cytogenetic assay following acute or repeated oral exposure (Stetka, 1979; Arco Chemical, 1980). In the acute study, the animals were killed 6, 24 or 48 hours after dosing; in the repeated-dose study, the animals were killed 6 hours after the final treatment. No increase in chromosome aberrations in tibial bone marrow was seen at any dose level following single or repeated administration of MTBE. A normal response was obtained with the positive control material (triethylene melamine). It was concluded that MTBE did not cause clastogenic damage to rat bone marrow under the conditions of the study.

In one MTBE vapour study (Vergnes and Morabit, 1989), the animals were killed 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 chromosome aberrations 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.

Vergnes and Kintigh (1993) investigated the effect of inhalation of MTBE vapour on the occurrence of micronuclei in bone marrow in mice. Animals were killed approximately 24 and 48 hours after the final exposure. There was a significant increase in the ratio of polychromatic to normochromatic erythrocytes in femoral bone marrow of the males from the 1,440 mg/m^3 exposure group at the 48 hour sampling point, although this appeared to be an isolated finding within the control range. No significant increase in the incidence of micronucleated polychromatic erythrocytes 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 the unscheduled DNA synthesis assay of Vergnes and Chun (1994), the animals were killed 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 (dimethylnitrosamine). It was concluded that MTBE did not induce DNA repair in mouse hepatocytes in this *in vivo*/*in vitro* assay.

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 3-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 sex-linked recessive lethal (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.

Evaluation

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

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. Nevertheless, 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. Closed suspension test results also proved negative and support these conclusions.

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+/- 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 (Galloway, 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 is 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 (1988) and a retention of 15%.

The animals from the highest treatment group (28,800 mg/m^3) received a daily dose of over 1,000 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. It is possible that the increase in the ratio of polychromatic to normochromatic erythrocytes found in the micronucleus study may support this. 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. Overall the available data strongly suggest that MTBE does not pose a genotoxic hazard.

The same conclusion is drawn with regard to the genotoxic activity of the metabolites TBA and formaldehyde. A summary of the available information is presented below.

Genotoxicity of formaldehyde

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

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 (IPCS, 1989).

Casanova and Heck (1997) investigated the ability of endogenously-derived formaldehyde to induce DNA and RNA cross-links and to become incorporated into nucleosides. Hepatocytes from female CD-1 mice (approx 30 g) were incubated *in vitro* with 0.33 to 6.75 mM MTBE, DNA and RNA samples were isolated and analysed for DNA-protein crosslinks (DPX) or RNA-formaldehyde adducts (RFA). (The highest concentration (6.75 *m*M) was 50% greater than the predicted concentration of female mouse liver following inhalation exposure to 28,800 mg/m^3 vapour, as derived from a PBPK model.)

While DPX and RFA were detected in hepatocyte cultures, the yields were very low and independent of MTBE exposure concentration (all values at or around the background of 1 pmol of formaldehyde bound per mg nucleic acid). In contrast, direct addition of formaldehyde to hepatocyte cultures gave a clear concentration-dependent increase in both DPX and RFA. Similar results were obtained using hepatocytes from male $B6C3F_1$ mice and F344 rats. The presence of DPX and RFA in hepatocytes was also determined after oral pretreatment of animals with MTBE (1.8 g/kgbw in corn oil on 3 consecutive days) to test if induction of MTBE metabolism was associated with an increased presence of covalently bound formaldehyde in liver tissue. Pretreatment had no discernable effect on the yields of nucleic acid adducts.

Incubation of hepatocytes from control (corn oil) or MTBE-pretreated CD-1 mice with ^{14}C-MTBE showed 5 to 10-fold greater incorporation of label into DNA nucleosides from pretreated animals versus controls, whereas incorporation into RNA was similar in both groups. Incorporation into DNA was due primarily to insertion into thymidine, while ^{14}C was incorporated into adenosine and guanosine in RNA.

The results of these studies indicate that the intracellular rate of formaldehyde production, formed during the *O*-demethylation of MTBE, was slow relative to its rate of oxidation to formate and subsequent incorporation into the one carbon pool. This formate was then used by the cell as a precursor for synthesis of thymidine, adenosine and guanosine, and did not lead to an increase in formation of DPX or RFA. The increased metabolic incorporation of labelled carbon fragments into DNA after gavage administration of MTBE (3 x 1.8 g/kg/d) appears consistent with the increased BrdU incorporation and cell proliferation seen in hepatocytes from mice following 5 daily exposures to 28,800 mg MTBE/m^3 (Chun and Kintigh, 1993).

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 unscheduled DNA synthesis in rat liver was seen, and the *in vitro* studies of Casanova and Heck described above. On this basis, production of formaldehyde from MTBE in the body is unlikely to cause genotoxic damage.

Genotoxicity of TBA

Tests on the mutagenicity of TBA *in vitro* are listed in Table 4.16.

Table 4.16: Mutagenicity of TBA in vitro

Test system	End point	Concentration (mg/plate)	Results [a]		Reference
			-S9 [b]	+S9 [c]	
Salmonella typhimurium TA 1535, 1537, 1538, 98, 100	Reverse mutation	Up to 1	-ve	-ve	Hüls, 1979
Salmonella typhimurium TA 1535, 1537, 98, 100	Reverse mutation	0.1 - 10	-ve	-ve	Zeiger *et al*, 1987; US-NTP, 1995
Mouse lymphoma cells L5178Y TK +/-	Forward mutation	0.625 - 5.0	±	ND	McGregor *et al*, 1988; US-NTP, 1995
		1.0 - 5.0	ND	-ve	
		1.0 - 5.0	-ve	ND	
		2.0 - 5.0	ND	-ve	
Chinese hamster ovary cells	Chromosome aberration	0.16 - 5.0	-ve	±	US-NTP, 1995
		2.0 - 5.0	-ve	-ve	
Chinese hamster ovary cells	Sister chromatid exchange	0.16 - 5.0	±	-ve	US-NTP, 1995
		2.0 - 5.0	-ve	-ve	

[a] -ve, no mutagenic activity; ±, equivocal

[b] In the absence of S9 metabolic activation

[c] In the presence of S9 metabolic activation

ND Test not performed

No mutagenic potential was observed in *Salmonella typhimurium* strains TA 1535, 1537, 1538, 98 or 100 after exposure to up to 10 mg/plate both in the presence and absence of S9 fraction (Hüls, 1979; Zeiger *et al*, 1987; US-NTP, 1995).

Results from a mouse lymphoma assay with L5178Y TK+/- cells 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; US-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 US-NTP (1995).

TBA has been tested *in vivo* for its potential to increase the frequency of micronucleated normochromatic erythrocytes in male and female mice administered drinking water containing 3,000 to 40,000 mg TBA/l for 13 weeks (US-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.*

4.1.2.6 Chronic toxicity and carcinogenicity

In order to avoid repetition of data, chronic toxicity and carcinogenicity data are presented together.

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; Bird *et al*, 1997) and F344 rats (Chun *et al*, 1992; Bird *et al*, 1997). These studies were designed in consultation with the US-EPA (40 CFR 799.5000) following conventional experimental protocols and conducted in accordance with GLP. Results are also available from a rat oral intubation study (Belpoggi *et al*, 1995). These studies are summarised below.

Mouse inhalation study

Groups of male and female CD-1 mice (50/sex/group) were exposed (6 h/d, 5 d/wk) to 0, 1,440, 10,800 and 28,800 mg/m^3 MTBE vapour for 18 months (Burleigh-Flayer *et al*, 1992; Bird *et al*, 1997). 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 killed moribund were sampled and subjected to microscopic histopathological examination.

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 mid- and high-dose 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. Body weight and body-weight gain were decreased in the 28,800 mg/m^3 exposed groups, for both sexes, with a 15 to 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 was noted for male mice at all dose levels (11%, 14% and 22%, respectively, relative to body weight). A small (approximately 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 26% males (14% control) and 18% females (0% control) exposed to 28,800 mg/m^3 MTBE. 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 none of the values 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 were 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 4.17).

Table 4.17: Incidence of endometrial cell hyperplasia in female CD-1 mice exposed to MTBE vapour *(Burleigh-Flayer et al, 1992)*

	Dose (mg/m^3)			
	0	**1,440**	**10,800**	**28,800**
Endometrial cell hyperplasia	3/50	3/50	3/50	0/50
Endometrial cell hyperplasia, cystic	26/50	17/50	15/50[a]	6/50[b]
Endometrial cell hyperplasia, polyploid	7/50	1/50	0/50[a]	3/50

[a] $p < 0.05$

[b] $p < 0.01$

Exposure 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.

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 4.18). No increase in malignant tumours was reported in females.

Table 4.18: Incidence of liver tumours in female CD-1 mice exposed to MTBE vapour *(Burleigh-Flayer et al, 1992)*

	Concentration (mg/m^3)			
	0	**1,440**	**10,800**	**28,800**
Hepatocellular adenoma	2/50	1/50	2/50	10/50[a]
Carcinoma	0/50	1/50	0/50	1/50
Adenoma and carcinoma	0/50	0/50	0/50	0/50
Adenoma and/or carcinoma	2/50	2/50	2/50	11/50

[a] $p < 0.01$

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 or statistically significant (Table 4.19).

Table 4.19: Incidence of liver tumours in male CD-1 mice exposed to MTBE vapour *(Burleigh-Flayer et al, 1992)*

	Concentration (mg/m^3)			
	0	**1,440**	**10,800**	**28,800**
Hepatocellular adenoma	11/49	11/50	9/50	12/49
Carcinoma	2/49	4/50	3/50	8/49
Adenoma and carcinoma	1/49	3/50	0/50	4/49
Adenoma and/or carcinoma	12/49	12/50	12/50	16/49

The study report noted that the combined tumour incidence for males was also within the range of the historic control data for CD-1 mice at 24 months, indicating that these findings were 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.

Evaluation

Chronic exposure of CD-1 mice to MTBE vapour for 18 months resulted in a significant increase in hepatocellular adenoma in high dose (28,800 mg/m^3) females only, with no effects in the other treatment groups in either sex (Burleigh-Flayer *et al*, 1992). No other tumour findings were seen in this study, although decreased endometrial cystic hyperplasia together with decreased relative uterine and ovarian weight and increased hepatic metabolism of 17-β-oestradiol (Moser *et al*, 1996), have also been reported in female mice exposed to MTBE vapour. 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 $B6C3F_1$ mice in chronic studies (37.3 g and 63 l/d respectively), and a retention of 30 or 15%, depending on the exposure concentration, an equivalent dose of 182, 648 and 1,824 mg/kgbw/d is obtained. The corresponding doses for female mice (35.3 g and 60 l/d respectively) are 184, 669 and 1,836 mg/kgbw/d.

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 cannot be considered to represent a full lifetime exposure, the design and 18-month duration were considered adequate to assess the chronic toxicity and carcinogenicity of MTBE in the CD-1 mouse.

Results indicate that the liver is a primary target for MTBE toxicity, based on 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 P450 activity noted in other studies.

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, two of which are outlined below:

- 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 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. It is unlikely, however, that this short-lived phenomenon can be held directly responsible for the subsequent appearance of liver tumours, since hepatocyte proliferation had returned to normal within one month after the start of treatment. Nevertheless 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. The stimulus required in the mouse liver to invoke cell proliferation in these circumstances may be increased damage occurring due to free radical production and uncoupling of microsomal cytochrome P450 activity (Parke and Ioannides, 1990). Enzymology studies have shown induction of cytochrome P450 oxygenase IIB1 (CYP2B1) in rats. However, definitive information for the mouse is not available.
- 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 P450 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 P450 activity) may be responsible. The decrease in endometrial cystic hyperplasia noted in female mice exposed to MTBE also points to a hormonal perturbation. Section 4.1.2.7 examines this hypothesis in more detail.

An alternate hypothesis, that formaldehyde formed as a consequence of the *O*-demethylation of MTBE might explain the appearance of female mouse liver tumours following long term inhalation exposure to MTBE vapour, has also been pursued since the previous review by ECETOC (1997). Casanova and Heck (1997) investigated the

ability of endogenously derived formaldehyde to form DNA and RNA cross-links and to be incorporated into nucleosides (Section 4.1.2.5). The results of these studies indicated that the intracellular rate of formaldehyde production (formed during the *O*-demethylation of MTBE), was slow, relative to its rate of oxidation to formate and subsequent incorporation into the one carbon pool. This formate was then used by the cell as a precursor for synthesis of thymidine, adenosine and guanosine, and did not lead to an increase in formation of DPX or RFA.

It seems, therefore, that formaldehyde formation from MTBE is closely coupled to cell intermediary metabolism, eliminating any potential for covalent binding to nucleic acids. Thus formaldehyde is not likely to be a critical factor in MTBE-induced liver tumours in female mice.

Rat inhalation study

Groups of male and female F344 rats (50/sex/group) were exposed (6 h/d, 5 d/wk) to 1,440, 10,800 and 28,800 mg/m^3 MTBE vapour for up to 105 weeks (Chun *et al*, 1992; Bird *et al*, 1997). 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 killed 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.

Increased mortality was observed in all groups of male rats, but was most common in the intermediate (88% at week 97) and high (82% at week 82) exposure groups. As a result, males from the 10,800 and 28,800 mg/m^3 groups were killed 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 were decreased in both males and females exposed to 28,800 mg/m^3, 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/m3, but remained 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 killing of the intermediate and high-exposed animals. Nevertheless, 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 was an increased frequency of chronic nephropathy in treated males, as determined microscopically from an increased incidence and/or severity of glomerulo-sclerosis, tubular proteinosis, interstitial nephritis and interstitial fibrosis (Table 4.20).

Table 4.20: Microscopic findings of nephropathy in rats exposed to MTBE by inhalation *(Chun et al, 1992)*

	Males [a]				**Females [a]**			
Concentration (mg/m^3):	**0**	**1,440**	**10,800**	**28,800**	**0**	**1,440**	**10,800**	**28,800**
Tubular proteinosis, total	49	49	50	50	47	23	9	49
Minimal	3	0	1	0	5	0	0	1
Mild	6	9	1	0	14	11	9	5
Moderate	18	18	14	5	20	9	15	23
Marked	21	9	10	12	8	3	8	14
Severe	1	13	14	33	0	0	7	6
Glomerulo-sclerosis, total	43	46	48	50	37	17	34	45
Minimal	3	1	1	0	9	1	4	1
Mild	19	19	9	4	22	11	15	26
Moderate	17	14	12	11	5	5	8	14
Marked	4	10	19	33	1	0	7	4
Severe	0	2	7	2	0	0	0	0
Interstitial nephritis, total	42	44	45	50	29	14	31	37
Minimal	7	3	0	0	9	2	3	7
Mild	15	17	15	3	17	12	19	15
Moderate	19	24	30	47	3	0	9	15
Marked	1	0	0	0	0	0	0	0
Interstitial fibrosis, total	27	35	43	48	13	7	25	23
Minimal	2	3	0	0	8	1	3	5
Mild	20	11	12	5	3	6	12	11
Moderate	5	20	29	40	2	0	10	7
Marked	0	1	2	3	0	0	0	0

[a] 50/group

Secondary (i.e. nephropathy-dependent) changes, such as increased weight of the parathyroid glands, stomach thickening, hyperinflation of the lungs, and mineralisation in the tubules, were also observed. A slight increase in nephropathy was diagnosed microscopically in females from the 10,800 and 28,800 mg/m^3 exposed groups, its magnitude and severity being much less than that seen in males.

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 mid- 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). (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 principal 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). No tubular cell tumours occurred in males from the low treatment group (Table 4.21).

Table 4.21: Incidence of renal cell tumours in male rats exposed to MTBE vapour *(Chun et al, 1992)*

	Dose (mg/m^3)			
Tumour type	**0**	**1,440**	**10,800**	**28,800** [a]
Renal tubular adenoma	1/50	0/50	5/50	3/50
Renal tubular carcinoma	0/50	0/50	3/50	0/50
Renal tubular adenoma and/or carcinoma	1/50	0/50	8/50[b]	3/50

[a] Early mortality may have influenced tumour incidence

[b] $p < 0.05$

Historic data for male F344 rats (Haseman *et al*, 1990) indicate the spontaneous occurrence of kidney tumours is in the range 0 to 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 (early mortality having not allowed for the development of more tumours).

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 to 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 4.22).

