INTERIM 1: 1/2003
INTERIM 2: 2/2005

INTERIM ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

METHANOL (CAS Reg. No. 67-56-1)

For
NAS/COT Subcommittee for AEGLs

February 2005

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicologic and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. AEGL-2 and AEGL-3 levels, and AEGL-1 levels as appropriate, will be developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and will be distinguished by varying degrees of severity of toxic effects. It is believed that the recommended exposure levels are applicable to the general population including infants and children, and other individuals who may be sensitive or susceptible. The three AEGLs have been defined as follows:

AEGL-1 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects, or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing odor, taste, and sensory irritation, or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL level, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL level. Although the AEGL values represent threshold levels for the general public, including sensitive subpopulations, it is recognized that certain individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL level.

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EXECUTIVE SUMMARY

Methanol (also known as wood alcohol) is a clear, colorless, volatile, flammable liquid with a sweet odor. It is used in paint removers, windshield washer fluid, automotive fuel, and antifreeze; as an industrial solvent; and as a raw material in the production of many commercially important organic compounds. Small amounts of methanol are produced over the course of normal body metabolism and are found in the exhaled air.

Methanol is rapidly absorbed after ingestion or inhalation. Percutaneous absorption is also considerable. Acute methanol toxicity varies greatly between species, primarily as a result of differential metabolism. At very high inhaled concentrations rodents exhibit much higher blood methanol concentrations than do primates. Primates accumulate greater amounts of the important toxic metabolite formic acid (found in equilibrium in plasma with its anion, formate). Primates are more susceptible than rodents because of the greater accumulation of formates in primates. Clinical experience with those who ingested methanol (often under the mistaken assumption that they were consuming ethanol) demonstrates marked variations in individual susceptibility and delayed onset of severe, overt toxicity. The initial phase of inebriation is similar to that seen after ethanol but is usually mild and transient and is generally followed by an uneventful initial recovery. The most important clinical consequences develop between 6 and 30 hours after the initial exposure.

Wide individual variations in response are most likely due to individual rates of formate production from methanol in the liver. People with pre-existing liver disease (e.g., cirrhosis) often appear resistant to methanol poisoning because of their relatively inefficient conversion of methanol to formic acid. Accumulation of formate in primates leads to depletion of the normal bicarbonate buffering capacity of the body, delayed-onset metabolic acidosis and death with acute cerebral edema, CNS depression, and coma. The severity of the poisoning and the patient's prognosis are related directly to the extent of formate and lactate formation, which account largely for this metabolic acidosis. Among victims who survive the initial phase, vision can become severely impaired and permanent bilateral blindness can follow formate-induced retinal edema, demyelination of the temporal retina, hemorrhagic necrosis in the basal ganglia, and nerve head pallor. Pancreatitis has been associated with acute abdominal pain. Occupational methanol exposures in confined spaces or in workrooms with inadequate ventilation have been associated with recurrent giddiness (mild inebration), headache, nausea, insomnia, blurred or dim vision, and conjunctivitis. The delayed onset of symptoms, the potent ocular degeneration, and the metabolic acidosis seen in primates poisoned with methanol are not observed in rodents. In rodents, methanol can cause fetotoxic and teratogenic effects. Preliminary studies provided some evidence of developmental effects in monkeys..

The AEGL-1 was based on a study in which human volunteers inhaled 800 ppm methanol for 8 hours (Batterman et al., 1998). As this was a pharmacokinetic study, health effects were not formally evaluated. In a personal communication the coauthor Dr. Alfred Franzblau stated that individual symptoms were asked of some subjects, other subjects were only asked generally if they had symptoms, and that in some exposure sessions subjects might not have been queried. According to Dr. Franzblau, none of the subjects reported symptoms. NIOSH (1980) and Frederick et al. (1984) reported significantly higher frequencies of headaches, dizziness, blurred vision after occupational exposure at 1060 ppm (mean concentration). NIOSH (1981) reported eye irritation in a worker after exposure at 1025 ppm for 25 minutes. Since the 1000-ppm level was considered already a discomfort level, the 800 ppm for 8 hour

exposure from the Batterman et al. (1998) study was chosen as a starting point for AEGL-derivation. Since the local irritation effects are determined by the concentration of methanol in air and not to the blood methanol level, calculation of AEGL-1 values was not done using a pharmacokinetic model (as done for AEGL-2 and -3) based on the end-of-exposure blood methanol level of 30.7 mg/l reported by Batterman et al. (1998). Instead, exposure to 800 ppm for 8 hours was used as the basis for AEGL-1 derivation. A factor of 3 was applied for intraspecies variability because interindividual variability with regard to slight central nervous system effects (e.g. headache) is likely to exist (although it cannot be quantified exactly from the existing experimental and epidemiological studies) and because subpopulations with a less than optimal folate status may be more susceptible to the health effects of methanol. The value was scaled to appropriate exposure periods according to the dose-response regression equation $C^n \times t = k$, using the default of n=3 for shorter exposure periods, due to the lack of suitable experimental data for deriving the concentration exponent. For the 10-minute AEGL-1, the 30-minute value was applied because no studies were available that demonstrated the absence of notable discomfort (with respect to irritation) in the general population, including susceptible subpopulations, at 970 ppm (which would be the extrapolated value for the 10-minute period).

A level of distinct odor awareness (LOA) for methanol of 8.9 ppm was derived on the basis of the odor detection threshold reported by Hellman and Small (1974). The LOA represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, about 10 % of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odor perception.

