Social Europe

Recommendation from the Scientific Committee on Occupational Exposure Limits for tert-butyl methyl ether

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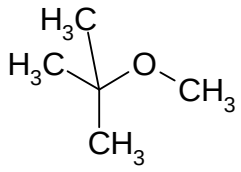
8 hour TWA:	50 ppm (183.5 mg/m^3)
STEL (15 min):	100 ppm (367 mg/m^3)
Notation:	none

Substance:

Tert-butyl methyl ether



Synonyms : tert-Butyl methyl ether, Methyl-1,1-dimethylethylether, 1,1,1-Trimethyl-dimethlether, Methyl-tertiary-butyl ether (MTBE)

EINECS No. : 216-653-1

EEC No. : 603-181-00-X

CAS No. : 1634-04-4

MWt : 88.15 g/mol

Conversion factor (20°C): 1 ppm = 3.67 mg/m^3

EU Classification : F, R11 Highly flammable;

Xi, R38 Irritating to skin

This document is based on the EU-RAR from 2001, the MAK-documentation from 2000 and the references based therein.

Physico-chemical properties

Pure MTBE is at 20 °C and 1013 hPa a colourless volatile liquid. MTBE has a terpene-like odour. The average detection threshold of MTBE in air is 0.053 ppm, the recognition threshold in air is 0.08 ppm (Vetrano et al, 1993). The unpleasant odour and the low odour thresholds have a premonitory effect. It is soluble in most organic solvents and it is also quite soluble in water. MTBE is flammable and combustible. The melting temperature is -109 °C and the boiling temperature is given as 55.3 °C. It has a flash point of -28.2 °C and the vapour pressure is 268 hPa at 20 °C.

1. Occurrence/Use

The annual production volume of MTBE in the year 1997 in the EU was 3030000 tonnes. About 187000 tonnes was imported and about 904000 tonnes was exported outside the EU in the year 1997 (EU-RAR, 2001). The annual consumption of MTBE within the EU was hence 2313000 tonnes in the year 1997. MTBE is typically manufactured in petroleum refineries but also in plants manufacturing industrial organic chemicals. MTBE is prepared principally by reacting isobutene with methanol over an acidic ion-exchange resin catalyst. It can also be prepared from methanol, tert-butyl alcohol (TBA) and diazomethane (EU-RAR, 2001).

The main use of MTBE is as an oxygenated additive/component in petrol. This usage covers more than 98% of the total quantity produced in the EU. Only a minor amount is used for other purposes, such as solvent instead of diethyl ether or diisopropyl ether in both chemical and pharmaceutical industry and laboratories.

2. Health significance

2.1. Toxicokinetics

Absorption of MTBE is well investigated both in animal studies and in human volunteer studies. There seem to be no major species differences.

MTBE is efficiently absorbed via the lungs in rats and humans.

Absorption via inhalation is in the range of 32-42 % in human studies (Nihlén et al., 1998a, Pekari et al., 1996), and 50 % in animal studies (Miller et al., 1997), showing neither considerable effects of concentration nor level of activity on the amount absorbed. The absorption occurs rather quickly, with a plateau after 2 to 4 hours.

Dermal absorption in male and female F344 rats was studied (Miller et al., 1997). MTBE is at least a moderate skin penetrant under occlusive conditions. Dermal absorption was 16 (lower dose levels) to 34 % (higher dose levels) under occlusive conditions in this experiment. However, under most practical exposure circumstances the high volatility of MTBE would strongly limit skin absorption because of competition between penetration and an efficient loss process by evaporation.

Oral absorption investigated in animal studies amounts to 100 % but seems to be slower than absorption by inhalation (Miller et al., 1997, Prah et al., 2000).

Based on its physical-chemical properties MTBE is likely distributed extensively in the mammalian body. Based on in vitro determined tissue/air partition coefficients in F344 rat (Borghoff et al., 1996) and human (Imbriani et al., 1997; Nihlen et al., 1995) tissues, MTBE is moderately soluble in blood, and 7-10 times more soluble in fat tissue. Rather similar solubilities to that in blood were found in rat liver and muscle. An exception was found for male rat kidney with 6 times higher solubility than in blood, due to specific binding (a2u-globulin-nephropathy). Directly exposure-related tissue MTBE concentrations were found after repeated dose whole-body exposure to Wistar rats for different dose levels and durations of exposure (Savolainen et al. 1985).