Table 4.22: Incidence of interstitial cell adenoma of the testis in male rats exposed to MTBE by inhalation *(Chun et al, 1992)*

Interstitial cell adenoma of the testis	**Dose (mg/m^3)**			
	0	**1,440**	**10,800**	**28,800**
Mild	0/50	3/50	3/50	2/50
Moderate	6/50	9/50	4/50	14/50
Marked	22/50	20/50	28/50	27/50
Severe	4/50	3/50	6/50	2/50
Total (%)	32/50 (64%)	35/50 (70%)	41/50 (82%)	47/50 (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, equivalent doses of 102, 384 and 1,023 mg/kgbw/d were obtained. The corresponding doses for female rats (229 g, 240 l/d) were 113, 425 and 1,132 mg/kgbw/d. The highest treatment resulted in a 20 to 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.

Histopathological changes seen in the testes were suggestive of a dose-related increase in benign Leydig cell tumours (Table 4.22). However, the biological significance of these findings is questionable since Leydig cell tumours occur commonly in untreated F344 rats (as evidenced from concurrent controls included in this investigation) with a spontaneous incidence of approximately 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 (Prentice and Meikle, 1995). There are also questions regarding the human relevance of this tumour type and incidence in rats; these are explored in more detail in Section 4.1.2.6.

Overall, it is concluded that nephropathy was the major cause of death in males in the intermediate and high exposure groups. The principal 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). The findings from this study suggested that a non-genotoxic mechanism, potentially involving the accumulation of the male rat-specific protein $\alpha_{2\mu}$-globulin, might be operating which would be consistent with the non-neoplastic and neoplastic changes seen in kidney tissue from the male rats (ECETOC, 1997). However, while the appearance and localisation of these lesions strongly suggested a role for this protein, other aspects of the data did not support a 'classical' $\alpha_{2\mu}$-globulin mechanism (reviewed by ECETOC, 1997).

Results from further research into the mechanism underlying this effect have since been reported, and are reviewed below.

Prescott-Matthews *et al* (1997) determined whether MTBE induced $\alpha_{2\mu}$-globulin nephropathy and enhanced renal cell proliferation in male, but not female, rats. Male and female F344N rats were exposed (6 h/d) to 0, 1,440, 5,400 or 10,800 mg/m³ MTBE vapour for 10 consecutive days. Microscopic examination of kidney tissue revealed epithelial cell necrosis, protein droplet accumulation and karyomegaly within proximal renal tubules from exposed male rats with a statistically significant trend for proximal tubular necrosis. These effects were not present in exposed females or in either control group. There was also a concentration-dependent increase, statistically significant following Fisher's exact test, in the accumulation of protein droplets (Mallory's Heidenhain stain) within the proximal tubules of treated male rats in the mid- and high-dose groups. Immuno-histochemical staining, specific for $\alpha_{2\mu}$-globulin, showed positive staining confined to protein droplets within renal proximal tubules from control and MTBE-exposed male rats. However, while the degree of immuno-staining was slightly greater in male rats exposed to MTBE in comparison to controls, no clear, linear exposure-related increase was apparent within the male treatment groups. No positive staining was seen in any of the female groups. Cell proliferation, as assessed with BrdU immuno-histochemistry, was increased in a dose-dependent manner in renal cortex (location of the P2 segment of the proximal tubule, considered a critical target for accumulation of $\alpha_{2\mu}$-globulin) from all male rats exposed to MTBE, whereas a less pronounced effect was seen when other (non-critical) segments of the proximal tubule were included in the analysis. No enhanced renal cell proliferation was detected in female rats. The results demonstrate that MTBE produced a mild $\alpha_{2\mu}$-globulin nephropathy and cell proliferation in selected regions of the renal proximal tubule in male rats after repeated inhalation of MTBE vapour.

In a related series of *in vitro* experiments, Poet and Borghoff (1997) isolated different kidney fractions from male and female F344 rats (age 10 - 12 wk) to better characterise their role in protein binding. A concentration-dependent, saturable partitioning of MTBE in kidney homogenate was seen in preparations from male rats. No partitioning was noted for female rat kidney (degree indistinguishable from that of Tris buffer), but it appeared after addition of exogenous $\alpha_{2\mu}$-globulin to the homogenate. The addition of β-lactoglobulin (a protein in the same family as $\alpha_{2\mu}$-globulin) or albumin did not influence the degree of partitioning of MTBE in homogenates of female rat kidney. Binding of MTBE to male rat kidney homogenate fractions or to purified $\alpha_{2\mu}$-globulin was heat sensitive and lost from both samples within 6 hours of the start of dialysis. Although these findings supported a role for $\alpha_{2\mu}$-globulin, somewhat surprisingly ^{14}C-labeled MTBE did not co-elute with this or any other protein when incubates of male rat kidney cytosol and proteins were separated using ion exchange chromatography. Dissociation from $\alpha_{2\mu}$-globulin and subsequent evaporative loss of MTBE from the ion exchange column was one possible explanation for this finding. This hypothesis was tested using a mathematical model to estimate a dissociation constant (K*d*) for MTBE and $\alpha_{2\mu}$-globulin, and a "best- fit result" of 2.15×10^{-4} mol/l was obtained for MTBE.

By comparison, *d*-limonene oxide (a "classical" male rat-specific kidney carcinogen, with a strong affinity for $\alpha_{2\mu}$-globulin) had a K*d* that was three orders of magnitude smaller (6.2×10^{-7} mol/l). Overall, while these data provide good evidence that MTBE binds to $\alpha_{2\mu}$-globulin present in male rat kidney, its loss from homogenates during equilibrium dialysis, together with the relatively high K*d* value, indicate that binding of MTBE to $\alpha_{2\mu}$-globulin is weak.

ECETOC (1997) noted that while renal lesions seen in male rats following repeated inhalation of MTBE vapour were consistent with many of the diagnostic criteria for $\alpha_{2\mu}$-globulin-induced nephropathy, others (in particular the absence of a demonstrable dose-related increase in immuno-staining for $\alpha_{2\mu}$-globulin, together with no $\alpha_{2\mu}$ globulin proteinaceous casts at the junction of the proximal tubules and the thin Loop of Henle) were not. The studies described above confirm that $\alpha_{2\mu}$-globulin is involved in the response of the male rat kidney to MTBE. However, the association between MTBE and $\alpha_{2\mu}$-globulin is comparatively weak, giving a tissue response that does not consistently conform to either the pattern or strength of that seen with more active $\alpha_{2\mu}$-globulin-binding agents such as *d*-limonene oxide or 2,2,4-trimethyl-2-pentanol. Since $\alpha_{2\mu}$-globulin-induced nephropathy and subsequent carcinogenesis are considered to be male rat specific effects, the kidney tumours seen in the rat bioassay are not considered to be relevant to human health risk assessment.

Rat oral intubation study

The carcinogenic potential of MTBE following ingestion was investigated by Belpoggi *et al* (1995). Groups of 60 male and female Sprague-Dawley rats (in-bred, Bentivoglio Castle colony) were administered 1 x/d, 4 d/wk (Monday, Tuesday, Thursday, Friday) MTBE in extra virgin olive oil by gavage at dose levels of 0, 250 or 1,000 mg/kgbw/d 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.

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/kgbw/d, compared with the control and low dose groups. In contrast, treated female rats, 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. No non-neoplastic changes were detected in any of the tissues from the treated animals.

These results support a NOEL for MTBE of at least 1,000 mg/kgbw/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/kgbw/d.

Neoplastic effects

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

A significant, dose-related increase in lymphomas and leukaemias (when combined) 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.

In 1997 this study was re-evaluated and the pathological material re-examined according to US-NTP criteria. This time, the authors concluded that there was sufficient evidence that the lymphomas and leukaemias in females were derived from the same stem cells, thus justifying the combining of the numbers of these tumours to produce a result of statistical significance (Belpoggi *et al*, 1998).

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

Evaluation

Due to deficiencies in the design and reporting of this study in 1995, the ECETOC Task Force considered the NOAELs 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.

The only significant treatment-related lesion noted in male rats after oral administration of MTBE for 104 weeks was an increase in Leydig cell tumours of the testes. However, interpretation of these findings is difficult, 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. A full discussion of the questions relating to the incidence of these tumours and their relevance to man is provided in Section 4.1.2.6.

In combination, the number of lymphomas and leukaemias in female rats was considered a significant tumour effect following oral administration of MTBE. However, evaluation of these findings is complicated by the apparently low control incidence recorded in the study, 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 analyses is given of the individual occurrences of different types of leukaemias and lymphomas are given. A discussion of the reporting of this tumour incidence, and of the doubtful nature of this as a treatment-related result, is provided in Section 4.1.2.6.

It is relevant to note that there was no evidence of genotoxicity for MTBE when tested *in vivo* (rat bone marrow cytogenetic assay), nor were any comparable effects seen following chronic inhalation exposure, at an equivalent retained dose.

Overall, the evaluation of the oncological findings from this lifetime study is complicated by the limited reporting of the findings. In particular, information on the historical control incidence for the principle lesions described by the authors is missing. Thus, the Task Force considers this study 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. The potential role of the metabolites formaldehyde and TBA is reviewed below.

Carcinogenicity of formaldehyde

Formaldehyde is a normal cell constituent that plays a pivotal role in cellular metabolism via the one-carbon pool. It is highly probable that the capacity of these various metabolic processes would effectively trap and utilise the formaldehyde released during catabolism of MTBE, and in this way prevent genotoxic or cell damage. This hypothesis is supported by *in vivo* studies following repeated administration of MTBE; no evidence was seen in rodents of cytogenetic damage in bone marrow or blood cells, or of unscheduled DNA synthesis in rat liver. On this basis, formation of formaldehyde from MTBE in the body can be considered as being unlikely to cause genotoxic damage.

Several long-term drinking water studies did not provide any evidence that formaldehyde is a systemic carcinogen. The first of these by Til *et al* (1989) was a 2-year drinking water study in Wistar rats with doses administered of 0, 1.2, 15 and 82 mg/kg/bw/d in males and 0, 1.8, 21 or 109 mg/kgbw/d in females. No evidence of systemic carcinogenicity was found. The second study by Tobe *et al* (1989) was also carried out over 2 years in Wistar rats, with drinking water formaldehyde concentrations of 0, 0.02, 0.10, and 0.50% (actual doses not reported). Various non-neoplastic lesions were observed in high dose animals, along with ulcerative lesions and hyperplasia in the forestomach; a few of these

lesions were also observed in the middle dose group. No abnormalities were noted in the lowest dose group and a NOAEL of 0.02% (equivalent to 10 mg/kgbw/d) was established. Takahashi *et al* (1986) reported a possible tumour promotion effect for formaldehyde in a 10-month study in Wistar rats following initiation with N methyl-N' nitro-N nitrosoguanidine. This is likely to be the result of localised irritation and necrosis in the stomach since it is confined to the gastro-intestinal tract. There was no evidence of systemic carcinogenic potential. However, Soffritti *et al* (1989) described haemolymphoreticular neoplasms in male and female rats after oral administration in drinking water (10 - 2,500 mg/l). On the basis of this latter investigation it has been suggested that formaldehyde may have been responsible for the increase in combined leukaemias/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 questioned (Feron *et al*, 1990, 1991) and were not considered 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.

Carcinogenicity of TBA

Groups of 60 B6C3F_1 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/kgbw/d in males or 0, 510, 1,015 or 2,105 mg/kgbw/d in females (US-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/kgbw/d in males, and 1,015 mg/kgbw/d in females.

TBA was administered to F344 rats (60/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/kgbw/d) in males and 0, 2.5, 5 or 10 mg/ml (approximately equivalent to 0, 175, 330 or 650 mg/kgbw/d) 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), 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/kgbw/d in males (renal tubular adenoma/carcinoma) and at least 650 mg/kgbw/d in females (no increased tumour response noted).

Evaluation

Results from genotoxicity studies with TBA (Section 4.1.2.5) showed no mutagenic activity in bacterial (*Salmonella typhimurium*, 4 strains) or mammalian (L5178 mouse lymphoma line) cells *in vitro*, either 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 (US-NTP, 1995), although no supporting data were available.

In rats, the nephropathy seen in males showed features consistent with hyaline droplet accumulation and $\alpha_{2\mu}$-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.

4.1.2.7 Overall discussion and evaluation of carcinogenicity studies

The genotoxicity of MTBE and its metabolites has been assessed in a comprehensive range of test systems (Section 4.1.2.5). The weight of evidence indicates that MTBE and TBA are not genotoxic *in vivo*. In contrast, formaldehyde has a proven potential to damage DNA, but when formed from MTBE in the body it will be detoxified rapidly, minimising its operational genotoxic potential. The Task Force has concluded that genotoxicity is unlikely to play a role in the neoplastic findings reported in chronic studies with MTBE.

For the purpose of the following discussion, the findings of the chronic/carcinogenicity studies (Section 4.1.2.5) are recapitulated in Table 4.23, together with an explanation of why the tumour incidences reported are not necessarily considered evidence of a carcinogenic hazard to humans. The rat and mice inhalation studies were terminated at 2 years and 18 months duration, respectively, whereas in the oral study, rats were treated for 2 years and then kept until they died or became moribund.

Table 4.23: Chronic/carcinogenicity studies with MTBE in experimental animals

Species, route / Duration/ Strain, group size, sex	Concentration or dose	Sex, tissue, tumour	Possible explanation	Reference
Rat, inhalation				
2 y				
F344, 50 M+F	0, 1,440, 10,800, 28,800 mg/m^3	M, kidney, tubular adenoma and carcinoma	$\alpha_{2\mu}$-Globulin accumulation, specific to male rat	Chun *et al*, 1992; Bird *et al*, 1997
		M, testis, interstitial cell adenoma [a]	Control incidence lower than historical control incidence, which is typically 64 to 98%. Relevance to man questionable.	
Rat, oral gavage				
2 y, then until death or moribund				
Sprague-Dawley, 60 M+F	0, 250, 1,000 mg/kgbw	M, testis, interstitial cell adenomaa	Higher tumour incidence in top dose animals, related to longer survival than controls. Relevance to man questionable.	Belpoggi *et al*, 1995
		F, haemolympho-recticular, lymphoma and leukaemia	Not raised above real spontaneous incidence in historical data (low incidence in controls). Analysis of combined tumour types questionable.	
Mouse, inhalation				
18 months				
CD1, 50 M+F	0, 1,440, 10,800, 28,800 mg/m3	M + F, hepatocellular adenoma and carcinoma	Liver toxicity and alterations in hormone balance at top dose. Responses only observed at exposures >> MTD. Proposed mechanism related to hormone imbalance.	Burleigh-Flayer et *al*, 1992; Bird *et al*, 1997

[a] Leydig cell tumour

Kidney tumours in male rats

In male F344 rats given MTBE by inhalation, the incidence of renal tubular cell adenomas combined with carcinomas was statistically different from controls (1/50) in the intermediate group (8/50 at 10,800 mg/m³) but not in the top dose group (3/50 at 28,800 mg/m³). There were no carcinomas in the top dose males. Hence the increase in kidney tumours in male rats given MTBE by inhalation is only marginal and weakly treatment-related. The evidence of involvement of $\alpha_{2\mu}$-globulin accumulation and renal cell proliferation in the proximal tubule suggests that this effect is not relevant to humans. No increase in kidney tumours was found in the oral study on MTBE.

Leydig cell tumours in male rats

Testicular interstitial cell tumours occur spontaneously at high incidence in F344 rats and have been reported to affect 90% of untreated rats on average (range 64 - 98%) in the US-NTP historical control database. Incidence is strongly age-related, with 24% and 63% of rats dying between 12 and 18 months and 18 months to 2 years, respectively (Haseman *et al*, 1990).

In the inhalation study on F344 rats the incidence of interstitial cell tumours of the testis was 32/50 (64%) in controls, 41/50 (82%) in the intermediate group (10,800 mg/m³) and 47/50 (94%) in the top dose group (28,800 mg/m³). Hence, compared with the background incidence of this tumour in rats from the same source, the incidence in controls was low and the incidence in the highest treatment group is within the historical range (64 - 98%). Thus, the significantly higher incidence of this tumour in the top treatment group is likely to be an artefact of the low incidence in controls and not a true treatment-related effect.

In the gavage study on Sprague-Dawley rats, the only significant treatment-related lesion noted in male rats after oral administration of MTBE for 104 weeks was an increase in Leydig cell adenomas. Incidences of interstitial Leydig cell adenomas were reported to be 2/26 in control rats, 2/25 in the low dose animals and 11/32 in the high dose animals. Although the incidence of tumours in the top dose animals was significantly increased compared with the controls, more of the high-dose animals survived longer than 2 years (the animals treated with MTBE in this study were only dosed for 4 d/wk because of toxicity). From survival curves provided by Belpoggi *et al* (1995), it would seem that 90% of the controls had died by 120 weeks whereas only 68% of the top dose animals had died by this time (i.e. 32% of the top dose animals were surviving compared to 10% of controls). In view of the increased susceptibility of male rats to this tumour with age, the increase in incidence in the top treatment group is not considered to be due to MTBE

treatment. Additionally, interpretation of these findings is difficult since no diagnostic criteria have been presented to allow small Leydig cell tumours to be distinguished from focal hyperplasia; this may have led to an overestimation of the incidence of this tumour type reported. Hence, the design of this study, combined with a failure to conduct mortality adjusted analysis, prevents any conclusions being drawn with respect to tumour incidence.

