HUMAN-TOXICOLOGICAL MAXIMUM PERMISSIBLE RISK LEVELS FOR METHYL-*tertiair*-BUTYLETHER (MTBE)

1.   INTRODUCTION [1])

Methyl-t-butylether (MTBE) is a synthetic ether that is mainly used as a fuel additive: it replaces lead in gasoline, increasing the anti-knock rating. Next to this it serves as an intermediate in the production of isobutylene and as a process solvent in the pharmaceutical industry. In clinical practice MTBE is used to dissolve gallstones.
At room temperature MTBE is a colourless liquid with a typical terpene-like odour.
Table 1 summarises the physical-chemical properties of MTBE.

Table 1.   Physical-chemical properties of methyl-t-butylether (data taken from ECB 2002 and ECETOC 2003)

Formula	$(CH_3)_3C–O–CH_3$
CAS no.	1634-04-4
Molecular weight	88.15
IUPAC name	2-methoxy-2-methylpropane
Melting point	–108.6 °C
Boiling point	55.2 - 55.3 °C
Density	0.741 g/cm^3 (20 °C)
Vapour pressure	270 hPa (20 °C), 330 hPa (25 °C)
Henry's law constant	43.8 Pa.m^3/mol (20 °C)
Log K$_{ow}$	1.06 (25 °C)
Water solubility	42 g/L (20 °C), 83 g/L (0 °C)
Odour threshold	0.19 mg/m^3
Conversion factors	1 ppm = 3.604 mg/m^3; 1 mg/m^3 = 0.278 ppm (25 °C) 1 ppm = 3.665 mg/m^3; 1 mg/m^3 = 0.273 ppm (20 °C)

In the scope of soil intervention values MTBE has been evaluated in 1994 and reported in 1995 (RIVM, 1995); a TDI of 900 µg/kg bw/day and a TCA of 500 µg/m^3 were derived.
The TDI was based on a 90-day study with rats orally dosed (gavage) with MTBE at 0, 100, 300, 900 and 1,200 mg/kg bw/day (Robinson et al. 1990). Decreased body weights and profound (reversible) anaesthesia were observed at the highest dose level; the NOAEL was 900 mg/kg bw/day.
The TCA was based on a 90-day inhalation study with rats exposed to MTBE at 0, 2,880, 14,400 and 28,800 mg/m^3 for 6 h/day, 5 days/week (Dodd and Kintigh 1989). At the medium and the high dose level slight growth retardation, decreased brain weight and length, and increased liver, kidney and ad-

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[1]) Abbreviations: bw   body weight
            LOAEL lowest observed adverse effect level
            NOAEL no observed adverse effect level
            TCA   tolerable concentration in air
            TDI   tolerable daily dose
            UF   uncertainty factor

renals weights were observed. Specific neurotoxic effects were not seen. The NOAEL (adjusted for exposure duration) in this study was 515 mg/m^3.

Both the TDI and the TCA were derived applying UFs of 10x10 for inter- and intraspecies extrapolation, and an additional UF of 10 for limited duration of the studies and database deficiencies.

Ad-hoc re-evaluations were done in 1997 (renewed TCA based on new data; RIVM 1997) and in 2002 (renewed TDI as the basis for a drinking water guideline; RIVM 2002).

In 1997 a TCA of 2.6 mg/m^3 was derived based on the NOAELs of 1,440 mg/m^3 (6 h/day, 5 days/week) in two chronic inhalation studies with rats and mice (Chun et al. 1992, Burleigh-Flayer et al. 1992). This NOAEL is equivalent with a NOAEL (adjusted for exposure duration) of 260 mg/m^3. With a UF of 100 (10 for inter- and 10 for intraspecies differences) this resulted in a TCA of 2.6 mg/m^3.

In 2002 a renewed TDI was derived based on the draft European risk assessment in which it was concluded that the 90-day study cited above (Robinson et al. 1990) actually showed slight kidney and liver toxicity at 900 mg/kg bw/day, with a NOAEL of 300 mg/kg bw/day. RIVM adopted this conclusion, and consequently derived a TDI of 300 μg/kg bw/day (applying the same UFs as was done in the evaluation of 1995).

The background exposure was unknown.

Relevant route in case of soil contamination: oral and inhalation.

Basis for the current evaluation were the evaluations by US-EPA (1993), Health Council of the Netherlands (GR, 1994), ATSDR (1996), IPCS (1998), IARC (1999), ECB (2002), and ECETOC (2003).

2.   TOXICOLOGY

2.1.   Toxicokinetics

Absorption

MTBE is rapidly and well absorbed following oral and inhalation exposure. In rats oral absorption is virtually complete, while inhalation absorption was approximately 50% (Miller et al. 1997, BRL 1990b). For humans no firm data on oral absorption are available, but the limited data indicate similar uptake kinetics compared with rats. Inhalation absorption by humans was reported to vary between 32 and 49% with an average of 38% (Pekari et al. 1996, Nihlén et al. 1998, Prah et al. 2000). Following occluded exposure, dermal absorption in rats is about one-third of the oral rate, but it is assumed that non-occluded skin contact results in lower bioavailability, mainly due to MTBE's high volatility (Miller et al. 1997, BRL 1990a-d, 1991, Prah et al. 2000).