In vivo studies on the metabolism of MTBE in rats (Savolainen et al., 1985, Miller et al., 1997, Bernauer et al., 1998, Amberg et al., 1999) and in humans (Nihlén et al., 1998a, 1999, Amberg et al., 1999) indicate qualitatively similar overall metabolism. MTBE is oxidatively demethylated by microsomal enzymes to formaldehyde (only detected under *in vitro* conditions) and t-butanol (TBA). *In vivo* Formaldehyde has not been measured following MTBE exposures, but it is known to be rapidly biotransformed further to formic acid, CO_2, or becomes incorporated into the one-carbon pool (McMartin et al., 1979). The biotransformation of TBA (by unidentified microsomal enzymes) yields 2-metyl-1,2-propanediol and α-hydroxyisobutyric acid. In addition, low concentrations of TBA-glucuronide, free TBA and probably TBA-sulfate, are formed. Variable amounts of acetone were also produced in MTBE metabolism.

In most experimental studies with rats and humans, after inhalation exposure, > 50 % of the MTBE retained in the body was biotransformed to urinary metabolites and < 50 % was exhaled unchanged (Miller et al., 1997, Nihlén et al., 1998a, Amberg et al., 1999). The main urinary metabolite in rats and humans was α-hydroxyisobutyric acid (accounting for about 70% of all urinary metabolites), followed by 2-methyl-1,2-propanediol and TBA conjugates (Miller et al., 1997, Amberg et al., 1999). The formation of these metabolites has been confirmed *in vivo* in humans after exposure to ^{13}C-labelled MTBE (Nihlén et al., 1999).

In very high MTBE concentrations (inhalation of 8000 ppm, 500 mg/kg i.p.) the main part is exhaled indicating metabolic saturation (Miller et al., 1997; Yoshikawa et al., 1994). Furthermore MTBE may induce its own metabolism, as shown in rats (Brady et al., 1990). But in the majority of studies the metabolic clearance of MTBE is proportional to the uptake in rats, mice and humans (Miller et al., 1997; Nihlen et al., 1998b; Pekari et al., 1996; Savolainen et al., 1985).

After exposure to MTBE, TBA is found in the blood circulation for a longer period and at higher concentrations than MTBE. The elimination half-time for TBA in blood was about 3 hours in the rat and about 10 hours in humans (Miller et al., 1997; Pekari et al., 1996). The elimination half-times for the different urinary MTBE metabolites varied between 2.9 – 5 hours in rats and between 7.8 – 17 hours in humans (Amberg et al., 1999). These data allow to conclude that MTBE or its metabolites will not accumulate in the human body significantly.

2.2. Acute toxicity

MTBE exhibits low acute toxicity via oral, dermal and inhalation in humans and test animals.

2.2.1. Human data

Several studies are available on patients, where MTBE has been used to dissolve gall stones. For this purpose about 1-15 ml MTBE was instilled into to gall bladder with a syringe. The duration of the treatment was up to seven hours per day, for one to three days. Mild complications during dissolution treatment (nausea, drowsiness, vomiting, local burning sensation) are frequent, and transient elevation of liver transaminases, fever and leukocytosis have occurred among 5-24 % of the patients (Janowitz et al., 1993; Leuschner et al., 1991). No symptoms were reported in 27 patients at blood levels of about 0.5 mmol/l. In the EU RAR it was calculated that some central nervous system

depression sets in at a dose corresponding to inhalation of 1000 ppm, and haemolysis at a level which is 2-3 fold higher.

In several studies with healthy non-smoking human volunteers no effects were recorded with chamber concentrations up to 50 ppm for 2 hours at slight activity state (Prah et al., 1994; Johanson et al 1995; Cain et al., 1996; Riihimäki et al 1996; Nihlen., 1998a).

In one study, 13 subjects were exposed to 0, 25, 75 ppm MTBE for 4 hours. Symptoms and other effects were assessed and reaction times and balance were tested during the exposure (after 1 and 3 hours) and 1 hour afterward. At the highest level and after 3 hours of exposure, there was a significant increase in reports of minor symptoms such as grogginess, and also to a lesser extent irritation of mucous membranes. Most of the symptoms had disappeared when assessments were made one hour after exposure was terminated. Six of the 13 persons reported MTBE-related symptoms. No effects related to the exposures were noted in the tests of balance and reaction time (Riihimäki et al. (1996).

In another study 10 volunteers were exposed to workplace-relevant concentrations (0, 5, 25 and 50 ppm) for 2 hours of light physical exercise (Johanson et al. 1995, Nihlen et al. 1998a). Subjective ratings for discomfort, irritative symptoms, CNS effects and measurements in eye (redness, tear film break-up time, conjunctival damage, blinking frequency) and nose (peak expiratory flow, acoustic rhinometry, inflammatory markers in nasal lavage) were recorded. There was no increase in the prevalence of reported symptoms. The parameters for eye irritation and the inflammation parameters in the nasal lavage fluid were unchanged. The nasal peak expiratory flow was significantly decreased, but not concentration-dependent.