It is concluded that neither study provides reliable evidence of carcinogenicity in rats based on interstitial cell adenomas (Leydig cell tumours) of the testes.

Relevance to humans

Approximately 1% of all human male neoplasms are tumours of the testis. Of this 1%, actual palpable Leydig cell tumours are rare, accounting for some 1 to 3% of testicular tumours. When Leydig cell tumours do occur, the vast majority (80 - 90%) are in the form of benign adenomas. This low level of Leydig cell tumour incidence in man would appear to be in marked contrast to that which is observed in rats. It has been noted that this type of tumour is frequently increased in rat cancer screening studies following treatment with approved pharmaceutical agents or dietary materials, yet is rarely reported in man, even after long-term exposure to the same substances (Bär, 1992; Prentice and Meikle, 1995). This would seem to indicate a difference in sensitivity or behaviour in Leydig cells between the two species.

There are several lines of evidence which suggest that human Leydig cells may indeed be less sensitive than rat Leydig cells in their proliferative response to chemicals, and this may explain the difference in incidence observed. The area has been comprehensively reviewed by Cook *et al* (1999). Their conclusions are as follows:

- "(1) the human incidence of Leydig cell tumours is much lower than in rodents even when corrected for detection bias; (2) several comparative differences between rat and human Leydig cells may contribute, at least in part, to the greater susceptibility of the rat to both spontaneous and xenobiotic-induced Leydig cell tumours; (3) endocrine disease states in man (such as androgen-insensitivity syndrome and familial male precocious puberty) underscore the marked comparative differences that exist between rats and man in the responsiveness of their Leydig cells to proliferative stimuli; and (4) several human epidemiology studies are available on a number of compounds that induce Leydig cell tumours in rats (1,3-butadiene, cadmium, ethanol, lactose, lead, nicotine) that do not demonstrate an association between human exposure to these compounds and induction of Leydig cell hyperplasia or adenomas."
- "…the weight of evidence suggests that human Leydig cells are quantitatively less sensitive than rat Leydig cells in their proliferative response to LH), and hence in their sensitivity to chemically induced Leydig cell tumours."

In addition to the above, another review of interstitial cell tumours of the testis (Prentice and Meikle, 1995) also concluded that increases in interstitial cell tumours in chronic rat studies "are most probably not predictive for man and human experience to date indicates that they cannot be considered as a relevant finding in terms of human safety assessment".

The testicular lesions reported in rats for MTBE could conceivably be consistent with an anti-oestrogenic activity, something to consider even though the actual incidence of the tumours is unlikely to be statistically significant (see above). It is also worth considering the results from a study by Williams *et al* (2000) (reported in Section 4.1.2.7). Administration of MTBE in rats produced mild perturbations in LH levels. However, even considering the likely sensitivity of the species to LH, it was impossible to ascertain whether this sort of hormonal change could result in induction of Leydig cell tumours on prolonged exposure.

Overall, the data would suggest that non-genotoxic compounds which induce these tumours in rats have low relevance to humans under most exposure scenarios.

Lymphoma and leukaemia in female rats

Belpoggi *et al* (1998) claimed that 20% and 10% incidence of the above combined tumours of in female Sprague-Dawley rats given MTBE orally at 1,000 mg/kgbw and 250 mg/kgbw, respectively, compared with the female control incidence of 3.3%, represent a tumorigenic effect of MTBE. Since the overall background incidence of these tumours is reported to be 10%, it would seem that the apparent increase in the females is due mainly to the low incidence in the female controls. In addition, it has been reported that the spontaneous incidence of leukaemia in Sprague-Dawley rats in the Bologna Institute of Oncology is 16 to 23% rather than 10% (Stern and Tardiff, 1997). This further substantiates the view that the apparent increase in incidence of these tumours in female rats is a chance finding, unrelated to treatment.

The practice of combining tumour types, such as lymphoma and leukaemia, for statistical analysis is considered to be highly questionable. It is known that a period of time of as little as 48 hours duration may be enough to make it impossible to differentiate between these two tumour types following cellular deterioration or autolysis. Hence, reliance must be placed on the protocols and on the experience of the interpreter.

In the criteria and guidelines for the analysis of tumour data from rodent carcinogenesis studies developed by the US-NTP (McConnell *et al*, 1986), it is indicated that tumours of different cellular origin should not be combined for the purpose of statistical analysis. However, it should be noted that the authors' use of a combined category for statistical analysis in this strain is supported by an IARC tumour classification scheme and also

by some historical data (California EPA, 1999b). In the subsequent pathology review of the 1995 oral study in rats, Belpoggi *et al* reported that all the haematopoietic neoplasms (lymphomas and leukaemias) observed in the original study were of lymphoid cell origin, supporting the combination of values to produce a statistically significant result. It is important to note that no independent re-evaluation has yet been carried out on these data, which would support the re-evaluation done by the group involved. Even if later analysis ultimately justifies combining these values, due to the limitations of the study this result in the cells of the immune system cannot be taken automatically to infer carcinogenic potential. Rather it merely suggests that such assumed potential cannot be ruled out in this sex, strain and species.

If these tumours were related to treatment with MTBE it would be reasonable to expect that they would be increased in both sexes. However, male rats show an opposite trend, with the control incidence being 16.7%, with the incidence in the low treatment group being 15% and that in the top treatment group 11.7%. Without a mode of action that adequately explains opposite trends in the two sexes, it should not be concluded this provides evidence of carcinogenicity for MTBE.

Additionally, the lack of increased lymphoma/leukaemia incidence reported in the inhalation study, in which relatively high doses of MTBE were given, would appear to cast doubt on MTBE being the cause of increased lymphoma/leukaemia in female Sprague-Dawley rats.

Thus, based on comparison to the historical control incidence, uncertainties in the scientific justification of combining the results, an opposite trend in male rats, and lack of substantiation in the inhalation study, it is unlikely that MTBE induces lymphoma/leukaemia in female Sprague-Dawley rats.

Liver hepatocellular adenoma and carcinoma in male and female mice

The mouse liver is known to be susceptible to spontaneous hepatocellular adenomas and carcinomas, though CD-1 mice are less susceptible than $B6C3F_1$ mice. When mice are treated with non-genotoxic agents that either produce liver damage or enzyme induction, an increased incidence of hepatocellular tumours often occurs, even though such materials do not appear to be carcinogenic to man. Hence if such tumours occur only at high dose levels at which liver enlargement, damage and/or enzyme induction are seen, this is not regarded as evidence for likely human carcinogenicity.

In the mouse study, involving inhalation of MTBE, the increase in liver tumours is confined to the top dose animals (28,800 mg/m^3 MTBE). In females, an incidence of 20% adenomas in the top dose, compared with an incidence of 4% in the controls. In males, an incidence of 16% carcinomas in the top treatment group compared with an incidence of 4% carcinomas in controls (the latter was not a statistically significant difference). The incidence of carcinomas was not increased in females and the incidence of adenomas was not increased in the males.

It has been reported that the incidence of adenomas in control CD-1 females ranges between 0 to 14% and the incidence of carcinomas in males ranges between 0 to 8%. Hence, the increased incidences reported are within, or barely outside, the spontaneous incidence range; furthermore both liver enlargement and cell enlargement (usually indicative of enzyme induction) were seen at this dose level. Hence the increased incidence of liver tumours in male and female mice was of questionable causation and, if treatment-related, it is most likely that a non-genotoxic mechanism (involving hormonal disturbances, enzyme induction and liver enlargement) was involved. As this process is not generally considered to be relevant to man, these marginal findings cannot be relied on as evidence of carcinogenicity to humans.

Conclusions

The tumours in two of the organs cited, the kidney tubular adenomas and carcinomas in male rats and the hepatocellular adenomas and carcinomas in mice, are unlikely to have arisen via a mechanism that is relevant to man.

The increase in testicular interstitial cell tumours is particularly doubtful as a treatment-related response; the incidence in the inhalation study utilising F344 rats was within the historical control range, and in Sprague-Dawley rats, increased survival and study deficiencies confound any observed increase. Importantly, there are also a number of physiological and toxicological reasons why this endpoint is of doubtful relevance to man.

The reported increase in incidence of leukaemias and lymphomas in female Sprague-Dawley rats is also doubtful as a treatment-related response as, in addition to being within the spontaneous tumour incidence range, the opposite trend was shown in males. It is more likely that this finding was due to a low incidence in the female control group. There are also doubts regarding the wisdom of combining these tumour types.

In addition to the above, at high dose levels, MTBE has been shown to cause some hormonal disturbances that could affect tumour incidences, e.g. testicular tumours and liver tumours. Such effects are unlikely to be relevant to man.

Accordingly, it is concluded that MTBE should not be classified as a Category 3 carcinogen, and that no classification for carcinogenicity is justified according to the criteria specified in Annex IV of Commission Directive 93/21/EEC (EC, 1993c).

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 $\alpha_{2\mu}$-globulin was 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 ECETOC Task Force believes that this plays no role in the induction of tumours seen following long-term exposure to MTBE.

IARC (1999) assessed the available data and came to the conclusion that "there is inadequate evidence in humans for the carcinogenicity of MTBE, that there is limited evidence in experimental animals for the carcinogenicity of MTBE, and that therefore MTBE is not classifiable as to its carcinogenicity to humans (Group 3)".

4.1.2.8 Toxicity for reproduction

The studies on developmental and reproductive toxicity are summarised in Appendix E.

Fertility

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

A 2-litter, single-generation inhalation study was carried out with Sprague-Dawley rats (Biles *et al*, 1987). To provide 2 litters/female at each treatment level, 15 F_0 males, pre-exposed for 12 weeks, (6 h/d, 5 d/wk) to MTBE vapours at 0, 900, 3,600 and 9,000 mg/m^3 were mated with 30 F_0 females exposed for 3 weeks prior to mating. Exposures were continued throughout a 15-day mating period, gestation (days 0 - 20) and lactation (days 5 - 21). 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 was 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.

In a 2-generation reproduction study 25 male and 25 female Sprague-Dawley rats were exposed (6 h/d, 5 d/wk) to MTBE vapours at target concentrations of 0, 1,440, 10,800, or 28,800 mg/m^3 (Neeper-Bradley, 1991). As with the previous study, animals were exposed during the pre mating, mating, gestation and postnatal periods. With both generations, exposures began 10 weeks before mating and were continued through mating until day 19 of gestation, and then during lactation (days 5 - 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. During certain periods of the pre-breeding 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 8-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 2 weeks of exposure. At 10,800 mg/m^3, weight gain in males was faster than that in 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 2 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 statistically significant 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 in days 7 to 14) was equivalent across groups. The total number of F_2 pups 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 to 4 and 4 to 28. Pup weight gains at 10,800 mg/m^3 were reduced on lactation days 7 to 14 and on 14 to 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.

A 2-generation reproductive study was carried out by Bevan *et al* (1997a) in which groups of 25 male and female Sprague-Dawley rats were exposed to MTBE by inhalation at 0, 400, 3,000 and 8,000 ppm (0, 1,430, 10,700 and 28,600 mg/m^3). The dose levels and dosing regime was similar to that of Neeper-Bradley (1991) above. There was parental toxicity at the upper two doses, and concomitant perinatal toxicity was also observed at these levels. Some toxicity was also recorded for pups in the F_1 and F_2 generations in terms of pup viability and survival, but this was not considered to be biologically significant.

Thus, the overall findings of this study were that no real treatment-related effects were recorded for any reproductive indices, i.e. there was no evidence of selective reproductive toxicity. These results support the results of the earlier multigeneration studies.

Development

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

In the first of these studies, groups of 25 pregnant CD-1 mice and Sprague-Dawley rats were exposed (6 h/d) to MTBE vapours at target concentrations of 0, 900, 3,600 and 9,000 mg/m^3 on days 5 to 6 of gestation (Schroeder and Daly, 1984a and b; Conaway *et al*, 1985). Food and water consumption were recorded and the dams killed 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 days 9 to 12 of gestation and in mice on days 12 to 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; Bevan *et al*, 1997b), groups of 15 pregnant albino rabbits were exposed (6 h/d) to MTBE vapour at target concentrations of 0, 3,600, 14,400 and 28,800 mg/m^3 on days 6 to 18 of gestation. Maternal clinical signs were recorded daily; body weights and food consumption were measured between days 0 to 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 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 28,000 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/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; Bevan *et al*, 1997b). Animals were exposed for 6 h/d on gestation days 6 to 15 and killed on day 18. Maternal clinical signs were recorded daily and body weights measured from day 0 to 18 of gestation. Maternal food consumption was measured throughout gestation. At scheduled killing, maternal body weight, gravid uterine weights and liver weights were recorded. Ovarian *corpora lutea* were counted, and all uterine implantation sites identified and assessed for early and late resorptions, dead 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. 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, hypoactivity and ataxia. Gestation parameters were affected at 28,800 mg/m^3 (reduced numbers of viable implantations/litter and altered sex ratio) and foetal body weights/litter (totals, males or females) 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 led to an increase in 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.

Conclusion

Effects of the inhalation of MTBE vapours on fertility and development have been evaluated in well-conducted inhalation studies with rats, mice and rabbits. In general, effects were the same as those described in repeat-dose sub-chronic studies.

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 and, reduced body weight, may have been secondary. No effects on fertility of parents and the F_1 generation were 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 organogenesis at 14,400 and 28,800 mg/m^3. It was not a developmental toxicant at any concentration examined, i.e. 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 concluded that these studies provided no indication that MTBE could adversely influence reproductive performance or foetal development at exposure levels that did not cause maternal toxicity.

Other effects on endocrine and reproductive systems

It has been proposed that a change in hormone homeostasis may have been responsible for the female mouse liver tumours seen in the inhalation bioassay (ECETOC, 1997), since oestrogens antagonise, and androgens promote, the expression of spontaneously arising hepatic tumours in female mice (Kemp and Drinkwater, 1990). This hypothesis has been tested subsequently by Moser *et al* (1998) who assessed endocrine-sensitive endpoints (weight of sex organs, oestrous parameters) from female B6C3F$_1$ mice (initial age, 8 wk) following exposure (6 h/d, 5 d/wk) to 28,800 mg/m^3 MTBE vapour for 3 days or 3, 12 or 32 weeks. The ability of MTBE to alter serum oestrogen levels and influence the expression and activity of the oestrogen receptor was also determined in a number of model systems. In view of its potential importance, results from this study are described and discussed in detail below.

There were alterations in the stages and length of oestrous cycle after 12 or 32 weeks of exposure to MTBE. At the latter time point, there was a significant increase in the mean number of days in both the oestrous and non-oestrous stages (both increased by around 50% relative to controls). Uterine weight relative to (decreased) body weight in mice exposed for 12 or 32 weeks was only 20% of that seen in the controls, and microscopic examination revealed a simplified structure (fewer uterine ducts and glands, less tortuous glands). Qualitatively, this effect was comparable to that seen in unexposed mice after ovariectomy. Measurement of uterine cell proliferation showed less DNA synthesis (as judged by BrdU incorporation), around 25% in luminal and 50% in glandular epithelial cells, respectively, after 12 or 32 weeks of treatment, versus 90% BrdU incorporation in both cell populations from controls. For comparison, less than 25%

of luminal and glandular epithelial nuclei in uteri from ovariectomised mice showed BrdU incorporation. Qualitatively similar effects on microscopic structure and BrdU incorporation were also detected in cervix and vagina from mice exposed to MTBE vapour for 12 or 32 weeks. Again, these findings were qualitatively similar to those seen in ovariectomised mice. Ovary weight relative to body weight was decreased by 55 to 60% after 12 or 32 weeks of exposure, but no significant changes in microscopic appearance or in BrdU uptake were reported.

Adrenal glands from exposed animals showed a loss of *zona reticularis* after 12 and 32 weeks of exposure (but not after 3 d or 3 wk), while there was an increase number of hyaline droplets in the *pars intermedia* of the pituitary which were immuno-reactive for adrenal corticotrophin hormone (ACTH). No differences were seen in BrdU staining in these two tissues. Neither was any significant difference in serum oestrogen levels apparent in treated and control animals matched for stage of oestrous cycle.