The AEGL-2 values were based on developmental toxic effects. In mice, repeated 7-hour/day exposures during gestational days 6 to 15 caused a dose-related, significant increase in cervical ribs at 2000 ppm or higher; other malformations, such as exencephaly and cleft palate occurred concentration-dependently at 5000 ppm or higher (Rogers et al., 1993). The same type of malformations was found after a single 7-hour exposure at 10000 ppm (no other concentrations tested) (Rogers et al., 1997). In another study, which has not been formally published up until know, Rogers and coworkers (Rogers et al. 1995, abstract; Rogers, 1999, personal communication) exposed mice on gestational day 7 to different concentration-time combinations. The most sensitive endpoint was cervical rib induction, which occurred at concentration-time products greater than or equal to 15000 ppm · h, but not at concentration-time products below 15000 ppm · h (i.e. no effects were observed at 2000 ppm for 5 h, 2000 ppm for 7 h or 5000 ppm for 2 h; authors expressed data only as CxT values). Thus, while 2000 ppm for 7 hours was a LOEL in the repeated exposure study (Rogers et al., 1993), it was a NOEL after single exposure. Although the single exposure study had shortcomings in the reporting, it was very consistent with the well-documented repeated exposure study. It was therefore considered adequate to use an exposure at 2000 ppm for 7 hours as a starting point for AEGL-2 derivation. At the NOEL of 2000 ppm for 7 hours (Rogers et al. 1995, abstract; Rogers, 1999, personal communication), the corresponding end-of-exposure blood methanol concentration was measured as 487 mg/l (Rogers et al., 1993). A total uncertainty factor of 10 was used. An uncertainty factor of 1 was applied for interspecies variability because a sensitive species was used for derivation of AEGL-2 values and because toxicokinetic differences between species were accounted for by using a pharmacokinetic model for calculating exposure concentrations. An uncertainty factor of 10 was used for intraspecies variability because no information on developmental toxic effects of methanol on humans is available and because also for other chemicals the variability in susceptibility of humans for developmental toxic effects is not well characterized. Moreover, pregnant women are a subpopulation with a less than optimal folate status and, thus, may be more susceptible to the health

effects of methanol. Using a total uncertainty factor of 10, a blood methanol concentration of 48.7 mg/l was derived as the basis for calculation of exposure concentrations. Application of the uncertainty factor to the blood methanol concentration was preferred because the calculated exposure concentrations in air stayed better in the concentration range for which the pharmacokinetic model was validated and the effect of methanol metabolism for longer exposure periods was more adequately taken into account. In contrast, first calculating exposure concentrations that would lead to a blood methanol level of 487 mg/l, and then applying a factor of 10 to the derived exposure concentration would result in calculation of extremely high concentrations in the fist step at which metabolic pathways would be saturated. After application of the uncertainty factor, concentrations would be below saturation level which would mean that the end-of-exposure methanol levels would vary for the AEGL-2 exposure concentration-time combinations. Using the pharmacokinetic model of Perkins et al. (1995a), inhalation exposure concentrations were calculated for appropriate time periods that would lead to a blood methanol concentration of 48.7 mg/l at the end of the time period. The calculated exposure concentrations were set as AEGL-2 values.

The AEGL-3 values were based on oral intoxications in humans. Several case studies (Naraqi et al., 1979; Erlanson et al., 1965; Bennett et al., 1955; Gonda et al., 1978; Meyer et al., 2000) reported measured blood methanol concentrations and time periods between intoxication and measurement. Given the time that elapsed until blood sampling, during which part of the methanol was metabolized, it can be concluded that peak blood methanol concentrations have been above 1000 mg/l in all fatal cases . Based on the extensive clinical experience with methanol intoxications, the American Academy of Clinical Toxicology (AACT, 2002) published clinical practice guidelines on the treatment of methanol poisoning. According to these guidelines, peak blood methanol concentrations >500 mg/l indicate serious poisoning for which hemodialysis is recommended. Based on the human experience, a peak blood methanol concentration of 500 mg/l was chosen as the basis for AEGL-3 derivation. A total uncertainty factor of 3 was used. An uncertainty factor of 3 was applied for intraspecies variability because clinical experience with methanol intoxications is mainly based on cases involving adult men while much less data is available for women, children or elderly persons, and because subpopulations with a less than optimal folate status may be more susceptible to the health effects of methanol. Using a total uncertainty factor of 3, a blood methanol concentration of 167 mg/l was derived as the basis for calculation of exposure concentrations. Application of the uncertainty factor to the blood methanol concentration was preferred because the calculated exposure concentrations in air stayed better in the concentration range for which the pharmacokinetic model was validated and the effect of methanol metabolism for longer exposure periods was more adequately taken into account. In contrast, first calculating exposure concentrations that would lead to a blood methanol level of 500 mg/l and then applying a factor of 3 to the derived exposure concentration would result in calculation of extremely high concentrations in the fist step at which metabolic pathways would be saturated. Using the pharmacokinetic model of Perkins et al. (1995a), inhalation exposure concentrations were calculated for appropriate time periods that would lead to a blood methanol concentration of 167 mg/l at the end of the time period. The calculated exposure concentrations were set as AEGL-3 values.

The proposed AEGL values are listed in the table below.

SUMMARY TABLE OF PROPOSED AEGL VALUES FOR METHANOL [a]						
Classification	**10-Minute**	**30-Minute**	**1-Hour**	**4-Hour**	**8-Hour**	**Endpoint (Reference)**
AEGL-1 (Nondisabling)	670 ppm (880 mg/m³)	670 ppm (880 mg/m³)	530 ppm (690 mg/m³)	340 ppm (450 mg/m³)	270 ppm (350 mg/m³)	No headache or eye irritation (Batterman et al., 1998; pers. commun. Franzblau, 1999; 2000; Frederick et al., 1984; NIOSH, 1980; 1981)
AEGL-2 (Disabling)	11000 ppm [b] (14000 mg/m³)	4000 ppm (5200 mg/m³)	2100 ppm (2800 mg/m³)	730 ppm (960 mg/m³)	520 ppm (680 mg/m³)	No developmental toxic effects in mice Rogers et al. (1993; 1995, abstract; 1997); Rogers (1999, personal communication)
AEGL-3 (Lethal)	#	14000 ppm [b] (18000 mg/m³)	7200 ppm [b] (9400 mg/m³)	2400 ppm (3100 mg/m³)	1600 ppm (2100 mg/m³)	Lethality in humans after oral exposure (AACT, 2002)