2.2.2. Animal data

In rats the average oral LD_{50} is 4000 mg/kg (ARCO, 1980; Mastri et al., 1969; Kirwin et al., 1993). After inhalation, an LC_{50} of approximately 100 mg/l has been determined (Mastri et al., 1969; ARCO, 1980). The dermal LD_{50} is over 10000 mg/kg (ARCO., 1980; Mastri et al., 1969; RBM, 1996b). In test-animals, the most typical symptom is decreased ability for muscle co-ordination and hypoactivity after oral and inhalative administration. Further clinical signs observed after inhalation were irritation in the eyes and nose, irregular and rapid breathing. After dermal exposure erythema and oedema were seen. According to EU classification criteria, classification is not necessary for any acute toxicity end-point.

2.3. Irritation

2.3.1. Human data

Pure MTBE vapours up to 50 ppm in air did not cause subjective symptoms of eye or nose irritation in young, healthy non smoking volunteers, and the objective measures of eye and nose functions as well as markers of mucous membrane inflammation were not significantly related to MTBE (Prah et al., 1994; Cain et al., 1996; Nihlen et al., 1998a).

2.3.2. Animal data

Skin irritation tests according to OECD-guidelines are somewhat equivocal. The best study showed, that MTBE is a skin irritant (Mürmann, 1985b). Further studies (partly also

according to other protocols) show no irritation or report only slight effects (RBM, 1992a; RBM, 1996a; ARCO, 1980; Hazleton, 1979).

The results of the eye-irritations studies performed according the OECD-guideline 405 (RBM, 1996c; RBM, 1992b; Mürmann, 1985a) show, that MTBE is no eye irritant. Only slight irritations were seen on rabbit´s eye.

The respiratory irritation property of MTBE in mice was investigated analysing the breathing frequency, respiratory waveform and lung lavage up to a concentration of 30000 mg/m^3/1 hour (Tepper et al., 1994). MTBE is not considered as a respiratory irritant.

2.4. Sensitization

A negative result was obtained in a Magnusson-Kligman maximisation test with guinea pigs. A further induction and challenge study and scoring according to Draize (1959) with ten guinea pigs was negative too. MTBE is not sensitising in guinea pigs. There are no observations available in humans.

2.5. Repeated dose toxicity

2.5.1. Human data

There was a greater increase of fatigue over the workweek in a group of petrol tanker drivers compared to milk delivery drivers with an apparent dose-response. Furthermore, 20% of the tanker drivers responded to an open question with symptoms similar to the ones reported earlier (mainly headache and nausea). Exposure measurements indicated that significant (up to 91 mg/m^3) peak levels of MTBE, on top of hydrocarbons (up to 551 mg/m^3), for about half an hour or less may arise in the loading phase (Hakkola et al., 1996).

In an epidemiology study performed in several petroleum factories in China, 96 workers occupationally exposed to MTBE for 1-10 years were investigated (Zhou and Ye, 1999). The time-weighted average concentrations of MTBE in workplaces ranged from 10 to 56 ppm (36 to 202 mg/m^3). The 102 controls were from the same factory and had not been exposed to any harmful chemicals besides MTBE. Exposed workers reporting health complaints were significantly more numerous than controls (62 versus 16 cases, odds ratio 9.8, 95% confidence interval 4.7-20.5). The most frequently reported symptoms in the exposed group (all sbeing significantly more common) were eye irritation (19 cases), dizziness (18), burning sensation in nose or throat (17), insomnia (13), nausea or vomiting (13), headache (12), fatigue (12), poor memory (12), irritability (6), and skin irritation or redness (5). The paper presents no details on MTBE measurements and no data on chemical exposures besides MTBE. It is therefore not possible to draw firm conclusions from this study.

2.5.2. Animal data

Inhalation

28-day study

Male and female F-344 rats and CD-1 mice were subjected to whole body MTBE vapour exposure at 0, 400, 3000, 8000 ppm (Chun et al., 1993). At concentrations ≥ 3000 ppm increased absolute and relative liver weights were seen in both sexes. At these concentrations proliferation of the kidney proximal tubular epithelial cells was seen in male rats, and increased kidney and adrenal weights were observed in females. Increased protein in the kidney tubuli was found in male rats at ≥ 3000 ppm. At 8000 ppm kidney and adrenal weights were increased in male rats also.

In female mice increased absolute and relative liver weights and slight increase in liver cell proliferation was seen at concentrations ≥ 3000 ppm and in males at 8000 ppm. Moreover, at 8000 ppm hepatocellular hypertrophy could be detected in both sexes.

A LOAEC of 3000 ppm and a NOAEC of 400 ppm based on the liver changes was determined.

90-day studies

A whole-body inhalation exposure with MTBE doses of 0, 250, 500, 1000 ppm was carried out with CD-rats (Greenough et al., 1980). At 1000 ppm slight reduction in lung weights was observed in females only and blood changes were seen in males (increased haemoglobin, blood urea nitrogen, LDH) and females (only reduced LDH).