Studies *in vitro* showed that MTBE did not displace or compete with bound oestradiol from purified human oestrogen receptor, nor did it bind to human oestrogen receptor present in transected HepG2 cells (luciferase assay), or antagonise the activity of oestradiol in the HepG2 system (inactive at concentrations to 0.1 mM MTBE in all instances). No difference in oestrogen receptor immuno-reactivity was seen in uterus, cervix or vagina sections from MTBE exposed animals (coincidental with nuclei in all tissues) (Moser *et al*, 1998).

Day *et al* (1998) reported that gavage dosing of Sprague-Dawley rats at 40 mg MTBE/kgbw/ resulted in an elevation of serum corticosterone levels after 14 days. With a dose of 800 mg/kgbw, the level remained higher after 28 days. Investigation into other hormone levels revealed that there was a decrease in serum testosterone at this higher dose level but no accompanying change in luteinising hormone (LH). This was unexpected, given that LH is involved in the regulation of testosterone production within the testis. The elevated corticosterone levels observed here have been noted in other studies involving both other rat strains (F344) and females, e.g. following 13 weeks of exposure to MTBE at 4,000 and 8,000 ppm (14,400 and 28,800 mg/m^3) (Lington *et al*, 1997). Elevated aldosterone was also observed.

Williams *et al* (2000) carried out a follow-up study to that of Day *et al* (1998) investigating the potential for MTBE-induced changes to the levels of certain hormones and to organs such as the kidney, liver, testis, adrenals and pituitary gland in rats following oral administration. The objective was to examine any changes in the hypothalamus-pituitary-testicular axis. Sprague-Dawley rats were administered doses of 0, 250, 500, 1,000 or 1,500 mg MTBE/kgbw/d by gavage for 15 or 28 days. In the 15-day dosing groups, only the high dose animals displayed increases in relative kidney, adrenal and pituitary

weight. Rats dosed for 28 days were found to have statistically significant increases in liver weight in the top two dose groups, while no testicular lesions were observed, and the relative weights of the testes were only increased in the high dose group. Effects observed through microscopy ranged from mild and potentially adaptive changes in the liver (centrilobular hypertrophy), to cellular necrosis in the kidney, characteristic of $\alpha_{2\mu}$-globulin nephropathy. At 28 days, serum triiodothyronine (T3) was significantly reduced in the 1,000 and 1,500 mg/kgbw animals, while serum LH and dihydrotestosterone (DHT) were decreased with respect to the controls at 1,500 mg/kgbw only. Prolactin levels, measured in serum, decreased in rats dosed with 1,500 mg/kgbw/d for 15 days but not 28 days.

Allgaier and De Peyster (1999) carried out studies that looked at the effects on certain endocrine parameters of high dose MTBE exposure to Sprague-Dawley rats by gavage. It was noted that the 800 mg/kgbw doses were high enough to cause sedation and ataxia in the animals. The studies were designed to investigate the potential for effects on the hypothalamic-pituitary unit in the rat with respect to LH production and release from the anterior pituitary. The animals had been gonadectomised, and a group were then provided with implants to maintain artificially the plasma testosterone levels. Radio-immuno assay was used to measure LH levels, and this was done at 2 to 5 hours after dosing, and then again at 5 days post-dosing. There were no consistent or statistically significant differences between MTBE-exposed and vehicle control groups of animals. The authors reported that previous experiments had shown some changes in testosterone levels over a longer time period and speculated that prolonged exposure and more frequent monitoring might be necessary to detect subtle cumulative effects.

Another short-term oral dosing study investigating toxicity to the testes was carried out in mice dosed with MTBE in vegetable oil at 0, 400, 1,000 or 2,000 mg MTBE/kgbw, 3 times over a 5-day period (Billitti *et al*, 1999). Testosterone levels were determined before, during and after exposure, the testes weights were recorded, as was serum testosterone, and histopathology was carried out. The only histological difference between high-dose treated animals and controls was a small increase in gross disruption of the tubules. Faecal analysis allowed the determination of LH-releasing hormone (LHRH) and testosterone levels, however no treatment-related differences were observed. In similar test procedures, TBA and *tertiary*-butyl formate were tested for the same parameters, but again no significant differences in histology or hormone levels were observed.

These study results indicate that MTBE is capable of producing some changes in the levels of hormones and in some endocrine tissues, however it remains unclear whether these changes could result in effects such as Leydig cell hyperplasia and/or tumours as might have been anticipated had the LH levels been suitably increased.

Evaluation

The tissue effects noted by Moser *et al* (1998) appear to be related to a change in oestrogen homeostasis in female mice following repeated exposure to high concentrations of MTBE vapour over several months. The authors suggest that this effect is consistent with "anti-oestrogenicity", i.e. that MTBE prevents oestrogen having an effect on oestrogen-dependent tissues. A decrease in DNA synthesis only in uterine epithelial cells, mimicking the changes seen in ovariectomised mice, was considered to support an anti-oestrogenic mechanism. However, *in vitro* studies showed that MTBE did not displace or compete with oestrogen for binding sites on the oestrogen receptor, nor did it decrease serum oestrogen levels, suggesting that an alteration in hepatic catabolism was not involved. No disturbance of the ovarian-pituitary axis (responsible for other aspects of oestrogen control) seemed to have occurred, since ovarian maturation and proliferation appeared normal in treated mice and there was a functional oestrous cycle (albeit of increased length). Normal litters had also been delivered in a rat 2-generation study. It is not known if the microscopic changes in structure of the adrenal and pituitary glands were linked to these changes in oestrogen-responsive tissues, or whether they were coincidental.

By analogy with US-EPA physiological constants for the $B6C3F_1$ mouse, it is possible to estimate the internal dose of MTBE in these studies. A body weight of 24.6 g, an inhalation rate of 0.04 m³/d and retention of 15% (ECETOC, 1997) equates to 1,756 mg/kgbw over a 6-hour exposure period. In contrast, worker exposure at an OEL of 90 mg/m³ would lead to a retained internal dose of 5.1 mg/kgbw (ventilation volume 1.25 m³/h, 8-h shift, 70 kgbw, lung retention of 40% of dose; ECETOC, 1997), with a 'worst-case' EU consumer exposure of 10 mg/m³ for 5 minutes giving an internal dose of 0.006 mg/kgbw (ECETOC, 1997). Hence the putative anti-oestrogenicity of MTBE in mice occurs at doses that are 344-fold greater than those experienced by workers, and around 30,000-fold greater than those experienced by consumers.

While it is not possible to derive a NOAEL from the Moser *et al* (1998) studies, the bioassay results showed no altered incidence of endometrial/uterine cystic hyperplasia in female $B6C3F_1$ mice following long term exposure to 10,800 mg/m^3 MTBE vapour. This is equivalent to a retained internal dose of 658 mg/kgbw, which is 129-fold or 110,000-fold greater than that likely to be experienced by workers or consumers, respectively.

In conclusion, although these findings may appear to indicate a putative role for anti-oestrogenicity of MTBE in female mice, it should be noted that these effects occur only after several months exposure to very high vapour concentrations (28,800 mg/m^3) of MTBE, a level in excess of the MTD found in chronic studies.

The results of the Williams *et al* (2000) study indicate that oral administration of MTBE at 1,500 mg/kgbw/d over 15 or 28 days can produce mild perturbations in the levels of circulating hormones and in certain hormone-sensitive tissues in the rat. It is not possible to ascertain from these results whether the continuation of treatment over a chronic period would result in the production of Leydig cell hyperplasia or tumours.

4.1.2.9 Neurotoxicity

Neurobehavioural effects have been reported in several studies that were designed to measure these effects and also in conventional repeated-dose studies.

Oral

In one acute oral gavage study (Arco Chemical, 1980) (Section 4.1.2.2 and Appendix C), some degree of central nervous system (CNS) depression occurred at all dose levels (1,900 - 6,810 mg MTBE/kgbw) ranging from slight at the low doses to marked at ≥ 4,080 mg/kgbw (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/kgbw/d, loss of righting reflex, ataxia at 3,160 and 2,450 mg/kgbw/d and CNS depression at 1,900 mg/kgbw. Onset of these effects was rapid, but they were transient, with animals returning to normal within a few hours.

In a conventional 14-day oral gavage study in Sprague-Dawley rats, high daily doses of ≥ 1,200 mg MTBE/kgbw 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 to 12 hours. Males given 1,428 mg/kgbw/d (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/kgbw/d for 28 days, caused occasional salivation in all groups and transient ataxia and/or hypoactivity at the two highest doses shortly after dosing. No histopathological lesions were observed in brain, spinal cord, or sciatic nerve (IIT, 1992).

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

Inhalation

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 hours exhibited lachrymation within 3 minutes. During the 4-hour exposure, signs progressed to ataxia, loss of righting reflex, hyperpnoea, lack of co-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 Chemical, 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 5 minutes (Hathaway *et al*, 1970b). Anaesthesia (loss of righting reflex) occurred at all doses between 125,000 to 800,000 mg/m^3, and all animals recovered except at 400,000 and 800,000 mg/m^3. 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) and Daughtrey *et al*, (1997) studied neurobehavioural effects in F344 rats (22/sex/group) exposed to 2,880, 14,400 and 28,800 mg/m^3 MTBE vapour for 6 hours. Motor activity was recorded and animals were subjected to a functional observation battery of tests (FOB). No changes in behaviour were 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/m^3, 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 at high doses corresponded with changes in the FOB at the mid- and high-dose levels, and suggested exposure-related but reversible CNS sedation. 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 for periods up to 6 h/d and the concentrations of MTBE in air were increased successively over 5 days from 12,400 to 341,000 mg/m^3. No behavioural 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 both 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 CNS effects (such as ataxia, prostration and tremors) 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 2 hours. 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 to 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 F344 rats and CD-1 mice, preparatory to a 13-week study (Dodd and Kintigh, 1989). Behavioural effects, including hypoactivity and periocular irritation were seen at all exposure levels, but principally at 28,800 mg/m^3. 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 FOB (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 (6 h/d, 5 d/wk) to MTBE vapours at 1,440, 10,800 and 28,800 mg/m^3 for 28 days, the daily neurological effects at ≥ 10,800 mg/m^3 were transient ataxia, hypoactivity, and lack of startle reflex (Chun and Kintigh, 1993).

Snamprogetti (1980) exposed (5 or 10 min/d, 5 d/wk) groups of Swiss mice at 288,000 mg/m^3 for 30 days 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; Neeper-Bradley, 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 liquid and vapour have been observed in several acute and repeated-dose oral gavage 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. Hyperactivity has also been reported at below 28,800 mg/m^3 (Gill, 1989; Daughtrey *et al*, 1997).

These behavioural effects are reversible when exposure ceases and have not been associated with gross structural changes to the nervous system. There was no evidence in animal studies of overt neurotoxicity due to exposure to MTBE at concentrations up to 10,800 mg/m^3 over 30 days (6 h/d, 5 d/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).

4.1.2.10 Human health effects data

Medical use and possible side effects

MTBE has been used successfully 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 up to approximately 500 ml (370 g) MTBE. The HEDSET contains some 30 references describing this clinical use of MTBE including its side effects (Arco Chemical Europe, 1997). The primary side effects reported in about 30% of the patients were nausea, vomiting and mild drowsiness. These signs disappeared quickly and completely and no lasting side effects were observed. Clinicians do not consider the side effects to be a serious disadvantage. Evidence of some systematic absorption is obtained from the detection of MTBE in blood and from the odour of MTBE in exhaled air.

During the introduction of MTBE into the bilary tract, spillages may occur and subsequent absorptions have been reported. For instance in one patient 21 ml (16 g) of 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.

Overall, 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. No cases of clinically significant haemolysis and renal failures have been reported from the standard application to several hundred patients.

Population studies following introduction of MTBE as oxygenate in gasoline

As a result of the Clean Air Act amendments of 1990 (US-EPA, 1990) the use of reformulated gasoline with MTBE at relatively high concentrations (10 - 15%, called oxygenated fuels, or simply oxyfuels) became mandatory in 1992 in 37 areas of the USA. Following the introduction of this new type of gasoline a number of health complaints were reported from users. Consequently, various Federal and State Health and Environmental Authorities commissioned a survey in Fairbanks (Alaska) of the potential health effects related to the introduction of the oxygenated fuel programme. This survey, and follow-up studies in Stamford, Albany, New Jersey and Wisconsin, are summarised in Table 4.24. Exposures were determined from MTBE concentrations in air at the workplace or from personal breathing zone samples.

Table 4.24: Survey and follow-up studies of potential health effects related to oxygenated fuels in the USA

General	Population Exposed	Reference	Exposure Assessment	Effects Assessment	Reference
Fairbanks, Alaska, 1992					
Questionnaire survey of 2 groups of volunteers considered to be exposed at work to gasoline; one group exposed to oxygenated fuel (Phase 1, Nov. 1992), the other surveyed after suspension of the oxygenated fuel programme (Phase II, February 1993)	18 individuals considered to be exposed to oxygenated fuel, including 10 service station/garage employees, plus 8 individuals who spent most of their workday with motor vehicles (Phase I).	28 individuals considered not to be exposed to oxygenated fuel, including 12 of the original participants (from Phase I), plus 16 additional volunteers (service station/garage employees).	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.	Moolenaar *et al,* 1994
Stamford, Connecticut, 1993					
Questionnaire survey of volunteers conducted during the first 2 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, 1993b

Table 4.24: Survey and follow-up studies of potential health effects related to oxygenated fuels in the USA (cont'd)

General	Population Exposed	Reference	Exposure Assessment	Effects Assessment	Reference
Albany, New York, 1993					
Questionnaire survey of volunteers conducted 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 no-oxygenated fuel, including 34 service station employees /mechanics, and 48 others, such as police man and tollbooth operators.	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.	CDC 1993c
New Jersey, 1992 - 1993					
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 1 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.	Mohr *et al*, 1994

Table 4.24: Survey and follow-up studies of potential health effects related to oxygenated fuels in the USA (cont'd)

General	Population Exposed	Reference	Exposure Assessment	Effects Assessment	Reference
Wisconsin, 1995					
Telephone questionnaire distributed to 3 randomly selected groups; 2 from areas using oxygenated fuel and 1 using traditional gasoline.	527 telephone responders from the Milwaukee metropolitan area and 485 from the Chicago metropolitan area, both with an oxygenated fuel programme in place during the study.	501 individuals from areas in the state of Wisconsin not having an oxygenated fuel programme.	MTBE was monitored in air from different, selected points; from 24-h samples in presumably high and low-level areas; 15-minute 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 3 groups.	Anderson *et al*, 1995

All studies used questionnaires that 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 (Table 4.25).

Table 4.25: Common symptoms that may be related to the use of oxygenated fuel in the USA *(Moolenaar et al, 1994)*

"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 or vomiting	Fainting or blackouts
Dizziness	Skin irritaion or redness
Disorientation[a]	Muscle aches
	Difficult breathing

[a] Reported as "spaciness"

Fairbanks (Alaska) study

Moolenaar *et al* (1994) studied 18 individuals exposed to gasoline with MTBE during their work (Phase I) of the study and 28 individuals, 12 of them participating also in Phase I, exposed to gasoline without MTBE (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 work shifts. Table 4.26 presents the scores of health complaints obtained from two groups of garage workers and service station attendants.

Table 4.26: Health complaints by individuals occupationally exposed to gasoline in Fairbanks Alaska *(Moolenaar et al, 1994)*

"Key" symptoms, considered related to MTBE	Phase I [a] Number	%	Phase II [b] Number	%
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
Disorientation c	6	33	0	0

[a] Individuals (n = 18) exposed to oxygenated fuel

[b] Individuals (n = 28) considered not exposed to oxygenated fuel

[c] Reported as "spaciness"

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 (Table 4.27). The maximum exposure concentration during Phase I was 370 mg/m^3 and in Phase II 130 mg/m^3.

Table 4.27: Concentration of MTBE (µg/l) [a] in blood of workers during Phase I and Phase II in Fairbanks, Alaska *(Moolenaar et al, 1994)*

	Phase I [b] Median	Range	Phase II [c] Median	Range
Pre-shift MTBE	1.15	0.1 - 27.8	0.21	< 0.05 - 4.35
Post-shift MTBE	1.8	0.2 - 37.0	0.24	< 0.05 - 1.44

[a] Approximate detection limit = 0.05 µg/l

[b] Individuals (n = 18) exposed to oxygenated fuel

[c] Individuals (n = 28) considered not exposed to oxygenated fuel

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 as follows:

- The authors consider the survey to be 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 had limited value as no personal monitoring measurements were carried out;
- the relatively high pre-shift MTBE-blood concentration given for Phase II might be due to problems of sampling and analysis (Moolenaar *et al*, 1994).