[a] Cutaneous absorption may occur; direct skin contact with the liquid should be avoided.
[b] The 10-minute AEGL-2 value and the 30-minute and 1-hour AEGL-3 values are higher than 1/10 of the lower explosive limit (LEL) of methanol in air (LEL = 55,000; 1/10th LEL = 5500 ppm). Therefore, safety considerations against the hazard of explosion must be taken into consideration.
[#] The 10-minute AEGL-3 value of 40,000 ppm is higher than 50% of the lower explosive limit of methanol in air (LEL = 55,000 ppm; 50% of the LEL = 27,500 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

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1. INTRODUCTION

Methanol is a clear, colorless, volatile flammable liquid with a characteristic pungent odor when pure.

Methanol is used in the industrial production as solvent and as raw material for the production of many important organic compounds, principally formaldehyde, methyl tert.-butyl ether, acetic acid, glycol methyl ethers, methylamine, methyl halides and methyl methacrylate. Methanol is a constituent of a large number of commercially available solvents and consumer products including paints, shellacs, varnishes, paint thinners, cleansing solutions, antifreeze solutions, duplicating fluids, denaturant for ethanol, and in hobby and craft adhesives. Potentially large uses of methanol are in its direct use as a fuel (in the future), in gasoline blends or as a gasoline extender. About 20 million tons of methanol were produced worldwide in 1991, principally by catalytic conversion of hydrogen, carbon dioxide and carbon monoxide (NLM, 1998; WHO, 1997). The world-wide production capacity was about 30 million tons in 1995 (WHO, 1997). Chemical and physical properties of methanol are listed in Table 1.

TABLE 1: CHEMICAL AND PHYSICAL DATA

Parameter	Value	Reference
Molecular formula	CH_3OH	NLM, 1998
Molecular weight	32.04	NLM, 1998
CAS Registry Number	67-56-1	NLM, 1998
Physical state	liquid	NLM, 1998
Color	colorless	NLM, 1998
Synonyms	Methyl-alcohol; carbinol; Methylalkohol; wood alcohol; EPA-Pesticide-Chemical-Code-053801	NLM, 1998
Vapor pressure	133 hPa (21.2 ˚C) 125 hPa (20 ˚C) 169 hPa (25 ˚C) 152 hPa (25 ˚C)	NLM, 1998 Rippen, 1998 NLM, 1998 Rippen, 1998
Density	0.8100 g/ml (0/4 ˚C) 0.7928 g/ml (20 ˚C)	NLM, 1998 WHO, 1977
Melting point	-97.8 ˚C	NLM, 1998
Boiling point	64.7 ˚C (1010.8 hPa)	NLM, 1998
Solubility	Miscible with ethanol, ether, ketones, benzene, most organic solvents and water; soluble in acetone, chloroform	NLM, 1998
Odor	Alcoholic odor; pungent odor when crude; pungent	NLM, 1998
Explosive limits in air	5.5% (lower) and 44% (upper) 6.7% (lower) and 36.5% (upper)	WHO, 1977 AIHA, 1994

Parameter	Value	Reference
Conversion factors	1 ppm = 1.31 mg/m³ (25 ˚C, 1010.8 hPa) 1 mg/m³ = 0.764 ppm (25 ˚C, 1010.8 hPa)	NLM, 1998 NLM, 1998

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

Almost all cases of acute methanol toxicity result from ingestion. Intoxication may result from methanol contamination of grain spirits, consumption of adulterated alcoholic beverages, suicidal ingestion of methanol containing products and unintended consumption of such products (ACCT, 2002, Buller and Wood, 1904, Becker, 1983, WHO, 1977). However, the majority of cases occurred at the end of the last and at the beginning of this century after introduction of wood alcohol as an industrial solvent, and no reliable exposure concentrations or durations are available for these cases. For example, Tyson and Schoenberg (1914) counted about 100 cases of impairment of vision and death from inhalation of methanol at the workplace. After early headache, dizziness, nausea, changes in color perception and blurred vision, delayed deaths follow, about one day after sufficiently high methanol exposure. Death and blindness (often bilateral) in those who survive are directly related to the extent of formate-induced metabolic acidosis.

In one methanol fatality by inhalation, a woman died after a 12-hour exposure at the workplace (Anonymous, 1932). The time between cessation of exposure and death was not stated. A postevent study of the exposure conditions revealed concentrations ranging from 4000 to 13000 ppm. No further details were reported.

Single Oral Exposure

From a large number of reports on methanol poisonings as a result of the consumption of adulterated beverages (WHO, 1977), it was concluded that the minimum oral lethal dose is about 1 g/kg (Buller and Wood, 1904; Röe, 1982). Buller and Wood (1904) concluded that an oral methanol dose of 1.4 g/kg would be lethal to 40 % of the victims.

The American Academy on Clinical Toxicology published practice guidelines on the treatment of methanol poisoning (AACT, 2002). The publication reviewed mechanisms of toxicity, clinical features and laboratory findings. Early after intoxication methanol may produce a significant osmolal gap. The osmolal gap is the difference between measured osmolarity in blood (usually 270-290 mOsm/kg water) and the calculated osmolarity (which is equivalent to $(1.86[Na^+]+[BUN]+[glucose])/0.93$). Early in the course of methanol poisoning the osmolal gap usually exceeds 20 mOsm/kg water; for example a blood methanol level of 1000 mg/l will cause an osmolal gap of 34 mOsm/kg water. At a later stage of methanol poisoning, the formic acid generated will produce metabolic acidosis and an anion gap. The latter is the difference between the sum of the sodium and potassium concentrations and the sum of the chloride and bicarbonate concentrations in blood (i.e. $([Na^+]+[K^+])-([HCO_3^-]+[Cl^-])$). The normal anion gap of 12-16 mmol/l can be attributed to negatively charged proteins, fatty acids, sulfates and phosphates. A significant anion gap will not be present early in the course of methanol intoxication when the serum bicarbonate concentration falls while the chloride concentration increases. When the bicarbonate buffer capacity is depleted, blood pH will start to decline and this is accelerated by the accumulation of lactate as a result of formate-induced inhibition of mitochondrial respiration. “Clinical symptoms correlate more closely to