Distribution

MTBE is distributed extensively in the mammalian body. It is moderately soluble in blood, and 7-10 times more soluble in fat tissues. Solubilities in rat liver and muscle were quite similar to that in blood, but in male rat kidney it was 6 times higher than in blood (Borghoff et al. 1990), which is believed to be species- and sex-specific: there is strong evidence that MTBE interacts with α2u-globulin (a protein specifically occurring in male rat kidney; Poet and Borghoff 1997, Prescott-Mathews et al. 1999).

In rats whole body exposed to MTBE at 50 - 300 ppm (180 - 1,100 mg/m^3) for periods from 2 to 15 weeks, tissue concentrations were directly exposure-related throughout the whole study period (Savolainen et al. 1985).

Biotransformation

In rats and humans MTBE is oxidatively metabolised to t-butanol (TBA) and formaldehyde (only observed *in vitro*); formaldehyde is known to be rapidly metabolised to formic acid and CO_2, or becomes incorporated into the one-carbon pool (McMartin et al. 1979, Savolainen et al. 1985, Miller et al. 1997, Nihlén et al. 1998, 1999; Amberg et al. 1999). TBA in turn is metabolised to 2-methyl-1,2-propanediol and 2-hydroxyisobutyric acid; next to these, low amounts of free TBA, TBA-glucuronide, and another conjugate (probably TBA-sulphate) were identified in urine. After inhalation exposure of rats and humans more than half of the MTBE retained in the body was biotransformed to urinary metabolites and less than half was exhaled unchanged, however, rats exposed to a high level (8,000 ppm

during 6 h, approx. 30 gram per m^3) the main part was exhaled indicating metabolic saturation (Miller et al. 1997, Nihlén et al. 1998, Amberg et al. 1999). The biotransformation of MTBE is depicted in fig. 1.
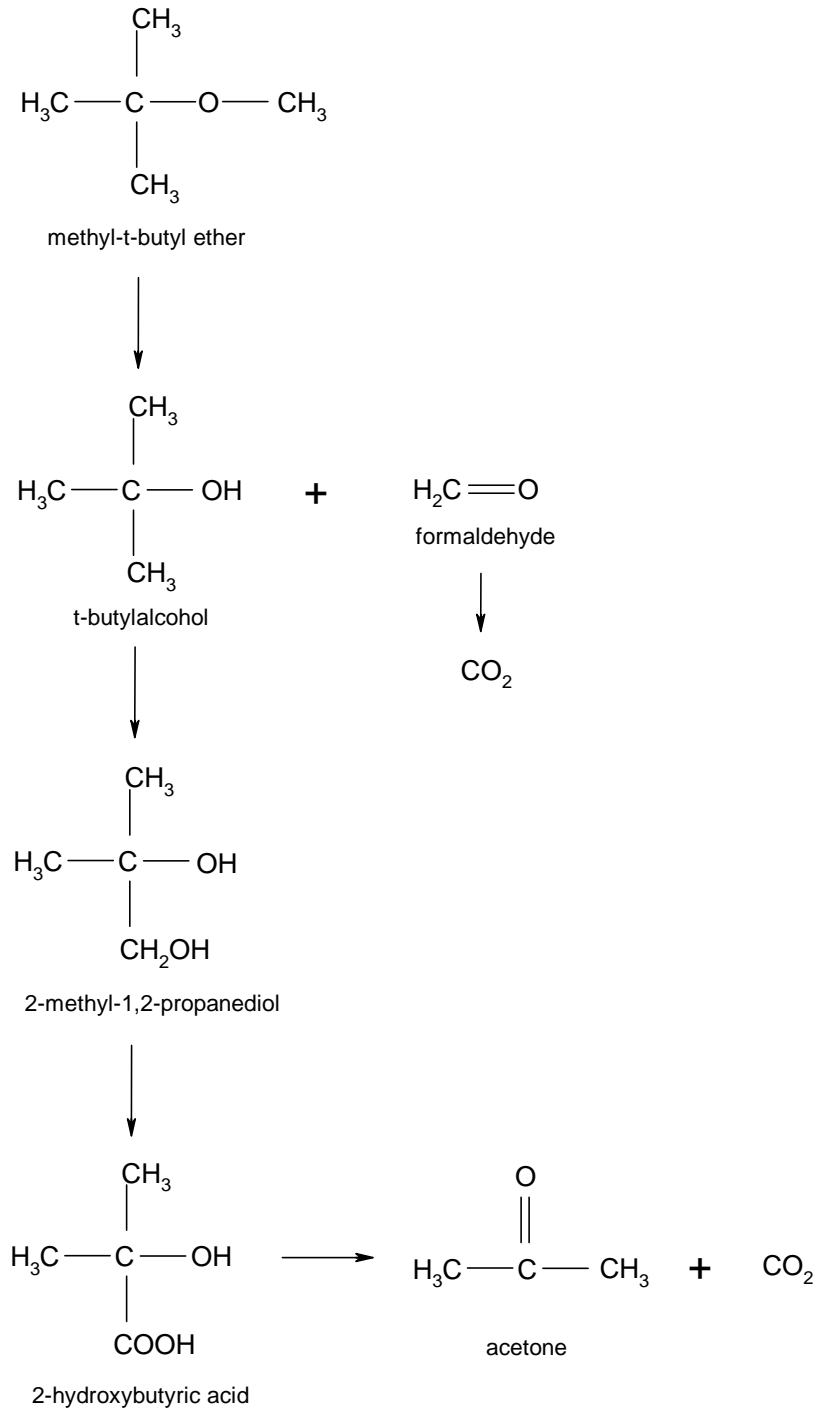