Other comments regarding this study are that the median exposure concentration reported for Phase I (37 mg/m^3) appears to be insignificant in the light of subsequent chamber studies (Section on human volunteer studies below). Furthermore, 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, no conclusions should be drawn on a possible causal association between the introduction of oxygenated gasoline and specific health effects.

Stamford (Connecticut) and Albany (New York) studies

In view of the shortcomings in the Fairbanks study, the US Centre for Disease Control (CDC, 1993b,c) undertook a follow-up survey in two other regions, Stamford (Connecticut) and Albany (New York) (Table 4.24). In Stamford, the oxygenated fuel programme had been introduced without any particular health complaints; in Albany oxygenated gasoline was not mandatory and was not used. The data obtained with the survey in Albany were used as a 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 groups of (male) subjects exposed to gasoline were composed of motor-car mechanics, professional (e.g. taxi) drivers and other professionals (e.g. meter readers) expected to be exposed regularly to gasoline vapour. The non-exposed group included commuters with a profession not related to car driving or fuel distribution, e.g. school teachers and students.

The results obtained with the questionnaire for the Stamford groups are presented in Table 4.28. The findings reported in Albany were similar.

Table 4.28: Prevalence of health symptoms potentially related to MTBE exposure in different jobs *(CDC, 1993b,c)*

"Key" symptoms	Control Commuters (n=59)	Exposed Professional drivers (n=57)	Motor mechanics/ service station attendants (n=48)	Others, e.g. meter readers (n=12)
	%	%	%	%
Headache:				
≥ 1 x	25.4	26.3	27.1	41.7
≥ 2 x	23.7	21.1	15.0	41.7
≥ 3 x	5.1	8.8	8.3	8.3
Eye irritation	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
Disorientation [a]	3.4	1.8	10.4	8.3
Nausea	0.0	0.0	2.1	8.3
≥ 1 "Key" symptoms	42.4	35.1	52.1	66.7
≥2 "Key" symptoms	13.6	7.0	22.9	50.0

[a] Reported as "spaciness"

In the Stamford study the median MTBE blood concentration was 0.12 µg/l in the non-exposed group, 1.73 µg/l for motor mechanics and 15.9 µg/l for service station attendants. The MTBE-blood levels correlated well 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 was detected in the blood of individuals not exposed to gasoline. Depending on the MTBE concentrations in blood, two groups were formed. 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.

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 (Table 4.28). 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. Another finding which sheds further doubt on a relationship between blood MTBE levels and occurrence of symptoms is the group of "others", with high reported symptom incidence but low blood MTBE concentrations.

New Jersey study

Mohr *et al* (1994) carried out a cross-sectional cohort study among gasoline-exposed garage workers in the state of New Jersey (Table 4.24). Beginning on 15 November 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. 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 (Alaska) and those noted in human clinical studies and in animal studies. The exposure concentrations were monitored as a control.

Exposure measurement over 1 hour revealed high MTBE concentrations in garages in the north (6 - 2 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 concern 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 each day. There were no differences in symptom reporting between these two groups.

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

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) (Table 4.24). The study was composed of (i) an air monitoring study, (ii) a study on the composition of gasolines on the market in Milwaukee and Chicago, (iii) health complaints received by the State Health Department and (iv) a random telephone enquiry about health complaints using a standardised questionnaire.

This review only deals with the results of the latter part (iv) 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 approximately 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 24 February and 19 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 and was highest in Milwaukee, followed by Wisconsin and then Chicago. The prevalence of other symptoms, such as backaches and fever not previously associated with gasoline exposures were also higher in the Milwaukee region. These symptoms were scored as "unexplained" health symptoms, meaning symptoms unrelated to cold, flu or to any other chronic health problem (Table 4.29).

Table 4.29: Awareness a of the "Oxygenated Fuel Programme" in Wisconsin *(Anderson et al, 1995)*

Question [b]	Chicago [b]	Milwaukee [b]	Wisconsin [c]
Do you live in an Oxygenated Fuel Programme area ?	105 (21%) [e]	409 (78%) [e,f]	13 (3%)
Purchased oxygenated fuel since 1 November 1994	51 (10%)	264 (50%) [e,f]	13 (3%)
Heard of MTBE	113 (23%) [e]	283 (54%) [e,f]	199 (40%)
Saw "Day One" on TV 19 January 1995	14 (3%)	29 (6%) [f]	25 (5%)
Saw week-long local TV series on MTBE	1 (0.2%) [e]	99 (19%) [e]	16 (3%)
Noticed different smells from gas	96 (20%)	275 (52%) [e]	77 (15%)
Saw other news stories about the Oxygenated Fuel Programme	40 (8%)	187 (35%) [e]	106 (21%)

[a] Number of respondents and percentage

[b] Directly or indirectly related to the introduction and media coverage of the Oxygenated Fuel Programme

[c] Required use of oxygenated fuel

[d] No oxygenated fuel requirement

[e] Different from Wisconsin, $p < 0.05$

[f] Different from Chicago, $p < 0.05$

The definitions of the symptoms were similar to the ones in other studies (Table 4.25). The symptoms were scored for potential correlation with exposure to gasoline and Milwaukee scored highest in all respects (Table 4.30).

Table 4.30: Prevalence a of health symptoms by region *(Anderson et al, 1995)*

Symptom	Since 1 November 1994			At time of interview		
	Chicago	Milwaukee	Wisconsin	Chicago	Milwaukee	Wisconsin
Headache	13 (3%) [b]	67 (13%) [c]	9 (2%)	9 (2%) [b]	58 (11%)	6 (1%)
Nausea	4 (1%) [b]	39 (7%) [c]	4 (1%)	2 (0.4%) [b]	27 (5%)	2 (0.4%)
Eye irritation	9 (2%) [b]	36 (7%) [c]	4 (1%)	6 (1%)	35 (7%)	3 (1%)
Dizziness	7 (1%) [b]	44 (8%) [c]	5 (1%)	6 (1%) [b]	34 (6%) [c]	1 (0.2%)
Diarrhoea	2 (0.5%) [b]	27 (5%) [c]	4 (1%)	0	20 (4%) [c]	3 (1%)
Rashes	3 (1%) [b]	23 (4%) [c]	2 (0.5%)	2 (0.4%) [b]	20 (4%) [c]	2 (0.4%)
Muscle ache	2 (1%) [b]	19 (4%) [c]	5 (1%)	2 (0.4%) [b]	15 (3%) [c]	5 (1%)
Throat irritation	8 (2%) [b]	47 (9%) [c]	4 (1%)	6 (1%) [b]	40 (8%) [c]	4 (1%)
Difficult breathing	6 (1%) [b]	27 (5%) [c]	2 (0.5%)	3 (1%) [b]	20 (4%) [c]	2 (0.4%)
Back pain	5 (1%) [b]	13 (2%) [c]	4 (1%)	2 (0.4%) [b]	10 (2%) [c]	4 (1%)
Fever	2 (0.5%) [b]	15 (3%) [c]	3 (1%)	2 (0.4%) [b]	7 (1%) [c]	0
Disorientation [d]	5 (1%) [b]	35 (7%) [c]	1 (0.2%)	4 (1%) [b]	29 (6%) [c]	1 (0.2%)
Sinus congestion	10 (2%) [b]	45 (9%) [c]	7 (1%)	7 (1%) [b]	40 (8%) [c]	6 (1%)
Funny smells	9 (2%) [b]	50 (9%) [c]	4 (1%)	6 (1%) [b]	44 (8%) [c]	2 (0.4%)
Other symptom	3 (1%) [b]	8 (2%) [c]	1 (0.2%)+	2 (1%) [b]	6 (2%) [c]	0 (0%)
Any unexplained sympton	30 (6%) [b]	119 (23%) [c]	32 (6%)	NA	NA	NA

[a] Number of respondents and percentage

[b] Not different from Wisconsin, $p < 0.05$

[c] Different from Wisconsin, $p < 0.05$

[d] Reported as "spaciness"

NA Not asked

Furthermore, 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 a cold, as presented in the last column (Table 4.31).

Table 4.31: Relative risk ratios a for symptoms among car owners and car owners with a cold *(Anderson et al, 1995)*

Symptom	Chicago	Milwaukee	Wisconsin [a]	Cold since 1 November 1994
Headache	1.5 [b]	7.9 [c]	1	2.2 [c]
Nausea	1.0 [b]	9.8 [c]	1	3.0 [c]
Eye irritation [b]	2.5 [b]	7.9 [c]	1	2.9 [c]
Dizziness [b]	1.5 [b]	10.6 [c]	1	1.9 [c]
Diarrhoea	0.5 [b]	6.5 [c]	1	3.2 [c]
Rashes	1.6 [b]	11.2 [c]	1	1.8 [b]
Muscle ache	0.6 [b]	3.7 [c]	1	1.7 [b]
Throat irritation	2.1 [b]	12.0 [c]	1	3.3 [c]
Difficulty breathing	3.1 [b]	13.3 [c]	1	12.0 [c]
Back pain	1.3 [b]	3.1 [c]	1	1.9 [b]
Fever [b]	0.4 [b]	4.0 [c]	1	9.7 [c]
Disorientation [e]	5.3 [b]	35.7 [c]	1	5.3 [c]
Sinus congestion	1.5 [b]	6.4 [c]	1	2.5 [c]
Funny smells	2.2 [b]	17.8 [c]	1	2.4 [c]
Other symptoms	3.2 [b]	7.5 [c]	1	2.2 [b]
Any unusual symptom	0.9 [b]	4.5 [c]	1	1.6 [c]

[a] Reference: Wisconsin car owners without a cold

[b] Not different from Wisconsin

[c] Different from Wisconsin, $p < 0.05$

[d] Age-adjusted

[e] Reported as "spaciness"

This table indicates that Milwaukee residents were between 3 and 35 times more likely to report "unexplained" symptoms that supposedly are 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 reported purchasing oxygenated gasoline and specific "unexplained" symptoms was only evident in Milwaukee.

In conclusion, there were clear differences in the reporting of symptoms 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 (Table 4.29). This heightened perception could have resulted in attributing any unusual health symptom to exposure to oxygenated fuel. 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.

This survey can be considered as a key study in design. Study and control populations were 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 those of Chicago and Wisconsin. 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.

Finnish studies at gasoline depots and service stations

The Finnish studies at gasoline depots and service stations (Hakkola and Saarinen, 1996 and 2000; Section 4.1.1.1 and 4.1.1.2) were designed to investigate the occurrence of neuropsychological symptoms and mood changes following occupational exposure to petrol vapour containing MTBE. The subjects, petrol tanker drivers and milk delivery drivers, were asked to fill in a questionnaire concerning moods (Profile Of Mood States).

The studies attempted to relate mood changes to exposure over the course of a working week. No differences in mood were observed between the two groups of petrol tanker drivers, however there was a greater increase in fatigue reported by the drivers which appeared to show a dose-response, and this was accompanied by symptoms such as headache and nausea. The loading phase for petrol tankers is believed to lead to levels of MTBE exposure high enough to account for any such effects, but no data are available at present, which tend to suggest that any non-transient effects are produced following such exposures to petrol vapour (Hakkola *et al*, 1997).

Population study related to exposure to MTBE via drinking and washing water

Vojdani *et al* (1997) examined the proportion of apoptotic lymphocytes present in blood samples from 60 human subjects (medical condition not defined, although the majority suffered from severe headaches, fatigue or exhaustion and cognitive dysfunction) in California (USA), previously exposed via drinking, bathing and showering to water containing MTBE (1 - 76 mg/l) and benzene (0.2 - 14 mg/l). The duration of exposure was 5 to 8 years, and the blood was analysed 10 to 12 months after exposure ceased. The control population comprised 32 unexposed healthy subjects. Lymphocyte DNA was labelled with fluorescein-deoxyuridine triphosphate and stained with propidium iodide, and apoptotic cells determined using flow cytometry.

The results showed that the rate of apoptosis in the "exposed" group was increased 80.5% over control, with arrest of the cell cycle from these individuals in the G2/M and S phases. A perturbation in molecular signalling, possibly involving nuclear factor Kappa-B, was identified as a possible underlying cause of these changes. The basis, and hence the reliability, of this exposure assessment was not reported. It is important to note that these exposure values resulted in doses which are well below the animal NOAELs. The biological significance of the findings cannot be interpreted, and any associative or causal relationship to MTBE exposure is unclear, given the likelihood of exposure misclassification bias occurring.

Human volunteer studies with controlled exposures to MTBE

A number of chamber studies 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 endpoints (Table 4.32).

Table 4.32: Human volunteer studies with MTBE

Concentration (mg/m^3)	Time(h) (h)	Number, sex	Ocular measurements	Nasal measurements	Other endpoints	Questionnaire	Reference
0, 6.1 [a]	1	22 M 21 F	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 (polymorphonuclear cells) in nasal lavage	Neurobehavioual testing, concentrations in blood	Profile of mood state, air quality, subjective symptoms	Cain *et al*, 1994
0, 5.0	1	19 M 18 F	Pre- and post exposure tear film break-up time, markers of inflammation from conjunctiva	Inflammatory markers from nasal lavage.	Concentrations in blood	33 standardised items and symptoms [b]	Prah *et al*, 1994
18, 90 and 180	2 [c]	10 M	Blinking frequency, conjunctival epithelial damage, eye redness, tear film break-up time	Acoustic rhinometry, nasal and mouth peak expiratory flow, inflammation markers in nasal lavage	Concentrations in blood, TBA in blood	Rating intensity of discomfort, irritation symptoms [b] and CNS-effects on a visual analogue scale	Johanson *et al*,1995
0, 90 and 270	1,3	13 M	None	None	Reaction time, posturography	Subjective symptoms and mood	Riihimäki *et al*, 1996
18, 90 and 180	2 [c]	10 M	Blinking frequency, conjunctival epithelial damage, eye redness, tear film break-up time	Acoustic rhinometry, nasal and mouth peak expiratory flow, inflammation markers in nasal lavage		Rating intensity of discomfort, irritation symptoms [b] and CNS-effects on a visual analogue scale	Nihlén et al, 1998 b

[a] Positive control: VOC

[b] Symptoms included so-called key symptoms mentioned in the population studies

[c] Light physical exercise

Cain *et al* (1994) exposed volunteers to MTBE in a simulated worst-case consumer exposure at a fuelling station, using positive (VOC) and negative (clean air) controls. The objective measurements included eye irritation, nasal lavage and CNS-function. The findings suggested an increase in eye irritation, but the effect was observed in both dose groups and thus was not related specifically to MTBE. Nasal lavage analysis revealed the polymorphonuclear cells to be unaffected by MTBE vapours. Standard neurobehavioural tests showed no differences between the exposed groups. Similarly, the subjective responses obtained through a questionnaire, did not score differences between eye-, nose- or throat- irritation. The authors concluded that exposures of normal healthy young people to 6.1 mg/m^3 MTBE for 1 hour, apart from the MTBE odour, did not induce any reaction.

Prah *et al* (1994) exposed healthy volunteers to MTBE for 1 hour in a double-blind experiment simulating consumer exposure at a fuel station. 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) exposed volunteers for 2 hours to MTBE concentrations matching occupational exposure levels. 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.

Riihimäki *et al* (1996) exposed healthy male volunteers in an exposure chamber. The MTBE concentrations reflected those at the workplace. 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. 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 for 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. (Pekari *et al* (1996) reported on the metabolic aspects of this study (see Section 4.1.2.1). Nihlén *et al* (1998b) exposed healthy male volunteers to MTBE during light physical work to simulate an occupational setting. By means of a questionnaire, subjects were asked to report the degree of irritant symptoms, discomfort, and CNS effects before, during and after exposure. Answers were given on a 100 mm visual analogue scale, graded from "not at all" to "almost unbearable". Ocular and nasal measurements were performed mainly at the highest exposure level. The results of the study suggested no or minimal acute effects of MTBE vapour upon short-term exposure at relatively high levels.

In all cases, the reported studies in human volunteers were carried out under well-controlled laboratory conditions. As such they eliminate many of the biases and 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.

All studies in human volunteers were well-conducted and are acceptable for further assessment. It may be concluded on the basis of the available data for subjective or objective endpoints that the studies do not reveal adverse effects of MTBE on humans. Though eye-irritation was reported at 6.1 mg/m^3 after one hour, it was not reported at 183 mg/m^3 after 2 hours exposure in another study.

Studies involving symptoms caused by odour

In a series of epidemiological studies, the relationship between objective exposures 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 (cacosmia) of the five following common environmental chemicals: i) pesticide, ii) automobile exhaust, iii) paint, iv) new carpet, and v) perfume. Sixty-six percent of 643 students reported feeling ill from inhalation of one or more of the five odours (Bell *et al*, 1993).