A LOAEC of 1000 ppm and a NOAEC of 500 ppm based on the lung changes in females was determined.

800, 4000 and 8000 ppm MTBE vapour concentrations were given to Fisher-344 rats via whole-body exposure (Dodd et al., 1989; Lington et al., 1997; Daughtrey et al., 1997). At concentrations ≥ 800 ppm absolute and relative liver and kidney weights were increased in males. Statistically significant decreased concentrations of liver transaminases (SGOT, SGPT) were seen at ≥ 800 ppm in males also. At 4000 and 8000 ppm increased absolute and relative liver and kidney weights were also seen in females and increased absolute and relative adrenal weights were observed in both sexes. Slightly increased kidney hyaline droplets were observed in males at 4000 ppm. The immunohistochemical analysis of the kidney slides showed the presence of a2u-globulin in the protein droplets found in the tubules (Swenberg et al., 1991).Furthermore in both sexes at 4000 ppm hypoactivity and at 8000 ppm ataxia was observed. At ≥ 4000 ppm reduced RBC haemoglobin levels and an increase in hematocrit, MCHC, MCV, reticulocytes and leukopenia was observed in males. At 8000 ppm both sexes showed increased blood corticosterone levels, the aldosterone and ACTH concentrations were not affected. Lymphoid hyperplasia in the lymph nodes, moderate haemosiderosis of the spleen and large hyaline droplets of the kidney was seen at 8000 ppm in males only. In this study also neurotoxicity was assessed by a functional observation battery, measurement of motor activity 20 h after last exposure and an examination of neuropathological signs. No significant effects have been found.

A NOAEC of 800 ppm was derived. Absolute and relative liver and kidney weights were significantly increased already at this dose level, however they were not considered adverse due to the absence of histopathological or clinical chemical findings.

Oral

28-day study

90, 440, 1750 mg/kg bw was administered to male and female Sprague-Dawley rats (IITRI, 1992). Relative kidney weights and hyaline droplet formation were significantly increased in males at ≥ 440 mg/kg bw. Statistically significant increased relative liver weights in males and females and relative adrenal weights in males were seen in the 1750 mg/kg bw dose group.

90-day studies

100, 300, 900 and 1200 mg/kg bw was administered by gavage to male and female Sprague-Dawley rats (Robinson et al., 1990). Blood urea nitrogen levels were slightly decreased (statistically significantly) but not in a dose-dependent manner in males and females at all dose levels. Relative kidney weights were increased at ≥ 300 mg/kg bw in females and absolute and relative kidney weights at ≥ 900 mg/kg bw in males. Increased relative liver weights were seen in males at ≥ 900 mg/kg bw. Increased AST levels were observed in males at 300 and 1200 mg/kg bw. Cholesterol was elevated at 900 mg/kg bw in males. Females exposed to all dose levels exhibited significant increases in serum cholesterol and a trend toward a decrease in LDH when compared with controls. The absolute and relative lung weights were increased in males at 1200 mg/kg bw, females had an increase in relative adrenal weight at 1200 mg/kg. The histopathological examination did not reveal any findings related to the treatment.

A LOAEL of 100 mg/kg bw based on increased liver weights as well as AST and cholesterol levels can be derived. I.

After dosing of 200, 600 and 1200 mg/kg bw to male Sprague-Dawley rats increased liver weights were observed at ≥ 200 mg/kg bw. At ≥ 600 mg/kg bw kidney weights were significantly greater than controls (Zhou et al., 1999). No changes in the liver were found by light microscopy. Electron microscopy demonstrated however at all dose levels nuclear condensation, fat droplet and lysosome appearance in cells and smooth endoplasmatic reticulum disintegration.

The LOAEL based on liver effects is 200 mg/kg bw.

Summary on repeated dose administration of MTBE

Mutagenicity in vitro

Bacterial tests

The majority of *Saccharomyces cerevisiae* D4 and Ames tests with MTBE and standard tester strains are negative with and without metabolic activation (EU-RAR, 2001). Only one positive test result was found with TA 102 (with S9).

Mammalian tests

Almost all genotoxicity tests (HPRT, chromosome aberration test with CHO cells) were negative (EU-RAR). Only one positive result was obtained in a mouse lymphoma test with metabolic activation – indicating probably formaldehyde involvement (ARCO, 1980). The indicator tests (UDS assay, SCE test, MNT) showed principally negative results too (EU-RAR).