Figure 1. Biotransformation of MTBE

Excretion

With all routes of administration, MTBE given to rats is rapidly removed from the blood by exhalation and by biotransformation to TBA. Elimination is rapid and largely completed within 24 h after administration. Metabolites are mainly excreted in the urine; less than 2% is found in the faeces. At higher doses a higher proportion of MTBE is exhaled, indicating saturation of metabolic pathways. The apparent plasma half life of MTBE is in the range of 0.45 to 2.3 h. Following administration of radiolabelled MTBE to rats, the excretion of radioactivity via expired air was virtually complete 3 h after treatment, and consisted mainly of parent compound. The results of all kinetic experiments indicate that the general elimination of MTBE is not route or sex dependent, and is rather similar in experimental animals and in humans (Leuschner et al. 1991, Johanson et al. 1995, Pekari et al. 1996, Miller et al. 1997, Nihlén et al. 1998, Dekant et al. 2001).

After exposure to MTBE, TBA is found in blood for a longer period and at higher concentrations than MTBE. The elimination half-life of TBA in blood is approximately 3 h in rats (BRL 1990a, b) and approximately 10 h in humans (Prah et al. 2000).

It can be concluded that MTBE or its metabolites will not accumulate in the human body to a significant extent (ECB 2002)+.

2.2. Acute toxicity

Animal studies

Symptoms of acute toxicity after oral exposure included hunching, piloerection, hypoactivity, hyperpnea, prostration and muscular weakness. High dose levels also provoke inflammation of the stomach and intestine. The oral LD$_{50}$ is 3.8 - 3.9 gram per kg bw (ARCO 1980, RBM 1996c).

Inhalation resulted in irritation of eyes and nose, lack of co-ordination, and irregular and rapid breathing. In another study ataxia, tremors, lacrimation, muscular contractions and hypoactivity were observed. In surviving animals congestion of blood in the lungs was seen. The LC$_{50}$ is 85 - 120 mg/L (4 h exposure; Mastri et al. 1969, ARCO 1980).

Dermal exposure of rats to 2 gram per kg bw resulted only in slight erythrema at the sight of application, signs of systemic toxicity were not noted (RBM 1996c). In two rabbit studies (doses of 6.8 and 10.2 gram per kg bw) erythrema and slight to moderate oedema were reported; mortality did not occur; the LD$_{50}$ was reported to be > 10 gram per kg bw (Mastri et al. 1969, ARCO 1980).

Human studies

In humans treated with MTBE for dissolution of gallstones or bile duct stones usually small quantities are instilled by a transhepatic or nasobiliary catheter during up to 7 days, to a total volume of 30 - 480 ml (Neoptolemos et al. 1990, Leuschner et al. 1991). During dissolution treatment in 5-25% of the patients mild complications were seen, including nausea, drowsiness, vomiting, and local burning sensations. In human volunteer studies with inhaled MTBE mild symptoms of central nervous system toxicity were reported (Prah et al. 1994, Nihlén et al. 1998).

2.3. Irritation

Skin

Rabbit skin exposed to MTBE for 4 h under occlusion (according to OECD guidelines) showed one h after exposure moderate to severe oedema and moderate erythrema, these effects lasted 8 days (Mürmann 1985). Another similar study with rabbits resulted in slight erythrema but no oedema (RBM 1996a). MTBE can be considered a skin irritant, but it is not corrosive (ECB 2002).

Eye

An eye irritation study with rabbits (according to OECD guidelines) resulted in redness of the conjunctiva and inflammation of the iris starting one h after application which lasted for 72 h (RBM 1996b). Several Draize tests showed transient corneal opacity and irritation to the iris and conjunctiva, these effects were all reversible (Mastri et al. 1969, Cuthbert 1979). Despite MTBE's slight eye-irritating properties, on the basis of the EU guidelines it is not classified as an eye irritant (ECB 2002).

Respiratory tract
MTBE vapours caused slight to transient irritation of the respiratory tract of experimental animals: a sensory irritation threshold (13% reduction of breathing rate) of 300 mg/m^3 was reported, there were no indications of lung injury (Tepper et al. 1994).

Sensitisation
A Magnusson-Kligman maximisation test with guinea pigs did not show hypersensitivity reactions (Cuthbert 1979); also another sensitisation study was negative (ARCO 1980). MTBE is considered not sensitising in guinea pigs (ECB 2002).