Steinheider *et al* (1993) 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 subjective complaints (e.g. sleeplessness, headaches and stomach aches), graded for effect. After including moderating factors in multiple regression analysis, highly significant associations were found between the level of cortisol and odour exposure.

A group of 62 humans were exposed for 2.75 hours to a mixture of 22 VOCs (0, 5, and 25 mg/m^3) known to be indoor air pollutants. Continuous evaluation of irritation in eyes, nose and throat showed a significant correlation with exposure at 5 and 25 mg/m^3. The effect was acute and there were no signs of adaptation. A digit span performance test showed decreased scores during exposure (Molhave *et al*, 1986).

4.1.2.11 Conclusions from human studies

Information on the effects of human exposure to MTBE is available from clinical applications, population studies and controlled 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 poor.

The population studies mainly scored subjective exposure data. The data may relate to acute as well as repeated exposures; no clear distinction between the two could be made. The quality of the information is variable and ranges 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 Fairbanks (Alaska) population studies suggested a relationship between exposure to MTBE and health complaints. An objective correlation between exposure concentration and subjective effects needed to be present to test this hypothesis, but this could not be demonstrated. Subsequent studies (New Jersey and Wisconsin) showed no increase in the subjective effects which could be legitimately related to MTBE exposure. This is in line with experimental studies showing no specific effects at similar exposure concentrations, and with 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.

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 of short-term duration and at levels which could be considered relevant for consumer exposure at service stations or for exposure at the workplace.

One consistent finding in the human volunteers studies was related to the odour of MTBE (and an odour threshold could be established). Odour might have been the cause of the subjective responses in the population studies. Eye and respiratory irritation have been of 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 MTBE/m^3 is derived. At 270 mg/m^3 (3 h), subjective symptoms were reported such as slight irritant effects on mucous membranes and feeling of heaviness in the head.

The volunteer studies were 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 such as elderly people or children. Fiedler *et al* (1994) interviewed 14 patients with multiple chemical sensitivities (MCS), 5 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 for both MTBE- and non-MTBE-related situations. The report is inconclusive with regard to any specific role for MTBE.

Furthermore, although no systematic survey on sensitisation in humans has been conducted, there are no reported cases of human skin or respiratory sensitisation.

It is concluded that occupational and/or environmental exposure to MTBE does not cause acute or medium-term health effects. Any alleged relationship between MTBE exposures and subjective health complaints seem to be due to factors such as smell (including the smell of gasoline in general), and media coverage of the introduction of the oxygenated fuels programme, and do not seem to be directly related to the presence of MTBE in gasoline.

4.1.3 Risk characterisation

4.1.3.0 General aspects / Summary of toxicological and human health aspects

Toxicokinetics

The toxicokinetic properties of MTBE have been studied extensively. It is readily absorbed 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. The one exception to this uniform distribution is a species- and sex-specific accumulation which occurs in the male rat kidney, due to the affinity of MTBE for the protein $\alpha_{2\mu}$-globulin. Metabolism leads to two principal metabolites, i.e. TBA and formaldehyde. These are further metabolised and show no tendency to accumulate to any significant extent. TBA excretion in humans proceeds relatively slowly with a half-life of 8 hours. 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.

It is concluded that 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 toxicity

MTBE is of low acute toxicity in experimental animals by oral, dermal and inhalation routes. LD_{50} values exceed 2,000 mg/kgbw 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. MTBE causes reversible moderate to severe skin 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.

Mild respiratory irritation occurred at a concentration of 270 mg/m^3 for 3 hours in human volunteers, while a concentration of 180 mg/m^3 for 2 hours did not cause such effects. 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.

It is concluded that 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.

MTBE has been classified as "irritant" (Xi) and labelled with the corresponding R-phrase 38 (irritating to skin) in accordance with the provisions of the Dangerous Substances Directive 67/548/EEC (Section 1.5).

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 found consistently 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.

It is concluded that 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.

Repeated-dose toxicity

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/kgbw was determined for the rat in an oral sub-chronic study (conducted according to GLP), based on increased kidney weight in both sexes. Effects were reported in females at this level, however these were not considered dose-related and were not supported by histopathology or clinical chemistry results. A study, in male rats only, to investigate disturbances in endocrine parameters, revealed increased kidney weights at below this NOAEL; this is likely to be due to the sex-specific accumulation of MTBE

known to occur in the rat kidney. The 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 NOAELs 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.

It is concluded that the principal effects identified for MTBE following repeated 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/kgbw. 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/kgbw/d for male and female F344 rats, and 182 and 184 mg/kgbw/d for male and female mice, respectively.

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 unscheduled DNA synthesis 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* unscheduled DNA synthesis 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 principal 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.

It is concluded that the available evidence does not raise concern with regard to genotoxicity of MTBE.

Carcinogenicity

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 F344 rats and CD-1 mice. In the oral study, an increase in combined lymphoma/leukaemia incidence in female rats was reported, but deficiencies in the design and initial reporting have made interpretation of the significance of these results difficult. However, a re-evaluation of the material from this study in 1998 produced a slightly clearer picture, which indicated that the combining of the lymphomas and leukaemias might indeed be scientifically justifiable, as it produced an apparently statistically significant increase above background levels for this strain in the high dose group. It remains doubtful if this is a true treatment-related response as, in addition to being within the spontaneous incidence range, the opposite trend was shown in males. Due to the limitations of the study, such a result for carcinogenicity in the cells of the immune system cannot be taken automatically to infer carcinogenic potential; it merely suggests that such potential cannot be ruled out in this sex, strain and species.

The effect on Leydig cell tumour incidence after ingestion is regarded as specific to Sprague-Dawley rats (taking into account historical control incidence within F344 rats). The inhalation study in F344 rats demonstrated a tumorigenic response in the male kidney at 10,800 and 28,800 mg/m^3 (corresponding to 384 and 1,023 mg/kgbw/d, 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 F344 rats, treated via inhalation, was considered a background event, within the historic spontaneous range for this rat strain, whereas in Sprague-Dawley rats, the increased survival time confounds any observed increase. 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 females at 28,800 mg/m^3. This concentration corresponds to a daily retained dose of 1,824 mg/kgbw, a level in excess of the MTD, and a non-genotoxic mechanism appears to be involved. This increase in the incidence of benign liver tumours at such a high dose is considered to occur by means of a mechanism not relevant to humans.

It is concluded that MTBE induces tumours in rodents at doses exceeding the MTD. Since genotoxicity does not appear 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.

MTBE does not require classification as a carcinogen according to the criteria presented in Annex IV of Commission Directive 93/21/EEC (EC, 1993c).

IARC has concluded that methyl *tertiary*-butyl ether is not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 1999).

Reproductive, developmental and endocrine 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 9,000 mg/m^3 or 28,800 mg/m^3, respectively. In mice, the NOAEL for developmental and maternal toxicity was 3,600 mg/m^3. At higher concentrations (14,400 and 28,800 mg/m^3) foetotoxicity was observed and the incidence of one malformation (cleft palate) was increased. These effects are not considered as direct effects on the foetus but secondary to concurrent maternal toxicity (reduced body weight and clinical signs of CNS effects such as hypoactivity, and ataxia).

Studies looking at the effects of MTBE on endocrine parameters showed some potential for changes to hormone levels in animals. However, these hormone level alterations were slight, well within the boundaries of homeostatic change, only occurred at high doses and their biological and toxicological significance remains unclear. No firm conclusions could be drawn from the data at this time.

It is concluded that MTBE is not a selective reproductive toxicant in animals and there is no evidence for a 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 the 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 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 2 hours. Exposure to 270 mg/m^3 for 3 hours caused mild mucous membrane irritation in some volunteers.

There are no indications that MTBE can act as a skin or respiratory sensitiser in humans. Although no systematic survey on sensitisation in humans has been conducted, no cases of human skin or respiratory sensitisation have been reported.

No objective CNS effects were observed in experimental volunteer studies at 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.

It is concluded that 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 occurring 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 endpoints and NOAELs

Table 4.33 summarises the conclusions with regard to MTBE-related effects observed after short- and long-term exposure of humans and experimental animals.

Table 4.33: Principal adverse effects and NOAELs in MTBE inhalation studies

Endpoint/ Species	Exposure time	Principal effects	NOAEL (mg/m^3)	Remarks	Reference
Acute toxicity					
Man	2 h (during light physical exercise)	Mucous membrane irritation	180	Subjective symptoms, e.g. slight irritation and heaviness in the head at 270 mg/m^3 for 3 h	Johanson *et al*, 1995; Riihimäki *et al*, 1996
Subchronic toxicity					
Rat	90 d	Liver and kidney toxicity (M)	2,880	Equivalent to 228 mg/kg bw/d (M)	Dodd and Kintigh, 1989
Chronic toxicity and carcinogenicity					
Rat	105 wk	Liver and kidney toxicity, kidney tumours (M)	1,440	Equivalent to 102 mg/kg bw/d (M)	Chun *et al*, 1992
Mouse	18 months	Liver and kidney toxicity, liver tumours (F)	10,800	Equivalent to 669 mg/kg) bw/d (F)	Burleigh-Flayer *et al*, 1992
Neurotoxicity					
Rat	6 h	Functional CNS effects	2,880	LOAEL 14,400 mg/m^3; effects reversible	Gill, 1989
Effects on fertility					
Rat	2 generations	No treatment-related effects	> 28,800	NOAEL 1,440 mg/m^3 for parental toxicity	Neeper-Bradley, 1991
Developmental toxicity					
Mouse	Gestation day 6 to 15	No direct effect on the foetus	3,600	Higher concentrations caused maternal toxicity and secondary developmental toxicity	Tyl and Neeper-Bradley, 1989

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 characterisation of MTBE. Mild respiratory irritation occurred at a concentration of 270 mg/m^3 for 3 hours in human volunteers, while a concentration of 180 mg/m^3 for 2 hours did not cause such effects. The lowest NOAEL for liver and kidney effects after chronic inhalation exposure was 102 mg/kgbw/d (retained dose in males).

4.1.3.1 Workers

MTBE production

Production is associated with relatively low exposures (< 10 mg MTBE/m^3 over 8 h), loading operations may result in higher concentrations (> 20 mg/m^3, peaks around 200 mg/m^3). Assuming an inhalation volume of 10 m^3/8-h shift and a relative respiratory uptake of 40%, exposure to 10 mg/m^3 would result in a retained amount of 40 mg MTBE/d corresponding to a dose of 0.57 mg/kgbw/d for a 70 kg adult. The lowest NOAEL from chronic animal inhalation studies is 102 mg MTBE/kgbw/d. Comparison of these two doses leads to an approximate 180-fold MOS for workers involved in production. Similarly, for loading operations, a 90-fold MOS is apparent. Comparison of the peak exposures of about 200 mg/m^3 with the concentration that caused mild mucous membrane irritation and slight heaviness in the head of some volunteers for a short period of time (270 mg/m^3 for 3 h) does not indicate concern.

Blending, distribution and handling of gasolines containing MTBE

Mean short-term exposure concentrations measured for loading and delivery of gasoline containing 10 to 15% MTBE were between 13 to 91 mg MTBE/m^3, with a maximum of 226 mg/m^3. These values were well in excess of the measured values for blending operations, which indicated maximal worker exposures of up to 9.5 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 loading and delivery operations lasted around 30 minutes, with mean short-term exposure concentrations 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 minutes would result in a retained amount of 25 mg MTBE/exposure period, corresponding to a dose of 0.36 mg/kgbw. Assuming a worst case of 4 exposures/d, this would lead to a daily dose of 1.44 mg/kgbw. The lowest NOAEL from chronic animal inhalation studies is 102 mg MTBE/kgbw/d. 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 that caused slight mucous membrane irritation in some volunteers (270 mg/m^3 for 3 h) does not indicate concern.

Service-station attendants and garage workers

Monitoring data from the USA show a highest mean exposure of approximately 3.5 mg MTBE /m^3, with a highest individual value of 7.56 mg/m^3. Assuming a ventilation volume of 10 m^3/8-h 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 MTBE/d,

corresponding to a dose of 0.2 mg/kgbw/d for a 70-kg adult. The lowest NOAEL from chronic animal inhalation studies is 102 mg/kgbw/d. Comparison of these two doses leads to an approximate 510-fold MOS 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.

Recommended occupational exposure limit

An occupational exposure limit of 90 mg MTBE /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/kgbw (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 exposed to a concentration of 180 mg/m^3 for 2 hours no effects were observed, while at 270 mg/m^3 for 3 hours only slight irritating effects on the mucous membranes were reported in some volunteers. Therefore, a concentration of 3 times the TWA (270 mg MTBE /m^3 or 75 ppm) is considered to be an appropriate short-term, peak exposure limit (15-min STEL).

Overall result statement

For the worker population exposed to MTBE it is considered that there is at present no need for further information and/or testing and for risk reduction measures beyond those already being applied.

4.1.3.2 Consumers

On the basis of the limited information available, mean short-term exposures during car refuelling are calculated as 6 mg MTBE /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 (Table 3.2). 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%. This results in a dose of about 0.006 mg/kgbw/d for an adult human (70 kg). Comparison with the NOAEL of 102 mg MTBE/kgbw/d obtained in chronic inhalation studies indicate a margin of safety of approximately 17,000 for consumers in this situation.

Overall result statement

For the consumer population exposed to MTBE, it is considered that there is no need at present for further information and/or testing and for risk reduction measures beyond those already being applied.

4.1.3.3 Humans exposed indirectly via the environment

Risk characterisation for indirect exposure of the general public has been carried out following the TGD (Section 2.4, p. 51 - 55 and Appendix VII) by calculating the MOS, i.e. the ratio between the total daily intake and the relevant exposure parameter, in this case the lowest subchronic oral NOAEL of 300 mg/kgbw/d. It is assumed that man is exposed throughout his lifetime. A subchronic NOAEL (or LOAEL) is only used if no chronic value is available, and such cases need extra attention in the evaluation of the MOS. The MOSs calculated for MTBE for the different scenarios are shown in Table 4.34.

Table 4.34: MOS for indirect human exposure to MTBE in different scenarios

Local scenario/Use Pattern	Total MOS (all routes and/or media)	MOS by air
1. MTBE used as a fuel additive		
Production	4,460	8,140
Formulation	4,460	8,140
Processing	5,230	7,810
Private use	35,200	45,900
2. MTBE used in production of isobutylene		
Processing	1,840	4,57
3. MTBE as a speciality solvent		
Processing	6,610	14,200
Regional scenario	45,600	46,500

The results show that all MOSs are several orders of magnitude greater than one, which implies that there is no inherent risk.

Overall result statement

For members of the general public exposed indirectly to MTBE it is considered that result ii) applies, i.e. there is at present no need for further information and/or testing and for risk reduction measures beyond those already being applied.

4.2 Human health (physico-chemical properties) [a]

4.2.1 Exposure assessment

4.2.1.1 Occupational exposure

Not discussed here.

4.2.1.2 Consumer exposure

Not discussed here.

4.2.1.3 Indirect exposure via the environment

Indirect exposure via the environment, and the influence on this of key physico-chemical properties, is dealt with in detail in other sections of the report. Assessment of the indirect exposure via the environment to other physico-chemical properties such as explosivity, flammability and oxidising potential is not relevant for this particular substance and its modes of use.

4.2.2 Effects assessment: hazard identification and dose (concentration) - response (effect) assessment

4.2.2.1 Explosivity

MTBE can form explosive mixtures with air. Autoflammability is recorded as occurring at 425°C (Table 1.1).

4.2.2.2 Flammability

MTBE is highly flammable and combustible, with the following flammability limits in air: 1.5 - 8.5% (Table 1.1).

4.2.2.3 Oxidising potential

No data are available.

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[a] Risk assessment concerning the properties listed in Annex llA to the ESR

4.2.3 Risk characterisation

4.2.3.1 Workers

For the worker population exposed to MTBE via the environment it is considered that result statement ii) applies.

4.2.3.2 Consumers

For the consumer population exposed to MTBE it is considered that result statement ii) applies.

4.2.3.3 Man exposed indirectly via the environment

For the human population exposed to MTBE via the environment it is considered that result statement ii) applies.

5. RESULTS

MTBE is produced in large amounts and is widely used, mainly as a fuel oxygenate and octane booster in gasoline. MTBE is readily detected by analytical methods and by taste and odour due to its low organoleptic thresholds in air and water. The solubility in water is high and it is also very volatile.

The available hazard data reviewed in this report indicate that, although not readily biodegradable, MTBE is inherently biodegradable. It is not dangerous to aquatic and other environmental organisms. The toxicokinetic data in experimental animals do not give any reasons for concern with regard to bioaccumulation of MTBE or its metabolites, effects on the CNS, genotoxicity or potential effects on reproduction. Skin and respiratory irritation are the only concern for human health. There is insufficient evidence for MTBE to be classified as a carcinogen.