metabolic acidosis rather than to serum methanol concentrations. Case series suggest that visual dysfunction occurs when formate concentrations exceed 200-300 mg/l. Poor prognostic indicators include serum formate concentrations >500 mg/l, a pH <7.0, and coma or seizures on admission to the emergency department." "A variety of factors complicate the correlation of serum methanol concentrations to clinical effects including differences in sample timing, individual variation, concentration of toxic metabolites, and the ingestion of ethanol. Clinical symptoms and mortality correlate more closely with metabolic acidosis rather than with serum methanol concentrations. Consequently, the clinical presentation and outcome of two patients with the same serum methanol concentrations may be substantially different." "Peak methanol concentraions below 200 mg/l usually are associated with asymptomatic individuals, but interpretation of the methanol concentration requires consideration of the time since ingestion, the co-ingestion of ethanol and the acid-base status. Peak methanol concentrations over 500 mg/l indicate serious poisoning, particularly if an anion gap metabolic acidosis is present." "If a patient presents with ophthalmological symptoms and signs or with significant acidosis in the context of a likely methanol ingestion, the initial priorities are to correct the acidosis with sodium bicarbonate, attempt to enhance metabolism of formate to carbon dioxide by administration of folinic acid [or folic acid], inhibit further metabolism of methanol to formate with either fomepizole or ethanol, and finally to arrange hemodialysis for further correction of metabolic abnormalities, if necessary." Treatment with fomepizole or ethanol is recommended at plasma methanol concentration >200 mg/l, or documented recent history of ingesting toxic amounts of methanol and osmolal gap >10 mOsm/kg water, or history or strong clinical suspicion of methanol poisoning and at least two of the following criteria: arterial pH <7.3, serum bicarbonate <20 mmol/l, osmolal gap >10 mOsm/kg water. Hemodialysis for removal of methanol and formate is recommended for the following conditions: significant metabolic acidosis (pH <7.25-7.30), abnormalities of vision, deteriorating vital signs despite intensive care support, renal failure, electrolyte imbalance unresponsive to conventional therapy, or serum methanol concentration >500 mg/l.

Naraqi et al. (1979) described 32 men (mean age 23, range 17-39) who drank pure methanol. The methanol was mixed with orange juice or soft drinks. The purity of the methanol was confirmed later by gas chromatography. The estimated amount of methanol consumed ranged from 60 to 600 ml (mean 275 ml). Three patients consumed ethanol immediately prior to drinking methanol. The first symptoms appeared 8-36 hours (mean 18 hours) after consumption and comprised blurred vision, pupillary changes, fundi changes, abdominal pain, vomiting, nausea, headache, dizziness, lethargy, restlessness, coma, seizures, and Kussmaul respiration. Circulating methanol and ethanol concentrations of 15 patients were measured in blood drawn within the first 48 hours after hospital admission. The treatment consisted of sodium bicarbonate infusion; ethanol, peritoneal or hemodialysis were not used. Of 28 patients admitted to hospital, 4 died (one of those had an elevated blood ethanol concentration) within 72 hours, 16 recovered without complications, 2 became totally blind, 4 developed severe visual impairment and 2 had severe visual disturbances as well as speech difficulties. Blood methanol concentrations in fatal cases (except for the case of concomitant ethanol exposure) are shown in Table 2. Blood methanol concentrations >500 mg/l were seen in only two non-fatal cases. Individual blood methanol concentrations of surviving patients were not reported.

Erlanson et al. (1965) described 4 patients that consumed pure methanol that had been sold as ethanol. Three patients died in spite of intensive care including ethanol therapy, bicarbonate infusion and hemodialysis. Blood methanol concentrations and symptoms are given in Table 2. The lowest concentration associated with fatal outcome was 275 mg/l measured 52 hours after methanol uptake; in this patient ethanol therapy was begun after 48 hours.

Bennett et al. (1953) reported on several cases of oral methanol poisoning. The cases in which no or only trace amounts of ethanol were detected in the blood are shown in Table 2. Of five cases, two with estimated oral doses of 0.6 and 5.6 g/kg died in spite of hospital treatment, while the other three cases survived ingestion of estimated doses of 1.1, 1.9 and 3.3 g/kg.

Gonda et al. (1978) described the consequences of ingestion of windshield washer fluid (90-95 % methanol). All cases were treated with ethanol, sodium bicarbonate and hemodialysis (except for 2 cases that did not receive ethanol). Of 9 patients, 2 died and 3 of the 7 survivors had permanent visual impairment. Measured blood methanol concentrations are given in Table 2.

Meyer et al. (2000) tabulated the time between methanol ingestion and hospital admission along with blood methanol concentrations for 4 cases (see Table 2).

Kahn and Blum (1979) described a fatal dermal methanol exposure in an 8-month-old boy. The child had been "treated" with methanol-soaked compresses during two nights (about 12 hours each) before he was admitted to hospital. A blood methanol concentration of 400 mg/l was determined in the early afternoon. The child died in that evening in spite aggressive medical intervention.

Although several other reports on fatal oral methanol exposures have been documented in the literature (e.g. Keeney and Mellinnkoff, 1949; Kane et al., 1968), these are not presented here because methanol exposure was combined with ethanol intake in most of these cases. Since ethanol at blood concentrations of about 1 g/l or higher can completely block methanol metabolism, reported methanol doses or blood methanol concentrations are not useful for the derivation of AEGL values.