Human studies
At 270 mg/m^3 during 2 h (with light physical exercise) MTBE caused subjective symptoms of irritation of the respiratory tract and heaviness in the head in young non-smoking healthy volunteers; these symptoms were not recorded at 180 mg/m^3. Objective symptoms of eye or nose irritation could not be found (Prah et al. 1994, Johanson et al. 1995, Cain et al. 1996, Riihimäki et al. 1996, Nihlén et al. 1998). Observations on sensitising potential in humans are not available (ECB 2002).

2.4.   Mutagenicity

MTBE
Out of nine bacterial gene mutation tests with *Salmonella typhimurium*, only one (with metabolic activation) was positive, but the metabolic activation could have been responsible for formaldehyde involvement. The same holds for the one positive result in two mouse lymphoma gene mutation tests. One *in vitro* UDS test (rat hepatocytes) was positive, but two other well-conducted tests were negative. Two gene mutation tests with CHV79 cells were negative, as was a chromosome aberration test with CHO cells. Two SCE tests with CHO cells and a mouse micronucleus tests were also negative (ECB 2002).
Of the reported *in vivo* tests, a sex-linked recessive lethal test in *Drosophila melanogaster*, a mouse UDS test, a mouse spleen lymphocyte gene mutation test, two mouse erythrocyte micronucleus tests, a rat cytogenetic bone marrow test, and a rat bone marrow chromosome aberration test were all negative. Only a Comet assay on rat lymphocytes was positive, but the biological significance of this test result is questionable (ECB 2002). Based on the available information, MTBE cannot be considered a mutagen (ECB 2002).

Formaldehyde and TBA
Formaldehyde is mutagenic in a number of *in vitro* assays, but this activity decreases when S9 is added, indicating rapid conversion of formaldehyde to non-mutagenic products (such as formate) (ECB 2002). Also experiments with endogenously produced formaldehyde suggested strongly that the rate of formation of formaldehyde from MTBE was slow relative to its rate of oxidation to formate and incorporation in the one-carbon pool of the organism (Casanova and Heck 1997).
TBA has been tested for its ability to induce gene mutations in several *Salmonella typhimurium* strains, but the results were negative, with as well as without S9 metabolic activation. A mouse lymphoma cell assay scored positive regarding forward mutations, but the results could not be reproduced. Chromosome aberration tests with CHO cells were negative. Also an *in vivo* micronucleus test in mouse erythrocytes was negative (NTP 1995, 1997).

2.5.   Subacute and subchronic toxicity

In several subacute and subchronic studies with rats and mice the principal target organs were liver and kidneys; generally adverse effects were seen at LOAELs for oral exposures $\geq$ 300 mg/kg bw/day and inhalation exposures $\geq$ 10 gram/m^3. The studies are summarised in table 2. Degenerative changes and protein droplet nephropathy of the male rat kidney proximal tubules are most commonly seen. In the inhalation studies up to 3.5 gram/m^3 only slight indications of kidney toxicity are seen: at 15 gram per m^3 male rats showed clear protein droplet formation. Of the oral studies, Williams et al. (2000) noted protein droplet nephropathy visible in light microscopy in male rats at 250 mg/kg bw/day. The

90-day study by Robinson et al. (1990) reported this only at 1,200 mg/kg bw/day, while Zhou and Ye (1999) mentioned no signs of nephropathy up to 1,000 mg/kg bw/day in the same rat strain. Most studies also reported increased liver weight; Zhou and Ye (1999) noted morphological changes of the smooth endoplasmatic reticulum visible only in electron microscopy, while Williams et al. (2000) observed centrilobular hypertrophy in light microscopy at 500 mg/kg bw/day. Hypertrophy and the subsequent weight increase and smooth endoplasmatic reticulum changes are typically adaptive liver responses which do not appear to result in irreversible or anatomical impairments. The kidney changes are most probably associated with α2u-globulin and its accumulation, this phenomenon is discussed in the next paragraph (2.6).

Table 2. **Summary of subacute and subchronic studies with MTBE (data taken from ECB 2002 and ECETOC 2003)**

Duration/route	Species	Effects	NOAEL	Reference
14 d, oral (gavage)	Sprague-Dawley rat	increased kidney weight, decreased lung weight, kidney hyaline droplet formation	<357 mg/kg bw	Robinson et al. 1990
28 d, oral (gavage)	Sprague-Dawley rat	increased kidney weight, hyaline droplet formation in kidney proximal tubular cells	90 mg/kg bw	IITRL 1992
28 d, oral (gavage)	Sprague-Dawley rat	nephropathy, kidney hyaline droplet formation, increased liver weight	<250 mg/kg bw (LOEL for minor effects)	Williams et al. 2000
90 d, oral (gavage)	Sprague-Dawley rat	kidney hyaline droplet formation, increased liver and kidney weight, AST, LDH and cholesterol, decreased body weight gain and blood urea nitrogen	300 mg/kg bw	Robinson et al. 1990
90 d, oral (gavage)	Sprague-Dawley rat	increased liver and kidney weight, morphological changes in hepatocyte structure	<200 mg/kg bw (LOEL for minor effects)	Zhou & Ye 1999
28 d, inhalation (6 h/d, 5 d/wk)	Fischer-344 rat	increased liver and kidney weight, kidney proximal tubular cell proliferation	1,440 mg/m^3 *)	Chun & Kintigh 1993
28 d, inhalation (6 h/d, 5 d/wk)	CD-1 mouse	increased liver weight, liver cell proliferation	1,440 mg/m^3 *)	Chun & Kintigh 1993
90 d, inhalation (6 h/d, 5 d/wk)	Sprague-Dawley rat	females: depressed lung weight; males: increased haemoglobin, blood urea nitrogen and LDH	1,800 mg/m^3 *)	Greenough et al. 1980
90 d, inhalation (6 h/d, 5 d/wk)	Fischer-344 rat	abnormalities in kidney proximal tubular cells, changed hormone levels, alteration in red blood cell parameters	2,900 mg/m^3 *)	Dodd & Kintigh 1989, Lington et al. 1997