5.1 Environmental risk assessment

An environmental risk assessment for all uses of MTBE was carried out in accordance with the TGD, and by means of EUSES for use of MTBE as a fuel oxygenate, as a production intermediate for isobutylene and as a speciality solvent. Where measured emission data were available, realistic worst-case emission factors were estimated and used in the EUSES calculations in place of the TGD default values. The assessment produced acceptable (< 1.0) RCRs and acceptable (> 1.0) MOSs for all stages of the life-cycle, except for two situations. These were the local water and sediment compartments for Use Pattern 2, processing in high-purity isobutylene synthesis. The action recommended was to consider carrying out sediment toxicity testing and to implement a statistically designed sampling and analysis programme to measure concentrations of MTBE in wastewaters from sites using MTBE for isobutylene synthesis.

Overall, the results of the risk assessment, using the factors described in this report, indicate that there is a low environmental risk of using MTBE as a fuel additive, process intermediate or solvent.

5.2 Human health risk assessment

Irritation after short-term exposure in humans, as well as liver and kidney toxicity after long-term exposure in experimental animals, are considered to be the critical effects for the health risk characterisation of MTBE. The basis for the risk characterisation is a comparison of three different doses/concentrations for these effects with occupational and consumer exposure data. This produced MOSs between 90 and 180-fold for workers involved in MTBE production, about 70-fold for workers handling gasolines containing MTBE, and between 250 and 510-fold for service station attendants and garage workers.

A 17,000-fold margin of safety was calculated for consumer exposure during car refuelling. Compliance by workers with the short- and long-term occupational exposure limits for MTBE of 270 mg/m³ (75 ppm) and 90 mg/m³ (25 ppm) respectively is considered likely to be protective regarding the above effects.

The risk characterisation produced here for occupational and consumer exposure to MTBE does not indicate concern for human health.

For humans exposed indirectly via the environment, all MOSs are greater than one, which implies that there is no inherent risk.

There are no risks to human health from the physico-chemical properties of MTBE.

In all, it is considered that for all human populations, exposed to MTBE via all exposure scenarios, there is at present no need for further information and/or testing and for risk reduction measures beyond those already being applied.

6. REFERENCES

This report combines an earlier health risk characterisation of MTBE (ECETOC, 1997) and an environmental risk assessment using EUSES (Watts and Mitchell, 2001) with a compilation of data collected and validated in the HEDSET and IUCLID (Arco Chemical Europe, 1997). These data sources were complemented by searches on the Internet, databases such as Aquire and US Geological Survey gasoline oxygenate bibliography [a], and the open scientific literature.

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APPENDIX A. GLOSSARY

Abbreviation/Acronym	Description
BCF	Bioconcentration factor: ratio between the concentration in an organism and the concentration in an environmental compartment. Bioconcentration is the net result of the uptake, distribution and elimination of a substance in an organism due to water-borne exposure.
BrdU	Bromodeoxyuridine (incorporation): immuno-histochemical technique
BUN	Blood urea nitrogen
EC_{50}	Median effective concentration: 1. Concentration resulting in a 50% change in a parameter (e.g. algal growth), relative to the control. 2. Concentration at which a particular effect (e.g. *Daphnia* immobilisation) is observed in 50% of the organism population, relative to the control.
EC_x	Similar to EC_{50}, but for x% effect; x usually is 0, 10, or 100%
ERA	Environmental risk assessment
EUSES	European Union system for the evaluation of substances
GLP	Good laboratory practice
HEDSET	Harmonised electronic data set: for data collection under the Existing Substances Regulation [a]
HPVC	High production volume chemical
IC_{25}	Inhibition concentration, estimated to cause 25% reduction in organism performance relative to control
IUCLID	International uniform chemical database: to manage the data collected under the Existing Substances Regulation [a]
kgbw	Kilogram bodyweight
kgww	Kilogram wet weight
LC_{50}	Median lethal concentration: A statistically derived concentration that can be expected to cause death in 50% of the animals exposed for a specified time. This can either be a concentration in the environment (water, soil, sediment, air) or a concentration in food (for bird or mammalian tests)
LD_{50}	Median lethal dose: statistically derived expression of a single administered dose of a material that can be expected to cause death in 50% of the dosed animals
LH	Luteinising hormone
LOAEL	Lowest-observed-adverse-effect level: the lowest concentration or amount of a substance, found by experiment or observation, which causes an adverse alteration of morphology, functional capacity, growth, development, or life span of the target organism distinguishable from normal (control) organisms of the same species and strain under defined conditions of exposure

[a] Council Regulation (EEC) 793/93 on the evaluation and control of the risks of existing substances

APPENDIX A. GLOSSARY (cont'd)

Abbreviation/Acronym	Description
Local PEC	As local PEC, the concentrations from the local distribution models are taken, adding the concentrations from the regional model as background level
LOEC	Lowest-observed-effect-concentration: the lowest concentration with adverse effects
log K_{ow}	Log of the octanol-water partition coefficient (K_{ow} or P_{ow})
LUST	Leaking underground (gasoline) storage tank
MOS	Margin of safety: the risk characterisation ratio (RCR) of a suitable effect or no-effect parameter value (e.g. an acute, oral LD_{50}, a sub-chronic, inhalatory NOAEL or a chronic LOEC in food) and a human exposure value of corresponding time scale and route of exposure. This is the inverse of the RCR for the environment
MTBE	Methyl *tertiary*-butyl ether
NOAEL	No-observed-adverse-effect level: the greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development, or life span of the target organism under defined conditions of exposure
NOEC	No-observed-effect concentration: the highest concentration without adverse effects.
PEC	Predicted effect concentration: the estimated amount of substance in a particular environmental compartment to which an organism is exposed
PNEC	Predicted no-effect concentration. A PNEC is regarded as a concentration (in environment or food) below which an unacceptable effect will most likely not occur
ppbv	Parts per billion (by volume)
RCR	Risk characterisation ratio: the ratio of the PEC to the PNEC for effects to environmental organisms
SCE	Sister chromatid exchanges
STP	Sewage treatment plant: across the European Union, taken as a whole, approximately 70% of the municipal wastewater volume (domestic and industrial loads) is treated in a biological wastewater treatment plan. In EUSES, the fate of a chemical in a communal STP is modelled by means of a non-equilibrium, steady state box model, known as SimpleTreat
TBA	*tertiary*-Butyl alcohol
TGD	Technical Guidance Document in support of EU risk assessment of new and existing substances (EC, 1996)

APPENDIX B. SAMPLING AND ANALYSIS PROTOCOLS FOR COLLECTION OF EMISSION DATA FOR PRODUCTION AND FORMULATION PLANTS

B.1 Introduction

The Finnish Competent Authorities issued a letter (dated 29th June 2000) to all companies manufacturing MTBE requesting more exposure data from MTBE production sites.

The ECETOC-EFOA Task Force for MTBE Risk Assessment compiled the following protocol to help ensure the precision and accuracy of the measurements of MTBE concentrations in samples from production sites.

B.2 General conduct and focus of monitoring

Monitoring is to be focused on the points on production and formulation sites representing combined emissions to air and water, and background samples.

- Existing monitoring programmes and the requirements of appropriate authorities are to be taken into account, refining these guidelines as needed.
- Site conditions are to be taken into account.
- Replicate samples have to be taken to quantify variations in sample composition.
- Appropriate quality assurance procedures are to be followed.
- Precise documentation on what measurements were taken, and how they were taken, is important (what has been measured, where and for how long, what method of sampling and analysis was used etc.).

B.2.1 Water samples

- In effluent monitoring, concurrent samples taken at points before and after any on-site WWTP are crucial.
- In receiving waters, it is advisable to take samples from points downstream of emissions from the site discharge pipe both before and after major dilution.
- The low odour/taste threshold (20 - 50 μg/l) may be used to focus sampling.
- Occupational monitoring data from stationary or personal measurements.
- MTBE concentration in ambient air at a distance of 100 m from the most significant point source of the industrial site and from the border of the industrial site during an emission episode is needed (2 - 8 hour averages).
- Additional information, (e.g. measured or estimated emission rates (g/s) from different point sources (e.g. five most important sources) as well as total annual MTBE emissions to air), is valuable. The emission rate allows to the average concentration of MTBE in the air at different distances to be estimated.

- Regular monitoring data for VOCs emitted from the site of concern may be added as supplementary information, particularly if data based on confirmed correlation with MTBE concentrations are available.
- Measurements of background samples (e.g. from upwind stations) may be informative also for quality control.

B.3 Frequency, timing and duration of monitoring

- For monitoring of surface waters, it is in many cases customary to take samples 4 - 5 times/year. For particular purposes, higher or lower frequencies may be used (e.g. control of highly variable fluxes, preliminary mapping of emissions or occurrence). Even in groundwater monitoring, coverage of the seasons may be needed.
- Sampling is to be focused on periods of high flow according to the process and hydrological variations to gain results representative of the main fluxes.
- The duration of the sampling programme varies according to the goals and context of monitoring; generally monitoring extending over several years is needed to discern developments and to account for year-to-year variation. However, shorter periods may be sufficient to establish the overall situation and for areal mapping of contamination.
- Duration of individual sampling incidents likewise varies; in many cases transient "grab" samples can be taken. Due to volatilisation, composite samples can be used only to a limited degree. However, some level of time integration may be achieved e.g. by using sorbents (in solid phase extraction).
- It is advisable to take samples at least over a period of 1 month, 2 replicate samples/week (=16 samples altogether) from each sampling location.
- For ambient air samples, the frequency and total duration of monitoring likewise varies according to the case (e.g. process variations), and to the context and purposes of monitoring.

B.4 Sampling, field measurements and sample pretreatment

- Avoid analyte losses and sample contamination
- In connection with sampling, measurements of important environmental conditions and sample properties (e.g. in case of water samples the flow rate, depth, suspended solids, pH and other physico-chemical parameters and in case of air samples the distance from the production facility) are important. For STPs, the size and capacity of the plant (e.g. in terms of average and maximum hydraulic flow rates) are needed in addition to instantaneous flows.

B.4.1 Water samples

- With groundwater, flushing of wells prior to sampling and attention to sampling equipment and techniques (e.g. minimisation of gas exchange) are essential to ensure representative samples.
- Samples are collected in clean, rinsed, low-sorbing glass containers, overflown and filled completely (with no headspace); or alternatively, if analysis using sampling from headspace is to be used, in special septum-capped vials. Sample containers, as well as sampling equipment and techniques, are selected and prepared in co-operation with the analytical laboratory analysing the samples.
- Samples are closed and stored in the cold and dark and are transported promptly (overnight) to the laboratory for analysis.
- Samples are marked sufficiently at all stages to ensure appropriate identification.

B.4.2 Air samples

Sampling can be performed according to the following procedure (Vainiotalo *et al*, 1998):

- Use KC 222-3 pumps (SKC, Eighty Four PA, USA) which are pulling air through charcoal tubes (SKC 226-01; 150 mg of absorbent) at 0.2 l/min (8-h air samples or 0.1 l/min (16-h air samples). Samples must be kept at a temperature of < 20°C.
- Sample pretreatment and analyses.
- The charcoal tubes are treated with carbon disulphide for desorption, and the eluate is analysed as described in following section.

B.5 Chemical analysis

- MTBE is commonly measured by gas chromatography with flame ionisation (GC-FID), or mass spectrometry (GC-MS) detection particularly when greater sensitivity and/or specificity is required (low concentration ranges, complex mixtures).
- GC-MS may be carried out after separation and concentration by purge and trap (US-EPA method 524.2 and its modifications and USGS method; detection limits ca. 0.06 - 0.09 μg/l) or by solid-phase extraction (Achten and Püttmann, 2000).

Using direct aqueous injection, screening-level detection limits of 1,000 μg/l have been obtained using GC-FID, while minimum concentrations in the linear range below 100 μg/l have been reached by GC-MS in the selective ion monitoring mode (Hong *et al*, 1999b; see also review by Achten and Püttmann, 2000).

APPENDIX C. ACUTE TOXICITY IN EXPERIMENTAL ANIMALS

Route/ Species	Result	Observations	Reference
Oral, gavage	**LD_{50} (mg/kgbw)**		
Rat	3,800	Deaths occurred at > 3,000 mg/kg (doses 2,000 and 10,200 mg/kgbw)	Hathaway *et al*, 1970a
Rat	3,866	Some degree of CNS depression at all doses (1,900 - 6,810 mg/kgbw). At 1,900 mg/kgbw diarrhoea; at 2,450 mg/kgbw ataxia, tremors, laboured breathing; at 3,610 mg/kgbw ataxia, loss of righting reflex, tremors; at 4,080 mg/kgbw as at lower dose, gastro-intestinal tract irritation and laboured breathing, 6/10 animals died; at 6,810 mg/kgbw all animals died	Arco Chemical, 1980
Mouse	4,000	None	Little *et al*, 1979
Dermal	**LD_{50} (mg/kgbw)**		
Rat	6,800 [a]	Transient hyperactivity	Shell, 1971
Rabbit	> 10,000	None	Arco Chemical, 1980
Rabbit	> 10,200	Initial pain followed by transient hyperactivity (3 - 5 min), local irritation, no deaths at all doses (6,800 and 10,200 mg/kgbw)	Hathaway *et al*, 1970a
Inhalation	**LC_{50} (mg/m^3), time**		
Rat	85,000, 4 h	At 44,000 and 395,000 mg/m^3, effects of treatment: hyperactivity, lachrymation, salivation, ataxia, tremors and unconsciousness	Hathaway *et al*, 1970a
Rat	120,300, 4 h	MTBE 99.1% pure, At all doses (68,100 - 230,500 mg/m^3) hypoactivity and ataxia in all animals within minutes. At 68,100 mg/m^3 all rats prostrated or ataxic by end of exposure. Upon cessation of exposure, survivors rapidly recovered. At > 68,100 mg/m^3 CNS effects, tachypnoea and nasal discharge	Arco Chemical, 1980
Rat	142,030, 4 h	MTBE 96.2% pure. Inco-ordination, hypoactivity, lachrymation and prostration at all concentrations (70,700 - 201,000 mg/m^3). Rapid recovery in survivors.	Arco Chemical, 1980
Mouse	400,000 [b], 5 min	Transient anaesthesia in some mice at all concentrations (125,000 - 800,000 mg/m^3). At 400,000 and 800,000 mg/m^3 2 and 4 animals, respectively, died. EC_{50} was 200,000 mg/m^3	Hathaway *et al*, 1970a

APPENDIX C. ACUTE TOXICITY IN EXPERIMENTAL ANIMALS (cont'd)

Route/ Species	Result	Observations	Reference
Inhalation	**LC_{50} (mg/m^3), time**		
Mouse	720,000	LT_{50} was 5.6 minutes	Snamprogetti, 1980
Mouse	648,000 [b], 10 min	Whole-body exposure. Toxicity was dose dependent	Snamprogetti, 1980
Mouse	141,000, 15 min	None	Marsh and Leake, 1950
Monkey, rhesus	0, 12,400 - 341,000, 6h / d, 5d	Up to 18,000 mg/m^3 no effects. At 38,800 mg/m^3 ataxia at 65 min. At 68,600 mg/m^3 ataxia, emesis, prostration, unconsciousness at 58 min. At 112,000 mg/m^3 ataxia, prostration, tremors, and bradypnoea at 50 min. At 173,000 mg/m^3 unconsciousness at 43 min. At 341,000 mg/m^3 unconsciousness at 24 min; apnoea after 85 min. All effects reversed to normal upon cessation of exposure.	Hathaway *et al*, 1970c
Intravenous	**LD_{50} (ml/kgbw)**		
Rat	0.56	Lethal within 5 min, preceded by hyper-salivation, urination, defecation and respiratory disorders. Survivors had CNS effects (various, as above), reversed within 15 - 20 min after cessation of exposure.	Snamprogetti, 1980
Subcutaneous [c]	**LD_{50} (mg/kgbw)**		
Mouse	3.6	None	Snamprogetti, 1980
Rat	6.7	None	Snamprogetti, 1980
Intraperitoneal [c]	**LD_{50} (mg/kgbw)**		
Mouse	1.36	None	Snamprogetti, 1980
Rat	1.67	None	Snamprogetti, 1980