Mutagenicity in vivo

A Sex-linked recessive lethal test in *Drosophila melanogaster* was negative (Sernau, 1989). In one UDS assay in CD-1 mouse hepatocytes, no increase in the net nuclear grain count and/or the percentage of cells in repair was observed after inhalational administration of MTBE for 6 hours daily up to 8000 ppm on two consecutive days (mice were sacrificed 16 hours after the second exposure) (McKee et al., 1997; Vergnes et al., 1994). A HPRT-locus test in CD-1 mice spleen lymphocytes was negative too (Ward et al., 1995). MTBE-concentrations up to 1000 mg/kg bw by gavage was administered 5 days/week for three weeks. Two micronucleus tests in mice bone-marrow erythrocytes were negative after single i.p. injection and inhalational administration for two days with a maximum concentration of 8000 ppm MTBE respectively (Vergnes et al., 1993; Kado et al., 1998). The chromosomal aberration assay in rat bone marrow cells after repeated dose administration of MTBE vapours up to 8000 ppm was negative too (McKee et al., 1997).

After oral administration of 0.04, 0.13 and 0.4 ml/kg MTBE, either as a single dose or five doses with 24 h intervals. no chromosomal aberrations in bone marrow cells of male rats were induced (Litton Bionetics Inc., 1979). One positive result was obtained in a comet assay on rat lymphocytes (animals received 40, 400, 800 mg/kg day for 28 days). Related parameters that are measures of DNA-strand breakage (e.g. tail length, tail moment) were significantly increased at the highest dose (Lee et al., 1998). However the biological significance of this result remains questionable.

Based on the available information, MTBE is considered to be a non-mutagen.

2.6. Carcinogenicity

Inhalation, 72 weeks

Male and female CD-1 mice were administered 0, 400, 3000 and 8000 ppm (Burleigh-Flayer et al., 1992). Kidney weights were significantly increased at ≥ 400 ppm males. Females at ≥ 3000 ppm had significantly elevated liver weights (9% and 39%). Hepatocellular hypertrophy was increased in the males of 3000 ppm and 8000 ppm dose groups and females of the 8000 ppm dose group. Hepatocellular adenomas and carcinomas (combined) was increased in the 8000 ppm group in females (f: 4%, 4%, 4%, 22%). Statistical significance was only seen in female liver adenoma at 8000 ppm. However, if all malignant tumours are summed, there is no difference from the controls. Furthermore the combined adenoma/carcinoma incidence does not differ from that usually seen in 24-month-old CD-1mice.

Female mice showed an increased kidney and spleen weight at 8000 ppm. The male adrenal weight was increased at 8000 ppm. Both sexes had an increased corticosterone level at 8000 ppm at week 79, but only males showed statistical significance. At 8000 ppm both sexes showed a significantly decreased urine pH at weeks 51 and 79, respectively. Female mice also had a concentration related and statistically significant decrease of uterine endometrium hyperplasia.

Inhalation, 104 weeks

In a two-year carcinogenicity study, groups of 50 Fischer-344 rats of each sex are exposed to MTBE concentrations of 0, 400, 3000, 8000 ppm in an exposure chamber for 6h/day, 5 days/week for 24 months (Bird et al., 1997). Chronic progressive nephropathy (CPN) increased mortality in all dosed groups and was dose related but it was most

severe in the 3000 and 8000 ppm treatment groups. Male rats had an increase in parathyroid gland adenomas at ≥ 3000 ppm (0/50, 0/50, 3/50, 1/50). Renal tubular cell tumours (adenoma and carcinoma combined) were increased only in male rats of the 3000 ppm and 8000 ppm groups (2%, 0%, 10%, 6%). A dose dependent, statistically significant increase of testicular interstitial cell (Leydig cells) tumours was observed at ≥ 3000 ppm (64%, 70%, 82% - $p<0.05$, 94% - $p<0.05$). The proportion of adenomas graded as "moderate" also increased in the 8000 ppm group. The spontaneous incidence of Leydig cell tumours Fischer 344 rats is high. Documented average percentages of control animals with tumours vary from 88%/89% (Chun et al., 1992, Haseman et al., 1990) up to 100 % (DFG, 2000). Nevertheless, a clear dose-response relationship was seen in this study. According to the EU-RAR (2001), the relevance of the development of Leydig cell tumours to man is probably not very significant.

When compared to the controls the male rats in the 3000 ppm group had twice as high corticosterone levels. The 8000 ppm males had 2½ times lower corticosterone levels than controls.

Groups of 50 male and female CD-1 mice were exposed to 0, 400, 3000 or 8000 ppm MBTE. The incidence of hepatocellular adenomas was increased in females (2/50, 1/50, 2/50, 10/50), while the incidence of hepatocellular carcinomas was not increased. In males no significant changes in hepatocellular tumours were observed.