AST: aspartate amino transferase
LDH: lactate dehydrogenase
*) for exposure of 6 h/day, 5 days/week

2.6. Chronic toxicity and carcinogenicity

The chronic toxicity and potential carcinogenicity of MTBE has been studied in well-conducted long-term inhalation studies in CD-1 mice (Burleigh-Flayer et al. 1992, Bird et al. 1997) and Fischer-344 rats (Chun et al. 1992, Bird et al. 1997). A Sprague-Dawley rat oral intubation study has been reported by Belpoggi et al. (1995, 1998).
F-344 rats (50 of each sex) were exposed to 0, 400, 3,000 or 8,000 ppm MTBE (equivalent with 0, 1,440, 10,800 or 28,800 mg/m^3) for 6 h/day, 5 days/week during two years. High dosed animals showed decreased body weight gain. Mortality occurred in all male dose groups and was dose-related. Relative liver and kidney weights were increased in the two highest dose groups (males and females). The predominant finding at necropsy was an increased frequency of chronic progressive nephropathy in treated males. Tumour incidences are summarised in table 3.

CD-1 mice (50 of each sex) were exposed to 0, 400, 3,000 or 8,000 ppm MTBE (equivalent with 0, 1,440, 10,800 or 28,800 mg/m^3) for 6 h/day, 5 days/week during 18 months. A slight increase in mortality was seen in high dosed males. Body weight, body weight gain, and brain weights were decreased in high dosed males and females. Kidney weights and relative liver weights were dose-relatedly increased in all treated males, but at the lowest dosage the increases were only minor and not considered adverse. Absolute and relative adrenal weights were increased in high-dosed males, and absolute kidney and spleen weights were increased in high-dosed females. At he end of the study both sexes had an increased corticosterone level at the highest dose (significant for males). Tumour incidences are summarised in table 3.

Sprague-Dawley rats (60 of each sex) were given MTBE in olive oil (gavage) at doses of 0, 250 or 1,000 mg/kg bw at all days except Wednesdays and the weekend (resulting in total weekly doses of 0, 1,000 or 4,000 mg/kg bw/week, or average daily doses of 0, 143 or 571 mg/kg bw/day), for two years. The test animals had no treatment-related adverse clinical signs, and neither gross examination at necropsy nor microscopic examination did reveal any adverse changes that were not tumour-related. In the high dose group mortality was lower than in the two other groups. Unfortunately the report of this chronic oral study is inadequate in many aspects, including the (lack of) observations on systemic toxic effects and the applied statistics (ECB 2002, ECETOC 2003). Tumour incidences are summarised in table 4.

Table 3. **Tumour incidences in F-344 rats and CD-1 mice inhalatory exposed to MTBE (data taken from ECB 2002 and ECETOC 2003)**

Tumour type	Dose (mg/m^3, 6 h/day, 5 days/week)			
	0	1,440	10,800	28,800
Fischer-344 rats (24 months)				
parathyroid adenomas	0/50	0/50	4/50	1/50
renal tubular cell adenomas and/or carcinomas	1/50	0/50	8/50 [#]	3/50
testicular interstitial cell adenomas	32/50	35/50	41/50 *	47/50 *
CD-1 mice (18 months)				
hepatocellular adenomas and/or carcinomas (males)	12/49	12/50	12/50	16/49
hepatocellular adenomas and/or carcinomas (females)	2/50	2/50	2/50	11/50 *
cystic hyperplasia of uterine endometrium (females)	26/50	17/48	15/50 [#]	6/50 *

[#]   statistically significantly different from controls (p<0.05)
*   statistically significantly different from controls (p<0.01)

Table 4. **Tumour incidences in Sprague-Dawley rats orally exposed to MTBE (data taken from ECB 2002 and ECETOC 2003)**

Tumour type	Dose (mg/kg bw, 4 days/week, 2 years)		
	0	250	1,000
lymphomas and leukaemias (females)	2/60	7/60	12/60 *
testicular interstitial cell adenomas	2/60	2/60	11/60 [#]

[#]   statistically significantly different from controls (p<0.05)
*   statistically significantly different from controls (p<0.01)