[a] Reported as > 5 ml/kgbw

[b] Reported as 18 % in air

[c] Single injection

APPENDIX D. REPEATED-DOSE TOXICITY IN EXPERIMENTAL ANIMALS

Route / Species, strain duration	Exposure regime	Dose or concentration	Result, NOAEL	Observations	Reference
Oral		**(mg/kgbw/d)**			
Rat, Sprague-Dawley	2 wk	0, 357, 714, 1,071, 1,428	714	NOAEL based on M kidney effects. All dose levels: diarrhoea, from d 3. At 1,071 mg/kgbw increased relative kidney weights (M); increased haemoglobin and haematocrit (M). Decreased monocyte numbers (M). At 1,428 mg/kgbw transient anaesthesia (recovery within 2 h); reduced body-weight gain; relative kidney weights; hyaline droplets; decreased blood urea nitrogen (F); absolute and relative lung weights decreased. No effects on other main organs, immune of reproductive tissues	Robinson *et al*, 1990
Rat, Sprague-Dawley	4 wk	0, 90, 440, 1,750	90	NOAEL based on M kidney effects. At 90 mg/kgbw: increased relative kidney weight (F). At 440 mg/kgbw hyaline droplets; increased relative kidney weights (M); mean red blood cell count increased (M); occasional CNS effects (salivation, hypoactivity, ataxia). At 1,750 mg/kgbw slight increase in relative liver weight; increased relative kidney weight (M and F) and relative adrenal weight (M); mean corpuscular volume (of erythrocytes) increased (F); increased serum cholesterol; localised gastro-intestinal irritation; no gross or histopathological effects on lung	IIT, 1992

APPENDIX D. REPEATED-DOSE TOXICITY IN EXPERIMENTAL ANIMALS (cont'd)

Route / Species, strain duration	Exposure regime	Dose or concentration	Result, NOAEL	Observations	Reference
Oral		**(mg/kgbw/d)**			
Rat, Sprague-Dawley	90 d	0, 100, 300, 900, 1,200	300	NOAEL based on M kidney effects. 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 d 3 decreased BUN and increased blood cholesterol at all doses. Occasional salivation, ataxia, hypoactivity in all treated groups immediately after dosing. At 300 mg/kgbw increased relative kidney weight (F), glucose and Co^{++} decreased. At 900 mg/kgbw increased absolute kidney weights (M); increased relative liver and kidney weights. At 1,200 mg/kgbw profound transient narcosis; increased monocytes, decreased mean corpuscular volume (of erythrocytes) (M); hyaline droplets with severe tubular changes (M); increased red blood cell count, haemoglobin, haematocrit and decreased white blood cell count (F); increased absolute and relative lung and kidney weight (M), relative liver weight (M), adrenal weights (F).	Robinson et al, 1990
Inh**alation**		**(mg/m^3)**			
Rat, F344	6 h/d, 13 d	0, 7,200, 14,400, 28,800	7,200	No deaths. Clinical signs in all groups but primarily at 28,800 mg/m^3. Effects included ataxia, hypoactivity, periocular irritation and reversible behavioural changes (ataxia, decreased startle and pain reflexes and/or muscle tone) in F+M. At 14,400 and 28,800 mg/m^3 decreased body-weight gain, and increase of relative weights liver, kidney and adrenals. No gross or histopathological changes.	Dodd and Kintigh, 1989
Rat, Charles River	6 h /d, 5 d/wk, 2 wk	0, 10,000, 30,000	> 30,000	No deaths, no clinical signs, no effects on body weight, no effects on haematology, clinical chemistry or urinalysis. No effects on main organs, including CNS.	Hathaway et al, 1970a
Rat, F344	6 h /d, 5 d/wk, 2 wk	0, 1,440, 10,800, 28,800	10,800	NOAEL based on decreased body-weight gain at 28,800 mg/m^3. No deaths. At 10,800 mg/m^3 increased tubular epithelial cell proliferation after 5 days (M).	Chun and Kintigh, 1993

APPENDIX D. REPEATED-DOSE TOXICITY IN EXPERIMENTAL ANIMALS (cont'd)

Route / Species, strain duration	Exposure regime	Dose or concentration	Result, NOAEL	Observations	Reference
Inh**alation**		**(mg/m³)**			
Rat, Sprague-Dawley	6 h/d, 5 d/wk, 9 d	0, 360, 1,080, 3,600, 10,800	360	At 360 and 1,080 mg/m³ no effects. At 3,600 and 10,800 mg/m³ chronic inflammation of nasal mucosa and trachea (27/40 rats). Serum phosphorus increased (F); blood urea nitrogen and urine analysis normal. Statistically significant increased relative liver weights (10,800 mg/m³, F+M).	Terrill and Daly, 1984
Rat, Wistar	5 - 10 min/d, 5 d/wk, 30 d	0, 180,000, 288,000	> 288,000	No treatment-related increase in deaths or clinical signs. Body weight, food intake, similar in all groups. No differences between control and treated groups in clinical chemistry, urine analysis, blood, organ weight and motor activity co-ordination.	Snamprogetti, 1980
Rat, Wistar	10 min/d, 5 d/wk 120d	180,000	> 180,000	Same end points as above, except for behavioural changes. No treatment-related changes.	Snamprogetti, 1980
Rat, Sprague-Dawley	6 h/d, 5 d/wk 13 wk	0, 900, 1,800 3,600	NS	No deaths. No treatment-related effects on haematology, clinical chemistry, urinalysis, organ weights (at 3,600 mg/m³ slight decrease in absolute and relative lung weight in F, but not treatment-related), gross or histopathology. Dose-related anaesthesia.	Greenough *et al*, 1980
Rat, Sprague-Dawley	6 h/d, 5 d/wk 13 wk	0, 2,880, 14,400, 28,800	NS	At 2,880 mg/m³ mild haematological changes, not treatment-related. At 14,400 mg/m³ hypoactivity, decrease in hind leg grip (M). At 28,800 mg/m³ 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 (M); haematocrit and neutrophils increased in F; increased incidence of lymphoid hyperplasia in lymph nodes (M).	Dodd and Kintigh, 1989

APPENDIX D. REPEATED-DOSE TOXICITY IN EXPERIMENTAL ANIMALS (cont'd)

Route / Species, strain duration	Exposure regime	Dose or concentration	Result, NOAEL	Observations	Reference
Inhalation		(mg/m³)			
Rat, F344	4 wk	0, 1,440, 10,800, 28,800	1,440	NOAEL based on liver, kidney effects. At 1,440 mg/m³ no treatment related effects. At 10,800 mg/m³ increased absolute and/or relative liver weight (both sexes); increased absolute and relative kidney and adrenal weight (F only); increased, reversible protein accumulation in kidney detected histopathologically in M; . At 28,800 mg/m³ ataxia; decreased body-weight gain in M (reversed during recovery); increased urine volume; increased absolute and/or relative liver weight (F + M), kidney weight (M), adrenal weight (F); decreased absolute and/or spleen weight (F + M); increased protein accumulation in kidney (M); reversible increase in renal cell proliferation in M (d 31 only). No evidence of $\alpha_{2\mu}$-globulin from antibody staining.	Chun and Kintigh, 1993
Mouse, CD-1	6 h/d, 1 - 2 d	0, 28,800	28,800	No deaths, no other adverse effects.	Vergnes and Kintigh, 1993
Mouse, CD-1	6 h/d, 5 d	0, 1,440, 10,800	1,440	No effects at 0 and 1,440 mg/m³. At 10,800 mg/m³ ataxia, hypoactivity and loss of startle response.	Chun and Kintigh, 1993
Mouse, CD-1	6 h/d, 13 d	0, 7,200, 14,400, 28,800	14,400	No deaths. Clinical signs in all groups but primarily at 28,800 mg/m³. Effects included ataxia, hypoactivity, and periocular irritation. No gross or histopathological changes.	Dodd and Kintigh, 1989
Mouse, CD-1	6 h /d, 5 d/wk, 4wk	0, 1,440, 10,800, 28,800	1,440 [a] (F) 10,800 [a] (M)	At 1,440 mg/m³ no treatment-related effects. At 10,800 mg/m³ increased absolute and relative liver weight (F). At 28,800 mg/m³ as before + CNS effects (ataxia, lack of startle response); decreased spleen weight (F); increased absolute and/or relative liver weight (F+M); centrilobular hepatocellular hypertrophy (F+M); reversible increase in hepatic cell proliferation (F, d 5 only). No kidney or thyroid effects. No deaths	Chun and Kintigh, 1993

[a] NOEL

APPENDIX D. REPEATED-DOSE TOXICITY IN EXPERIMENTAL ANIMALS (cont'd)

Route / Species, strain duration	Exposure regime	Dose or concentration	Result, NOAEL	Observations	Reference
Inhalation		(mg/m^3)			
Mouse, Swiss	5-10 min/d, 5 d/wk, 30 d	0, 180,000, 288,000	> 288,000	No treatment-related increase in deaths or clinical signs. Body weight, food intake, similar in all groups. No differences between control and treated groups in clinical chemistry, urine analysis, blood, organ weight and motor activity co-ordination.	Snamprogetti, 1980
Monkey, rhesus	6 h/d, 5 d, 2 wk	7,000 - 10,600	NS	No deaths. Weight loss in 6 of the 8 animals. Some sluggishness in high-dose group each test day after 3 hours of exposure. Normal haemaotogy, clinical blood/urine chemistry and gross or microscopic pathology parameters.	Hathaway *et al*, 1970d
Intraperitoneal		**(mg/kgbw/d)**			
Rat, Wistar	1 x/d, 15 d	0, 185 [b]	185	No deaths. Body-weight gain depressed (75 % of controls). Urinary parameters and organ weights at termination similar to controls. No treatment-related histological changes	Snamprogetti, 1980

[b] 0.25 ml/kgbw/d

APPENDIX E. NEUROTOXICITY IN RATS

Exposure route, time	Result, LD_{50} / LC_{50}	Observations	Reference
Oral	NS	At 40 mg/kgbw no effects; 400 mg/kgbw drowsiness	Bio-Research Laboratories, 1990a
Oral	NS	At 440 mg/kgbw ataxia	IIT, 1992
Inhalation, 6 h	NS	At 2,880 mg/m^3 no effects. At 14,400 mg/m^3 ataxia, duck walk; decrease in rectal temp; reduced hind leg grip (F); significant decrease in motor activity. All effects apparent after 1 h, absent by 6 - 24 h. Highest dose tested 28,800 mg/m^3	Gill, 1989; Daughtrey *et al*, 1997

NS Not Stated

APPENDIX F. DEVELOPMENTAL TOXICITY IN EXPERIMENTAL ANIMALS EXPOSED BY INHALATION

Species, strain	Concentration [a](mg/m^3)	NOAEL(mg/m^3)	Observations	Reference
Rat, Sprague-Dawley	0, 900, 3,600, 9,000	9,000	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 weight, crown-rump distance, sex distribution or ossification). Incidences of soft tissue malformations of foetus and litter were comparable between control and treated groups. At 900 mg/m^3 lachrymation.	Schroeder and Daly, 1984a; Conaway *et al*, 1985
Mouse, CD-1	0, 900, 3,600, 9,000	9,000	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 (F).	Schroeder and Daly, 1984b; Conaway *et al*, 1985
Mouse, CD-1	0, 3,600, 14,400, 28,800	3,600	No effects on mortality. At 14,400 and 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. At 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.	Tyl and Neeper-Bradley, 1989
Rabbit, Albino	0, 3,600, 14,400, 28,800	3,600 (maternal), ≥ 28,800 (developmental)	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. At 14,400 mg/m^3 significantly reduced body-weight gain and food intake. At 28,800 mg/m^3 significant increase in relative liver weights; laboured breathing; ataxia; hypoactivity and ataxia (d 6).	Tyl, 1989

[a] Applied 6 h/d on gestation days 6 - 15

APPENDIX G. REPRODUCTION TOXICITY IN SPRAGUE-DAWLEY RATS EXPOSED BY INHALATION

Exposure regime, duration	Concentration[a] (mg/m^3)	NOAEL) (mg/m^3)	Observations	Reference
6 h/d, wk 16 - 28	0, 900, 3,600, 9,000	9,000	1 generation, 2 litters. No significant effects on dam or foetal parameters. Nasal discharge at 9,000 mg/m^3. Minor effects on pups: weights slightly reduced at 9,000 and 3,600 mg/m^3 on d 14 - 21; gross observations at pup necropsy; most frequent observation: hollow kidney, similar in treated and control groups, frequently seen in this strain of animal.	Biles *et al*, 1987
6 h/d , 5 - 7 d/ wk, wk 14-19	0, 1,440, 10,800,	1,440, 28,800 1,440 (pup development)[a]	2 generations, 2 litters. At 10,800 mg/m^3 (M) hypoactivity; lack of startle response; increased liver weight in F_1 but no associated lesions, reduced F_1 and F_2 pup weights, reduced weight gains, reduced body-weight gain (M); periocular encrustation and ocular discharge. Overall, no effects on respiratory, renal, immune or reproductive systems, heart, gastro-intestinal tract or associated tissues	Neeper-Bradley, 1991

[a] LOEL 10,800 mg/m^3

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Joint Assessment of Commodity Chemicals (JACC) Reports

No. Title

No. 1 Melamine
No. 2 1,4-Dioxane
No. 3 Methyl Ethyl Ketone
No. 4 Methylene Chloride
No. 5 Vinylidene Chloride
No. 6 Xylenes
No. 7 Ethylbenzene
No. 8 Methyl Isobutyl Ketone
No. 9 Chlorodifluoromethane
No. 10 Isophorone
No. 11 1,2-Dichloro-1,1-Difluoroethane (HFA-132b)
No. 12 1-Chloro-1,2,2,2-Tetrafluoroethane (HFA-124)
No. 13 1,1-Dichloro-2,2,2-Trifluoroethane (HFA-123)
No. 14 1-Chloro-2,2,2-Trifluoromethane (HFA-133a)
No. 15 1-Fluoro 1,1-Dichloroethane (HFA-141B)
No. 16 Dichlorofluoromethane (HCFC-21)
No. 17 1-Chloro-1,1-Difluoroethane (HFA-142b)
No. 18 Vinyl Acetate
No. 19 Dicyclopentadiene (CAS: 77-73-6)
No. 20 Tris-/Bis-/Mono-(2 ethylhexyl) Phosphate
No. 21 Tris-(2-Butoxyethyl)-Phosphate (CAS:78-51-3)
No. 22 Hydrogen Peroxide (CAS: 7722-84-1)
No. 23 Polycarboxylate Polymers as Used in Detergents
No. 24 Pentafluoroethane (HFC-125) (CAS: 354-33-6)
No. 25 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) (CAS No. 2837-89-0)
No. 26 Linear Polydimethylsiloxanes (CAS No. 63148-62-9)
No. 27 n-Butyl Acrylate (CAS No. 141-32-2)
No. 28 Ethyl Acrylate (CAS No. 140-88-5)
No. 29 1,1-Dichloro-1-Fluoroethane (HCFC-141b) (CAS No. 1717-00-6)
No. 30 Methyl Methacrylate (CAS No. 80-62-6)
No. 31 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2)
No. 32 Difluoromethane (HFC-32) (CAS No. 75-10-5)
No. 33 1,1-Dichloro-2,2,2-Trifluoroethane (HCFC-123) (CAS No. 306-83-2)
No. 34 Acrylic Acid (CAS No. 79-10-7)
No. 35 Methacrylic Acid (CAS No. 79-41-4)
No. 36 n-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9)
No. 37 Methyl Acrylate (CAS No. 96-33-3)
No. 38 Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3)
No. 39 Tetrachloroethylene (CAS No. 127-18-4)
No. 40 Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions
No. 41 *n*-Butanol (CAS No. 71-36-3)
No. 42 Tetrafluoroethylene (CAS No. 116-14-3)
No. 43 *sec*-Butanol (CAS No. 78-92-2)

Special Reports

No.	Title
No. 8	HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances
No. 9	Styrene Criteria Document
No. 10	Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1)
No. 11	Ecotoxicology of some Inorganic Borates
No. 12	1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0)
No. 13	Occupational Exposure Limits for Hydrocarbon Solvents
No. 14	n-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document
No. 15	Examination of a Proposed Skin Notation Strategy
No. 16	GREAT-ER User Manual

Documents

No.	Title
No. 32	Environmental Oestrogens: Male Reproduction and Reproductive Development
No. 33	Environmental Oestrogens: A Compendium of Test Methods
No. 34	The Challenge Posed by Endocrine-disrupting Chemicals
No. 35	Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances
No. 36	Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals
No. 37	EC Classification of Eye Irritancy
No. 38	Wildlife and Endocrine Disrupters: Requirements for Hazard Identification
No. 39	Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach
No. 40	Comments on Recommendation from Scientific Committee on Occupational Exposure Limits for 1,3-Butadiene
No. 41	Persistent Organic Pollutants (POPs) Response to UNEP/INC/CEG-I Annex 1
No. 42	Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction
No. 43	Contact Sensitisation: Classification According to Potency, A Commentary