TABLE 2: ACUTE ORAL METHANOL INTOXICATIONS IN HUMANS

Clinical outcome	Sex, age	Blood methanol conc. (mg/l) at time postexposure (h)	Latent period, symptoms, remarks	Reference
death after 48 h	male 27	730 (< 48 h)	8 h coma (admission)	Naraqi et al., 1979
death after 36 h	male 19	1110 (< 48 h)	36 h coma (admission)	Naraqi et al., 1979
death after 36 h	male 20	3260 (< 48 h)	12 h coma (admission)	Naraqi et al., 1979
death after 136 hours	male 49	275 (52 h)	15 h failing vision, 24 h vomiting, hearing disturbances, 28 h restlessness, 29 h coma, 48 h (admission and ethanol therapy)	Erlanson et al., 1965
death after 79 h	male 65	277 (53 h)	15 h nausea, vomiting, headache, 19 h failing eye sight, 30 h severe visual disturbances, cyanosis, 42 h coma, 48 h (admission and ethanol therapy)	Erlanson et al., 1965
death after 110 h	female 49	860 (53 h)	42 h unconsciousness, 43 h respiratory standstill, 44 h (admission and ethanol therapy)	Erlanson et al., 1965
survived	female 39	194 (50 h)	9 h vomiting, 36 h failing eye sight, 44 h blindness, 45 h clouding of consciousness (admission and ethanol therapy)	Erlanson et al., 1965
death during treatment of relapse	male 41	4000 (18 h)	blind, headache; estimated oral dose about 50 ml	Bennett et al., 1953
death on 4th day	male 48	1300 (24 h)	blind, headache, abdominal pain, blind, stupor; estimated oral dose about 500 ml	Bennett et al., 1953
death during treatment of relapse	male 26	2500 (48 h)	cloudy vision, headache, nausea, abdominal pain, vomiting	Bennett et al., 1953
recovered	male 34	1500 (18 h)	cloudy vision, headache, abdominal pain, weakness, vomiting, stupor; estimated oral dose about 100 ml	Bennett et al., 1953
recovered	female 29	2700 (18 h)	impaired vision, retinal edema, headache, dizziness, nausea, vomiting; estimated oral dose about 150 ml	Bennett et al., 1953

Clinical outcome	Sex, age	Blood methanol conc. (mg/l) at time postexposure (h)	Latent period, symptoms, remarks	Reference
recovered	male 43	1600 (48 h)	cloudy vision, retinal edema, headache, abdominal pain	Bennett et al., 1953
died	male 30	5600 (12 h)	comatose	Gonda et al., 1978
died	male 48	3700 (24 h)	confusion, progressing coma	Gonda et al., 1978
survived, eye damage	male 43	5700 (4 h)	comatose	Gonda et al., 1978
survived, eye damage	male 42	250 (40 h)	blurred and greenish vision	Gonda et al., 1978
survived, eye damage	male 45	30 (100 h)	weakness, dyspnea, vomiting, abdominal pain, visual impairment developed after 3 days	Gonda et al., 1978
survived	female 51	530 (24 h)	dizziness, headache, nausea	Gonda et al., 1978
survived	male 15	740 (24 h)	stupor, nausea, vomiting	Gonda et al., 1978
survived	female 48	560 (24 h)	slurring speech	Gonda et al., 1978
survived	male 36	1020 (40 h)	profound weakness, photophobia, blurred vision, slurred speech	Gonda et al., 1978
died	male 30	2050 (36 h), 970 ethanol	coma	Meyer et al., 2000
survived	male 28	1150 (36 h)	nausea	Meyer et al., 2000
survived	male 25	990 (36 h)	visual impairment	Meyer et al., 2000
survived	female 41	192 (36 h)	no symptoms	Meyer et al., 2000

2.2. Nonlethal Toxicity

The signs and symptoms of methanol poisoning include initial headache, dizziness, nausea, weakness and insomnia, shooting pains, paresthesia, prickling and numbness in the extremities. Changes in color perception and blurred vision (Browning, 1965; NIOSH, 1976; Becker, 1983; Kavet and Nauss,

1990; ACCT, 2002) develop as formate concentrations increase over time. After a latency period (cf. Section 4.2) life-threatening metabolic acidosis and permanent bilateral blindness can develop.

2.2.1. Experimental Studies

Batterman et al. (1998), studied 4 healthy women (aged 41-63 years) exposed at 800 ppm for 30, 60 and 120 min. Each of these exposures was repeated with the same subjects. Additionally, 3 other women and 12 men (age not stated) were exposed at 800 ppm methanol for 8 hours. All volunteers were healthy, non-smoking individuals. In the article, the authors made no statement on the presence or absence of any signs or symptoms of the methanol exposure. In a personal communication, the second author, Dr. Alfred Franzblau, stated that although no formal mechanism of recording symptoms was used, the subjects were generally asked during exposure if they experienced any discomforts. Dr. Franzblau wrote "individual symptoms were certainly asked of some subjects" and that "none of the subjects reported odor, irritation, headache or other non-specific symptoms"; likewise "none of the subjects reported any difficulties or alterations of visual function". Dr. Franzblau wrote that it is possible that some subjects were not queried in that no written notes were made. Both, investigators and subjects, knew the methanol concentrations during each of the sessions. Dr. Franzblau recalled that a meter was set up outside the window of the exposure chamber so that the subjects could see directly the concentration of methanol inside the chamber. The investigators also had exposure to methanol at the various levels, either because they spent some time in the chamber during the experiments, or because they conducted trail runs on themselves before conducting the studies on other subjects (Franzblau, 1999; 2000; personal communication).