Relevance of tumours following chronic exposure to MTBE
Kidney tumours in F-344 rats
The male rat kidney secretes several milligrams of α2u-globulin per day which is normally cleared via the urine. A similar protein has never been found in human kidneys. Many xenobiotics cause an overload of α2u-globulin in male rats, the so-called α2u-globulin syndrome, which is initiated by binding of the xenobiotic to α2u-globulin, making it more resistant to hydrolysis (Borghoff et al. 1990, Borghoff 1993). This leads to an accumulation of hyaline droplets and damage to the proximal convoluted tubule, finally resulting in chronic progressive nephropathy and in the long term urothelial hyperplasia and ultimately tubular neoplasia. Many studies indicate that α2u-globulin-associated nephropathy is the mechanism in MTBE induced kidney tumour formation in male rats (Robinson et al. 1990, Bird et

al. 1997, Prescott-Mathews et al. 1997, 1999). Hence for man these tumours are considered irrelevant (ECB 2002).

Leydig cell tumours in F-344 and Sprague-Dawley rats
Testicular interstitial cell adenomas are characteristic for Leydig cell tumourigenesis, and occur spontaneously at high rates (up to >90%) and strongly age-related in ageing rats, particularly (but not limited to) F-344 rats. A disturbance of the hormonal balance (reduced testosterone or estradiol followed by compensation through increasing levels of LH) has been suggested as a possible mechanism. Although decreased testosterone (and increased corticosterone) levels were found following exposure to MTBE (Day et al. 1998, Allgaier and De Peyster 1999, Williams et al. 2000), increasing LH levels were not found. Historical data record a spontaneous incidence of Leydig cell tumours in F-344 control rats of at average 89% (Haseman et al. 1990). In the MTBE-studies of Chun et al. (1992) and Belpoggi et al. (1995) cited above, significant increases are only seen at the highest or two highest doses, and the dose-response relationship is rather weak or even absent. Moreover, in the oral study mortality in the high dose group was lower than in the two other groups, which might have skewed the incidences of Leydig cell tumours. In conclusion, there is evidence that MTBE causes an increased incidence of Leydig cell tumours in rats. The interpretation of these results is hampered by the limited data, and also because it is not clear if and how the differences in anatomy and physiology between rat and human testes influence the susceptibility to Leydig cell tumourigenesis. In humans testicular cancer is rare: about 1% of all human neoplasms. Of these testes cancers only 2-3% are Leydig cell tumours. This suggests an important difference in sensitivity or behaviour in Leydig cells between rat and man. In conclusion the relevance to humans of the observed increase in Leydig cell tumourigenesis is probably low (ECB 2002).

Haematopoietic neoplasms in Sprague-Dawley rats
In the oral rat study an increasing incidence of lymphoma/leukaemia in females was reported, but there were no signs of neoplastic lymphoid cell changes in the carcinogenicity inhalation studies with F-344 rats and CD-1 mice. The report of the oral Sprague-Dawley rat study is inadequate in many aspects (ECB 2002, ECETOC 2003), resulting in a low level of confidence of the results. The tumours may be of relevance, but on the basis of this low-quality study firm conclusions are not possible (ECB 2002).

Parathyroid neoplasia in F-344 rats
The proliferative changes seen in the parathyroid of male F-344 rats are likely due to hyperparathyroidism which is commonly seen in cases where the parathyroid compensates for hypercalcaemia caused by, e.g., chronic renal failure (ECB 2002).

Liver tumours in CD-1 mice
The increase in adenoma incidence was only seen in females at the highest dose, suggesting female specificity. It has been postulated that interference of MTBE in oestrogen-affected tissues may play a role in mouse liver tumour formation. MTBE, however, was shown not to have tumour-promoting activity (Moser et al. 1997). In conclusion, MTBE causes changes in oestrogen-sensitive tissues without affecting serum oestrogen levels. There may be a connection with these changes and the increased incidence of liver adenomas seen in female mice at high dose, but there is no evidence to corroborate such a theory. The relevance of these tumours to man is questionable (ECB 2002).

Conclusion on chronic toxicity/carcinogenicity
MTBE produces tumours in mice and rats at doses $\geq$ 10,800 mg/m^3 (6 h/day, 5 days/week) after chronic inhalation exposure, and in rats at chronic oral doses $\geq$ 250 mg/kg (4 days/week). There is no evidence of a genotoxic mode of action, and the tumours appear mostly at very high and systemically toxic doses. Based on the weight of evidence on carcinogenicity and genotoxicity, the threshold approach can be applied.

2.6.   Reproductive and developmental toxicity

Reproductive toxicity
In a one-generation inhalation study with Sprague-Dawley rats whole body exposed to 0, 900, 3,600 or 9,000 mg/m^3 MTBE (6 h/day, 5 days/week before, during and after mating and lactation), parent animals showed no signs of toxicity. Pup viability was significantly lower in the mid- and high dose

groups of the F1b litter, but no such changes were seen in the F1a litter (Biles et al. 1987). However, the viability of control F1b litter was 99.0% while the viability of the control F1a litter was 97.6%, which may have skewed the significant difference seen.