Oral, 104 weeks

Male and female Sprague-Dawley rats were given, by gavage, 250 and 1000 mg/kg bw of MTBE in olive oil (Belpoggi et al., 1995, Belpoggi et al., 1998). Female rats at ≥ 250 mg/kg bw had an increase in the incidence of lymphoimmunoblastic lymphomas and lymphoblastic leukaemia (3,3%, 11,7% - $p<0.1$, 20% - $p< 0.01$). In addition an increase of dysplastic proliferation of lymphoreticular tissues (DPLT, from various body sites) was observed in females at ≥ 250 mg/kg bw. There was an increase in lymphoimmunoblastic dysplasia seen in females at ≥ 250 mg/kg bw (with higher incidence in the 250 mg/kg bw dose group). The most frequently found neoplasm in both dose groups was lymphoimmunoblastic lymphoma localised in the lungs, with a proportion of >85% of the combined incidence.

Male rats showed an increase in incidence testicular interstitial cell adenoma (Leydig cell tumours) at 1000 mg/kg bw. There were no signs of increase of testicular degeneration or atrophy. The spontaneous incidence of Leydig cell tumours in Sprague-Dawley rats is about 10% (Greim, 2000). Females had a dose dependent decrease of fibroma and fibroadenoma of the mammary gland. Statistical significance at level $p<0.01$ was reported in Leydig cell tumours and lymphomas and leukaemia only when the incidences were counted against the number of animals *alive* at the time of observation of the first tumour.

In this study the dosing schedule was unsusual. Futher, animals were allowed to live out their natural lifespan and mortality adjusted analysis was not performed. Therefore, estimates of effective group numbers and tumour incidences were difficult to analyse (IARC 1999).The publication's reporting was inadequate in many aspects, such as toxicological findings, statistical testing and methods, resulting in a low level of confidence of the results. The tumours in the haematopoietic system may be of relevance but based on the information given in this study makes it difficult to interpret.

Mode of action

Several attempts have been made to investigate a possible □2u-globulin nephropathy mechanism to explain the kidney tumours in male rats. First, an accumulation of protein droplets related to MTBE exposure has been observed in the kidney of male not female rats after repeated dose administration. Second, MTBE induced protein droplets staining was positive for □2u, but there was no concentration-dependent linear increase in the intensity of □2u staining after repeated dose administration. Third, MTBE induced kidney lesions, characterised by tubular necrosis and protein droplet accumulation. The lesions were mild especially when compared with strong □2u inducers. Additionally MTBE caused enhanced cell proliferation in male but not in female kidney (Borghoff et al., 1996). Even if the supporting evidence is not as strong as for other known, stronger □2u-inducers it seems quite likely that this is the principal mode of action in the kidney tumorigenesis and consequently not relevant for humans (EU-RAR, 2001).

To test the hypothesis that female mouse tumour response in the liver was associated with hormonal changes, female mice were exposed to MTBE vapor (8000 ppm) for 3 or 21 days or 4 or 8 months Moser et al. 1998). MTBE seems to have an antioestrogen-like effect but does not have specificity for oestrogen receptor. The authors concluded that the relevance of the endocrine alterations in mice exposed to high concentrations of MTBE for extended periods of time to human risk assessment is unknown. In another study changes in oestrogen sensitive tissues were observed in CD-1 mice (Okahara et al., 1998) and it is concluded, that there may be a connection with these changes and the increased amount of liver adenomas seen in female mice at 8000 ppm.

Leydig cell tumours have been induced by non-genotoxic carcinogens that disturb the hormonal balance of testosterone, luteinizing hormone and luteinizing hormone releasing factor in rats. Due to differences between rats and humans in the regulation of gonadotropins, it is questionable that a similar effect will occur in humans. But, there are no studies with measurements of these hormones after MTBE exposure (WHO 1998).

Conclusion on Carcinogenicity

MTBE induces tumours in mice and rats at doses ≥ 3000 ppm after inhalation exposure. Tumours have also been reported in rats at oral doses ≥ 250 mg/kg. The tumours seen at the high doses are also observed in the controls with the exception of the proliferative changes seen in the parathyroid of male Fischer-344 rats. These changes are likely due to hyperparathyroidism, which is commonly seen in cases where parathyroid compensates in hypocalcaemia caused by, e.g., chronic renal failure.

Neither the kidney tumours caused by alpha-2-microglobulin and, consequently, the parathyroid tumours seen in male rats nor the liver tumours in female mice which were seen also in the control animals seem to be of relevance for human health. The same is true for the Leydig-cell tumours.

There is no evidence of a direct genotoxic mode of action. MTBE is considered not-classified for carcinogenicity in the EU. According to the IARC working group evaluation, MTBE is not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 1998)

2.7. Reproductive toxicity

Fertility

There were no adverse changes observed in the gonads in any of the sub-chronic or long-term toxicity studies.