Chuwers et al. (1995) allowed 26 healthy subjects (15 men, 11 women) in an exposure chamber to inhale methanol at 200 ppm for 4 hours. The exposure concentration was continuously monitored by an infrared spectrophotometer and, in addition, by gas chromatography. The measured exposure concentration was 199±7 ppm. Immediately before and upon conclusion of exposure several visual (Vistech contrast sensitivity test, Lanthony 15 Hue desaturated panel color discrimination test), neurophysiological (P-300 auditory evoked potentials) and neurobehavioral (2-and-7 visual scanning performance, Stroop test, Symbol Digit substitution test, Sternberg memory task) tests were performed. Because the time to complete all tests required one hour, some of the tests (2-and-7, Stroop and Symbol Digit tests) were started during the last half hour of exposure. Each subject was once exposed to methanol and once to water vapor in random order in a double-blind fashion. Methanol and formate concentrations in serum and urine were measured during exposure 0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes after beginning and 1, 2, 3 and 4 hours after the cessation of exposure. The effect of methanol was significantly only on two outcomes: the P300 amplitude when alcohol consumption and smoking accounted for between-subject variability and on the Symbol Digit test with age accounting for between-subject variability. A correlation with the area under the serum methanol curve was found for P300 amplitude, but not for the Symbol Digit test. Although no odor detection was reported by the subjects, 18/26 subjects (13 expected) guessed correctly the methanol exposure session. The possible unblinding of test subjects potentially could have affected the subjects‘ performance. The authors concluded that a 4-hour exposure to 200 ppm methanol did not significantly affect neurobehavioral, neurophysiological and visual performance in a healthy normal population. An accompanying paper about the same study did not find a significant increase in urinary or serum (14.3±8.9 mg/l vs. 12.7±1.7 mg/l in controls) formate concentrations (D‘Alessandro et al., 1994).

In a similar experiment, Cook et al. (1991) exposed 12 healthy young men, each serving as his own control, for 75 minutes to 250 mg/m³ (190 ppm) methanol. The mean analytical concentration (±SD) measured using an infrared gas analyzer and by gas chromatography was 249±7 mg/m³. Each subject was twice sham-exposed and twice exposed to methanol under double-blind control conditions. 22 neurobehavioral and neurophysiological tests were administered before, during, and after exposure to measure visual, behavioral, reasoning, and hearing functions. Methanol exposure had no effect on the subjects' performance on most of the tests. However, some methanol-exposed subjects reported fatigue and lack of concentration. Performance was also slightly impaired in the Sternberg memory task. There were also changes in the latency of the P200 component of the visual- and auditory-event related potential. These effects were small and did not exceed the range of results measured in filtered air-exposed subjects.

Muttray et al. (2001) exposed 12 male, healthy, right-handed students by inhalation in an exposure chamber for 4 hours to 20 or 200 ppm methanol (cross-over designed study). Analytical concentrations were 20.3±3.8 (±SD) ppm and 203.5±2.5 (±SD) ppm, respectively. Electroencephalographic examinations were performed immediately after conclusion of exposure with closed and open eyes and during the color word stress test. Significant alterations in the encephalograms between exposure to 20 or 200 ppm were found only in measurements performed with eyes shut. No effects were found in the color word stress test. A German version of an Swedish Performance Evaluation System questionnaire was administered before, 2 h and 4 h after exposure. It contained the following 17 items: headache, dizziness, nausea, tiredness, pain or pressure over the chest, coughing spells, shortness of breath, irritation to the eyes, watering eyes, blurred sight, irritation to the nose, running nose, sensation of a bad smell, irritation to the throat, sensation of an unpleasant taste, irritation to the skin, and feeling of faintness or vertigo. Subjects were requested to check off the degree of their symptoms of an ordinal scale from 0 (no symptom) to 5 (severe symptom). None of the symptom scores increased significantly during the exposure to 20 or 200 ppm methanol. The authors considered the electroencephalographic alterations not as an adverse effect, but as a subclinical, excitatory effect of methanol.

The American Industrial Hygiene Association critiqued odor threshold studies and reported a range of 4.2-5960 ppm with a geometric mean of 160 ppm for the odor detection threshold and a range of 53-8940 ppm with a geometric mean of 690 ppm for the odor recognition threshold (AIHA, 1989). Other review articles reported ranges of 10-20500 ppm (Ruth, 1986), 382-15280 ppm (O'Neill and Phillips, 1992) and 3-7640 ppm (Verschueren, 1983). In a review article, Amoore and Hautala (1983) reported a geometric mean odor detection threshold of 100 ppm (range 10-20500 ppm) using odor thresholds reported in the literature, but "omitting extreme points and duplicate quotations". Several of the reviewed studies (Scherberger et al., 1958, May, 1966) cannot be considered adequate for deriving a reliable odor threshold because of insufficient exposure conditions (sniffing at a bottle opening), unstated purity of the methanol used, lack of presentation of technical details and analytical procedures.

Hellman and Small (1974) measured the absolute and recognition thresholds of methanol in air. In this study odor thresholds were determined for 101 petrochemicals using a trained odor panel in the Union Carbide Technical Center, South Charleston, WV. Details of the procedure used were not reported. The absolute odor threshold (detection limit) for methanol was 4.26 ppm. At this concentration "50 % of the odor panel observed an odor". The odor recognition threshold was the concentration at which 50 % "of the trained odor panel defined the odor as being representative of the odorant being studied". The air odor recognition threshold was 53.3 ppm (at this concentration all subjects recognized the odor, the 50 % recognition level was not established).

Leonardos et al. (1969) used a combination of a test room and an antechamber, which was held odor-free using an air filter system, and a trained panel of four staff members of the Food and Flavor Section of Arthur D. Little, Inc., determined the air odor threshold for methanol. At least 5 different concentrations were tested. The individual concentrations tested were not reported. An odor recognition threshold of 100 ppm was determined for methanol. A similar value was also reported in an experimental study by Ryazanov (1961).