In a two-generation inhalation study with Sprague-Dawley rats whole body exposed to 0, 1,440, 10,800 or 28,800 mg/m^3 MTBE (6 h/day, 5 days/week before, during and after mating and lactation), there were general signs of (parental) toxicity at the two highest dose groups in both generations of parent animals. No significant changes in the reproduction parameters could be seen even at the highest dose. Some toxicity was also recorded for pups in the F1 and F2 generations in terms of pup viability and survival, but this was not considered of biological significance (Bevan et al. 1997a). The NOAEL for maternal toxicity was 1,440 mg/m^3.

It can be concluded that MTBE does not cause significant toxicity to reproduction in Sprague-Dawley rats (ECB 2002).

Developmental toxicity

Effects of MTBE on development have been investigated in a number of studies in mice, rats and rabbits, by exposure of pregnant animals at certain days of gestation to MTBE vapours ranging from 900 to 28,800 mg/m^3.

In a rat and a mouse study MTBE was not maternally toxic, embryotoxic or teratogenic at exposures up to 9,000 mg/m^3 (which was the highest exposure in these two studies; Conaway et al. 1985).

In another mouse study animals were exposed to 0, 3,600, 14,400 or 28,800 mg/m^3 MTBE vapour. Maternal toxicity was seen at 14,400 and 28,800 mg/m^3; also gestation parameters were affected at these concentrations. At the highest dose there was a significant increase in the incidence of cleft palate (Tyl and Neeper-Bradley 1989, Bevan et al. 1997b). The NOAEL for maternal and developmental toxicity was 3,600 mg/m^3.

In a rabbit study pregnant animals were exposed to similar MTBE concentrations. The two highest exposure levels were maternally toxic, but there were no treatment-related effects on gestation parameters (Tyl 1989, Bevan et al. 1997b). The NOAEL for maternal toxicity was 3,600 mg/m^3, the NOAEL for developmental toxicity was at least 28,800 mg/m^3.

In conclusion it can be stated that MTBE does not appear to induce adverse foetal development at exposure levels that do not cause maternal toxicity (ECB 2002).

3.   EVALUATION

RIVM adopts the conclusions of the recent European risk assessment report (ECB 2002). The principal studies are summarised in tables 5 and 6.

The weight of evidence indicates that MTBE is not genotoxic. The substance induced testicular (Leydig cell) tumours in male rats (F-344 and Sprague-Dawley), renal tumours in male rats (F-344), liver tumours in female mice (CD-1) and lymphomas and leukaemias in female rats (Sprague-Dawley).

All investigations on nephrotoxicity are consistent with the renal tumours observed in F-344 rats being related to α2u-globulin nephropathy, an effect considered specific to male rats and thus of no relevance to humans (IPCS 1998, ECB 2002).

Leydig cell tumours have been induced in two strains of rats. This tumour type has been reported to be induced by non-genotoxic carcinogens that disturb the hormonal balance of testosterone, luteinizing hormone and luteinizing hormone releasing factor in rats. Due to the differences between rats and humans in the regulation of gonadotropins it is questionable that a similar effect will occur in humans.

Liver tumours have been induced in female mice. The effect was modest and occurred only at high doses and in association with hepatocellular hypertrophy and altered oestrogen metabolism. The relevance of these mouse liver tumours for human risk assessment is considered questionable (IPCS 1998, ECB 2002).

In an oral study with rats (Sprague-Dawley) the frequency of lymphomas and leukaemias were increased in the high dose group females, but this effect was not supported by any indication of relevant effects of the lymphoid system in other studies. Moreover, the description of the study makes it difficult to evaluate the results in an adequate way (IPCS 1998, ECB 2002).

In line with the previous RIVM-evaluation, the two-year chronic toxicity/carcinogenicity inhalation study with rats (Chun et al. 1992) is considered the pivotal study for derivation of the TCA. The NOAEL in this study is 1,440 mg/m^3 for a chronic exposure of 6 h/day, 5 days/week. Corrected for exposure duration this results in a NOAEL of 6/24 x 5/7 x 1440 = 260 mg/m^3. Application of a UF of 100 (10x10 for inter- and intraspecies variation; RIVM 1997) results in the TCA of 2.6 mg/m^3.

In previous RIVM-evaluations the TDI has been derived from the subchronic (90-day) oral (gavage) study with rats of Robinson et al. (1990). Evaluation of the chronic (2-year) oral (gavage) study of Belpoggi et al. (1995) shows that this study does not provide the solid information which is needed to justify a correction of the previously derived TDI. The study of Zhou and Ye (1999) does not report detectable changes by light microscopy - in contrast to many other studies - and only observed nephro-toxicity at higher dosages ($\geq$ 1,000 mg/kg bw/day). By electron microscopy some hepatocellular effects were seen (with a LOEL of $\leq$ 200 mg/kg/day), but these are considered not to be adverse. Williams et al. (2000) focussed mainly on endocrine effects with less attention for other toxicologically relevant endpoints, observing at the LOEL ($\leq$ 250 mg/kg bw/day) only an increase in kidney weight. Consequently the study of Robinson et al. (1990) is still considered the pivotal one. The NOAEL of 300 mg/kg bw/day and the application of a UF of 1000 (10x10 for inter- and intraspecies differences, and an additional 10 for limited duration of the study and database deficiences; RIVM 2002) results in the TDI of 0.3 mg/kg bw/day.