Inhalation, One-generation reproduction study

In an one-generation study Sprague-Dawley rats inhaled 250, 1000, 2500 ppm (whole body) 6 h/day, 5 days/week. Male rats were exposed for a total of 12 weeks. The premating exposure period was 3 weeks, the mating period 5 days. Females were exposed during the premating and mating period for 5 days per week, then every day from day 1 to day 20 of gestation, and from day 5 to day 20 of lactation. The second mating began after a two-week rest period (WHO 1998).

The only effect seen in the parent generation was an increased incidence of dilated renal pelvis at ≥ 250 ppm in females. No effects were found in histopathologic examinations of male and female reproductive organs.

A slight, not statistical significant reduction in the pregnancy index was found for both litters.

The pup viability was significantly lower at ≥ 1000 ppm groups of the F1b litter (96 % compared to 99 % in controls. No such change was seen in the F1a litter, but there the viability of the controls was somewhat lower (97.6%) There was also a significant reduction in pup survival index in days 0-4 of lactation at 250 ppm and 1000 ppm only in the F1a litter. The pup body weights in the mid and high dose groups of both litters were slightly, but not significantly lower than in controls during days 14 and 21. The most frequent post-mortem observation for pups sacrificed on day 21 was dilated renal pelvis.

Inhalation, Two-generation reproduction study

Groups of 25 Sprague-Dawley parent rats were exposed whole-body to 400, 3000, 8000 ppm MTBE (Bevan et al., 1997). The rats were exposed during the pre-mating period over 10 weeks, The exposure of the females continued gestation and lactation starting day 5 until the day of sacrifice, which followed the weaning of the offspring. Male exposure continued until the delivery of F1-litter. The new parents were randomly selected from the F1-litter at weaning on postnatal day 28 (exposure start). The exposure procedure was the same as it was for the P-animals.

There were general toxicity signs at 3000 and 8000 ppm in both generations of parental animals (reduced bodyweight, hypoactivity, blepharospasms). Gross examination of the parent F1 animals revealed at necropsy statistically significantly increased relative liver weights in males at ≥ 3000 ppm and at 8000 ppm in both sexes. There was a statistically significant increase of dead pups in both F1 and F2 generation litters with no change in survival indices. The authors did not consider the deaths in the F1 generation to be related to MTBE because the increase was due to a death of an entire litter of 16 animals.

In conclusion: MTBE does not cause significant toxicity to reproduction in Sprague-Dawley rats.

Developmental toxicity

Inhalation, Sprague-Dawley rats

MTBE was administered to pregnant Sprague-Dawley rats in an inhalation exposure chamber at concentrations of 0, 250, 1000 and 2500 ppm during the gestation days 6-15 for 6/hours/day (Conaway et al. 1985). No changes in body weight gain could be detected in dams, however, there was a significant reduction in food consumption in all treatment groups during the treatment interval on days 9-12. No changes were found in dam liver weights. No treatment related findings were found in pregnancy rates, in number of resorptions or live foetuses compared to controls. No compound related effects were observed in foetuses too. There was a 58% predominance of male foetuses in the treated dams, which authors contributed to biological variability.

Inhalation, CD-1 mice

MTBE was administered to pregnant CD-1 mice in the same way as described above (Conaway et al., 1985). No changes in body weight gain were seen in dams but food and water consumption was decreased in the treated groups. The only adverse effect in foetuses was a slight increase in skeletal tissue abnormalities in the 2500 ppm dosed group (Incidence of fused sternebrae 0, 0.6, 1.2, 2.1 %).

Pregnant CD-1 mice were exposed to 1000, 4000, 8000 ppm MTBE during gestation days 6-15, 6 h/day (Bevan et al., 1997). There were no deaths or abortions and the pregnancy rate was over 93% in all groups. Clinical signs like ataxia, hypoactivity, prostration, laboured respiration and lacrimation were mainly noted in the animals of ≥ 4000 ppm groups. Reduced body weights, and uterine weights (50% lower than controls) were observed at 8000 ppm. Total post-implantation loss was more than three-fold when compared to other groups. This resulted mostly from significantly ($p<0.01$) increased incidences of late resorptions and dead foetuses. About 30% reduction in live foetuses/litter was seen at 8000 ppm and sex distribution was female dominant by almost 10 % when compared to control.

Foetal body weights were reduced significantly ($p<0.01$) by 8% and 27% in the 4000 and 8000 ppm dose groups, respectively. Poor ossification was seen in concentrations ≥ 4000 ppm. After visceral examination the incidence of cleft palate was significantly increased in the 8000 ppm group. Also in the 8000 ppm group, there was a significantly reduced incidence of partial foetal atelectasis, a failure in the inflation of at least one region of the lung. Skeletal variations were increased at 8000 ppm and were represented by reduced ossification in the skull, cervical, thoracic, and caudal centra, limbs and sternebrae.