Flury and Wirth (1933) exposed 2 to 4 individuals for 5 minutes to methanol concentrations of 1, 10 or 86 mg/l (760, 7600 or 65400 ppm; nominal concentrations). Methanol was sprayed into the exposure chamber and dispersed by a ventilator; analytical measurements of the exposure concentrations were not performed. Only a weak odor perception was reported at 760 ppm. 7600 ppm was associated with very weak nasal irritation, while 65400 ppm induced a very strong (unbearable) nasal irritation, which made deep respiration impossible, and marked ocular irritation. From the study report it remains unclear whether the test subjects were examined for symptoms other than irritation.

Leaf and Zatman (1952) studied the pharmacokinetics of methanol exposing themselves up to four times to methanol concentrations between 0.7 mg/l (530 ppm) for about 3.3 hours and 1.43 mg/l (1090 ppm) for about 3 hours. The authors stated that under the conditions of the experiment exposures of 3-4 hours were as long as could reasonably be tolerated. They did not state, however, whether this limitation was due to effects caused by methanol or the experimental design.

2.2.2. Occupational Exposure

Studies with repeated inhalation exposure

NIOSH (1980) (data also published in Frederick et al., 1984) studied the exposure relationship and possible health effects of methanol exposure from spirit duplicators in teacher aides. Fifteen-minute breathing zone samples from 21 of 58 duplicators in 12 schools were analyzed using a Wilkes Miran 1A® gas analyzer. Measured methanol concentrations ranged from 365 to 3080 ppm (mean 1060 ppm, median 1040 ppm). Fifteen of 21 measurements exceeded 800 ppm. 11 measurements were between 1000 and 1500 ppm and only one was above this range. The authors reported that additional exposure as a result of skin absorption during the handling of paper wet with methanol was likely. A health questionnaire survey was conducted among 84 female teacher aides, of whom 66 (mean age 39.8 years, range 24-60) responded. Exposure times varied widely from 1 hour/day for 1 day/week to 8 hours/day for 5 days/week during about 3 years. 302 teachers from the same schools served as a comparison group. Of the teachers responding, 66 female (mean age 37.5 years, range 24 to 59 years) were randomly selected for comparison. Part of the teachers also spent some time in the duplicator rooms (the reports do not provide exact exposure information for the teachers). Among the aides, 4 of the 22 symptoms listed in the questionnaire were reported significantly ($p<0.05$ using Mantel-Haenszel Chi-square test) more frequently: headache (34.8% in aides vs. 18.1% in controls), dizziness (30.3% vs. 1.5%), blurred vision (22.7% vs. 1.5%) and nausea/upset stomach (18.0% vs. 6.0%). Similar prevalences were found for symptoms, such as trouble sleeping, unusually tired, irritable, giddiness, poor memory/confusion, muscle weakness and dry/sore throat. No information on the exact exposure duration and the time between start of exposure and occurrence of symptoms was provided. The data indicated that the prevalence of methanol toxicity cases increased with the percentage of time spent at duplicators per week. The authors defined a methanol toxicity case by any of the following four symptom aggregations: 1) visual changes or blurred vision, 2)

one acute symptom (headache, dizzines, numbness, giddiness, nausea or vomiting) and one chronic symptom (unusually tired, muscle weakness, trouble sleeping, irritability or poor memory), 3) two acute symptoms or 4) three chronic symptoms.

Kawai et al. (1991) analyzed 48 personal samples of breathing-zone air from 31 different subjects, using tube-type diffusive samplers and gas chromatography: 5 samples indicated time-weighted average methanol concentrations during an 8-hour work shift between 3000 and 5500 ppm, 10 samples were between 1000 and 2000 ppm, 4 samples were between 500 and 1000 ppm and 19 below 500 ppm. Exposed workers were grouped into a group exposed to higher methanol concentrations (22 workers; geometric mean exposure concentration 459 ppm) and a group exposed to lower methanol concentrations (11 workers; geometric mean 31 ppm) (the authors did not report the concentration used as the criterion for grouping). The following subjective complaints were given significantly more in the high-exposure group compared to the low-exposure group: dimmed vision during work (11/22 vs. 0/11) and nasal irritation during work (7/22 vs. 0/11).
The symptom of 'dimmed vision' has been questioned by the authors who stated that "Further questioning disclosed that the workers in fact saw fog in the workroom air, especially on humid days when the factory was especially busy; the fog was probably produced by the reaction of methanol vapor with humidity in the air. No visual problems were noted when the windows were kept open and fresh air was allowed to flow in, suggesting that this symptom might not be of direct medical significance, although it should indicate the presence of dense methanol vapor." The fact that headaches did not occur more frequently supports the author's interpretation that the 'dimmed vision' was a physical rather than a health-related problem because in other occupational studies, headaches occurred at lower concentrations than effects on vision (Kingsley and Hirsch, 1955) or, at higher exposure concentrations, as a more frequent symptom than blurred vision (NIOSH, 1980; Frederick et al., 1984). In conclusion, the reported 'dimmed vision' is considered most likely not to be a methanol-caused health effect.
The authors did not try to correlate the symptoms with the measured breathing-air samples. No significant differences between the two groups were found for the following symptoms: dimmed vision off work, unusual feeling in the throat, unusual smell during work, headache off work, increased sensitivity of the skin in the extremities off work, forgetfulness off work, fainting after suddenly standing up off work, and chill sensation in the extremities off work. On ophthalmologic examination, 3/22 vs. 0/11 subjects showed clinical signs: in two subjects a slow light reflex of the pupils was observed and one person showed slightly mydriatic pupils. The geometric mean of methanol exposure of the 3 subjects was 1017 ppm. One of the two subjects showing a slow light reflex had a habit of drinking an equivalent of 75 g ethanol per day. No information on the exposure duration and the time between start of exposure and occurrence of symptoms was provided.