Table 5.   Principal adverse effects and NOAELs in MTBE inhalation studies

Species	Exposure	Effects	NOAEL (mg/m^3)	Reference
Man	0.19 mg MTBE per m^3	odour threshold	-	Prah et al. 1994
Man	2 h	slight irritation of respiratory tract, head heaviness	180	Johanson et al. 1995, Riihimäki et al. 1996
Rat	90 days subchronic toxicity	liver and kidney toxicity	2,880 *)	Dodd and Kintigh 1989
Rat	24 months chronic toxicity and carcinogenicity	liver and kidney toxicity, kidney tumours (m)	1,440 *)	Chun et al. 1992
Mouse	18 months chronic toxicity and carcinogenicity	liver and kidney toxicity, liver tumours (f)	1,440 *)	Burleigh-Flayer et al. 1992
Rat	2 generations reproductive toxicity	Parental toxicity (no reproductive toxic effects)	1,440 *)	Bevan et al. 1997a
Mouse	gestation day 6-15 developmental toxicity	Maternal toxicity, secondary developmental toxicity	3,600 *)	Tyl & Neeper-Bradley 1989, Bevan et al. 1997b

*) for exposure of 6 h/day, 5 days/week

Table 6.   Principal adverse effects and NOAELs in MTBE oral studies

Species	Exposure	Effects	NOAEL (mg/kg bw/day)	Reference
Rat	90 days (gavage)	kidney and liver toxicity	300	Robinson et al. 1990
Rat	90 days (gavage)	liver and kidney toxicity	<200 (LOEL for minor effects)	Zhou & Ye 1999
Rat	24 months chronic toxicity and carcinogenicity	haematopoietic neoplasms (f), testicular interstitial cell adenomas	250 *)	Belpoggi et al. 1995

*) for exposure during 4 days/week

The background exposure was estimated by ECB (2002) in a reasonable *worst case* exposure scenario for a person who is exposed to MTBE at the petrol station during and after refueling his/her car and who lives near to (50 m) a petrol station, and amounts to 70 - 470 µg/day, equivalent to 1 - 7 µg/kg bw/day. This scenario includes also the exposure due to commuting by car and bus. Exposure to MTBE in drinking water would in a *worst case* scenario add at most approximately 30 µg/day, equivalent to 0,5 µg/kg bw/day.

4. EVALUATIONS BY OTHER ORGANISATIONS

ATSDR derived a chronic inhalation MRL (minimal risk level; equivalent to a TCA) of 2.5 mg/m^3 based on the chronic toxicity/carcinogenicity study by Chun et al. (1992), extrapolating the NOAEL of 1,440 mg/m^3 to the MRL by applying correction for exposure duration and a UF of 100 [2]) (ATSDR 1996).

ATSDR derived an oral MRL for intermediate duration (comparable to a TDI) of 0.3 mg/kg bw/day based on the subchronic study by Robinson et al. (1990), extrapolating the LOAEL of 100 mg/kg bw/day (LOAEL for decreased blood urea nitrogen)[3]) to the MRL by applying a UF of 100 [2]) and an additional UF of 3 for the use of a LOAEL for minor effects (ATSDR 1996).

US-EPA derived a RfC (equivalent to a TCA) of 3 mg/m^3 based on the chronic toxicity/carcinogenicity study by Chun et al. (1992), extrapolating the NOAEL of 1,440 mg/m^3 to the MRL by applying correction for exposure duration and a UF of 100 [2]) (and rounding off) (US-EPA 1993).

ECB (2002) did not derive a TDI or TCA but took the oral NOAEL of 300 mg/kg bw/day from Robinson et al. (1990) and the inhalation NOAEL of 1,440 mg/m^3 from Chun et al. (1992) as the basis for their calculations of margins of safety in which these NOAELs are compared with exposures estimated according to several exposure scenarios.

IARC assessed the available data in 1998 and concluded that "there is limited evidence in experimental animals for the carcinogenicity of MTBE, and there is inadequate evidence in humans for the carcinogenicity of MTBE - *MTBE is not classifiable as to its carcinogenicity to humans*" (group 3; IARC 1999).

5. CONCLUSION

Substance	TDI	TCA	Background exposure	Odour threshold
Methyl-t-butyl ether	0.3 mg/kg bw/day	2.6 mg/m^3	0.004 - 0.005 mg/kg bw bw/day	0.19 mg/m^3

TCA   tolerable concentration in air
TDI   tolerable daily dose
Background exposure: the mean of the reasonable *worst case* exposure scenarios as estimated by ECB (2002)

Relevant routes of exposure in case of soil contamination: oral and inhalation

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[2]) Viz. 10x10 for inter- and intraspecies extrapolation.
[3]) In all other evaluations this effect is not considered relevant.

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