Inhalation, New Zealand White rabbits

Pregnant rabbits were administered 1000, 4000, 8000 ppm MTBE during gestation days 6-15, 6 h/day (Bevan et al., 1997). The pregnancy rates remained between 80%-100% in all groups. The only treatment related effect found on maternal rabbits was 15% increased liver weight in the 8000 ppm group and over 70% reduced food consumption. Gestation parameters and other reproduction data were similar along all the groups.

The foetuses had no significant differences in weight, in the incidence of external, visceral or skeletal malformation or variation. While all other groups had zero skeletal malformations, the highest dose group had skeletal abnormalities in 3/12 litters. These were mainly malformations of the thoracic part of the body.

Conclusion on developmental toxicity

The malformations seen at 8000 ppm in CD-1 mice are considered to occur at a dose level of marked maternal toxicity. Also the skeletal abnormalities occur only at high maternal toxic dose levels. Therefore MTBE is not considered to have specific developmental toxic effects.

Summary on the endocrine and reproductive system

Twelve female B6C3F1-mice were exposed to 8000 ppm MTBE for 4 or 8 months (6 h/d, 5 d/w) (Moser et al., 1998). Several effects consistent with endocrine modulation were seen (decreased uterine, ovary and pituitary weight, fewer uterine glands, decreased number of cervical and vaginal epithelial layers). Additionally, alterations in the stages and length of the oestrous cycle and increased ACTH-immunoreactivity of the pituitary and a loss of zona reticularis of the adrenal cortex were seen. Changes in endocrine hormones included an increase in corticosterone levels were seen at ≥ 4000 ppm after 13 weeks in Fisher-344 rats (Lington et al., 1997) at ≥ 3000 ppm after 2 years in Fisher-344 rats and CD-1 mice (Bird et al., 1997). There was also an increased corticosterone level seen in rats at 40 mg/kg already after 14 days of MTBE administration (Day et al., 1998).

Male Sprague-Dawley rats were treated with up to 2500 mg MTBE/kg/day for 15 and 28 consecutive days respectively, in order to study possible hormonal changes (Williams et al., 2000). The 1000 mg/kg bw/d group had a lower serum testosterone (15 d) and a significant serum LH decrease (28 d). At ≥ 1000 mg/kg (28 d) the triiodothyronine was significantly decreased and at 1500 mg/kg (28 d) the rats showed a decreased dihydrotestosterone. Decreased serum testosterone levels were also observed at 800 mg/kg after 28 days in Sprague-Dawley rats (Day et al., 1998.The database is insufficient to conclude on the true significance rendering the derivation of a meaningful NOAEL impossible.

Recommendation

Animal studies in mice and rats reveal a spectrum of tumours induced by MTBE in liver, kidney, parathyroid and Leydig-cells that occur only at high doses (> 250 mg/bw after oral administration and > 3000 ppm in inhalation studies). Neither the kidney tumours caused by alpha-2-microglobulin and, consequently, the parathyroid tumours seen in male rats, nor the liver tumours in female mice which were seen also in the control animals seem to be of relevance for human health. The same is true for the Leydig-cell tumours. There are neither epidemiological studies addressing a possible association of MBTE with human cancer, nor grounds for assuming there to be a concern. MTBE is not genotoxic. Therefore, a threshold for the carcinogenic potential of this compound is assumed.

Studies on toxicokinetics concluded that MTBE or its metabolites do not accumulate in humans.

Experimental animal studies involving repeated inhalation exposure indicate no effects of toxicological significance for human health below 1000 ppm, effects having been reported only at 3000 ppm and above. There are no relevant data for the toxicity of MTBE in man after repeated exposure.

In a study by Rihimäki et al. (1996), volunteers reported mild symptoms, mainly feeling of heaviness in the head, and mild mucosal irritation. The frequency of symptoms was related to the exposure level (0, 25, 75 ppm) and reached statistical significance at 75 ppm after 3h of exposure to MTBE. No effects were seen on simple reaction time or body sway posturography. This study suggests a LOEL of 75 ppm.

In another volunteer study Nihlén et al (1998b) found no increased ratings of either irritation, or CNS effects. Further, none of the objective measurements indicated any irritative or inflammatory response (blinking frequency, eye redness score, tear-film break-up time, conjunctival epithelial damage score, inflammatory markers in nasal lavage, nasal acoustic rhinometry, nasal blockage index). This study suggests a NOEL for short-term exposure of 50 ppm.

Taking into account the low inhalation toxicity in animal studies, the NOEL of 50 ppm for irritation in humans and the mild effects seen after several hours at 75 ppm, SCOEL proposes a 8-h TWA of 50 ppm and a 15-min STEL of 100 ppm.

The limited available data suggest that the dermal route is of minor importance, thus there is no need for a skin notation.

There should be no measurement difficulties at the proposed limits.

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