Kingsley and Hirsch (1955) reported that an unspecified number of employees working in the immediate vicinity of direct process duplicating machines complained of frequent and recurrent headaches. The duplicating machines used duplicating fluids containing 5-98 % methanol. Since the other ingredients were not identified, exposure to other volatile compounds cannot be ruled out. The authors stated that those individuals situated closer to the machines experienced more severe headaches, those who actually operated the equipment suffered the most, and that with the onset of cold weather, when doors and windows were closed, the severity and frequency of the headaches increased. Methanol concentrations measured in the breathing zone of the workers ranged from 15 to 375 ppm and generally were in excess of 200 ppm. The method of analysis was not reported. No information on exposure duration was provided.

2.2.3. Case Studies

Cases of methanol poisoning after inhalation have been reported in the literature (Tyson and Schoenberg, 1914; NIOSH, 1976; IUCLID, 1996). However, reliable information about exposure concentrations or durations is lacking and the incidents very often involved repeated or long term exposure to methanol.

NIOSH (1981) reported the results of an environmental evaluation of a spirit duplicating machine workplace. Measurement was done by collecting breathing zone samples for 5 consecutive 5-minute periods. The measured concentration range was 950-1100 ppm (mean 1025 ppm). The operator experienced eye irritation at the end of the 25-minute period. No information is given regarding sex and age of the operator and whether this operator had experienced more or less symptoms in the past compared to other duplicating machine operators in the same school.

Humperdinck (1941) reported a case of methanol poisoning during handling of damp nitrocellulose (35-40 % methanol) in a nitrocellulose plant. The worker had been on this job for 4 years and had not previously reported any symptoms. He became ill following the institution of wartime blackout measures which impaired plant ventilation. The worker became blind in the right eye with marked narrowing of the visual field in the left eye. Examination of the workplace air revealed methanol concentrations ranging from 1600 to 10900 mg/m³ (1200 to 8300 ppm). These symptoms were not reported in another 22 workers exposed to methanol. No statement was made on whether these workers experienced any other symptoms.

689 **TABLE 3: SUMMARY OF EFFECTS ON HUMANS AFTER INHALATION OF METHANOL**

Concentration (ppm)	Exposure Time	Study type and effects	Reference
4000-13000 (probable range)	12 h (workplace)	case study; fatal case after occupational exposure	Anonymous, 1932
1200-8300 (probable range)	unknown (workplace)	case study; visual disturbances, blindness on one eye	Humperdinck, 1941
65400	5 min	experimental study; very strong (unbearable) nasal irritation, strong eye irritation	Flury and Wirth, 1933
7600	5 min	experimental study; very weak nasal irritation	Flury and Wirth, 1933
760	5 min	experimental study; weak odor perception, no irritation	Flury and Wirth, 1933
1060 (mean)	1 h/d to 8 h/d (repeatedly at workplace)	occupational study; more frequent headaches, dizziness, blurred vision, nausea/upset stomach	NIOSH, 1980; Frederick et al., 1984
1025 (mean)	25 min	eye irritation	NIOSH, 1981
800	8 hours	experimental pharmacokinetic study with no statement on effects; in a personal communication, a coauthor stated that the subjects did not report any symptoms	Batterman et al., 1998; Franzblau, 1999; 2000
459 (mean)	8 hours (repeatedly at workplace)	occupational study; dimmed vision (the authors suggested that visibility was temporarily reduced by fog in the workroom) and nasal irritation	Kawai et al., 1991
200-375	unknown (repeatedly at workplace)	occupational study; recurrent headaches	Kingsley and Hirsch, 1955
200	4 hours	experimental study; no significant CNS effects	Chuwers et al. 1995
190	75 minutes	experimental study; no significant CNS effects	Cook et al., 1991

2.3. Developmental/Reproductive Toxicity

Very little information is available regarding developmental or reproductive effects of methanol in humans (NTP-CEHRH, 2003; WHO, 1997).

Lorente et al. (2000) investigated the role of maternal occupational exposure in occurrence of cleft lip and palate. Data from the study was obtained from a multicenter European case-referent study utilizing 6 congenital malformation registers between 1989 and 1992. Occupational exposures during the first

trimester were studied in 851 women; 100 cases had infants with oral clefts and 751 referents had infants without oral clefts. The subjects were interviewed to determine occupational history and the types of products used on the job. An industrial hygienist reviewed interview responses to determine the probability of chemical exposures. Confounding factors considered included maternal age, socioeconomic status, residence, urbanization, country of origin, and medical history. Subjects were interviewed about smoking, and alcohol intake but it is not clear if the analyses considered those factors. Data were analyzed by estimating an adjusted odds ratio for each type of exposure. Analyses determined that at least 10 % of the subjects were likely exposed to methanol during the first trimester of pregnancy. Odds ratios of 3.61 (95% C.I. 0.91-14.4) and 3.77 (95% C.I. 0.65-21.8) were calculated for methanol exposure and occurrence of cleft palate only and cleft lip with or without cleft palate, respectively. Although these ratios are elevated, they are consistent with the null hypothesis of no increased risk for orofacial clefts after occupational exposure to methanol. It should be noted that for methanol, the numbers were quite small (only 2 cases with cleft palate and 4 with cleft lip with or without cleft palate exposed methanol).

2.4. Genotoxicity

No studies documenting genotoxic effects of methanol in humans were identified (WHO, 1997).

2.5. Carcinogenicity

No studies documenting carcinogenic effects of methanol in humans were identified (WHO, 1997).

2.6. Summary

Although several case reports on lethal methanol poisoning of humans due to exposure by inhalation have been published in the literature, data on exposure concentration and exposure duration are usually lacking. Information about lethal effects on humans after oral uptake of methanol is available: The conclusion drawn by several authors (Buller and Wood, 1904; Röe, 1982) that the minimum lethal oral dose is about 1 g/kg is supported by three studies reporting on intoxication incidents in which humans drank pure methanol (i.e. no concomitant ethanol consumption). Bennett et al. (1953) reported two lethal cases after uptake of estimated oral doses of 0.6 and 5.6 g/kg, while another three cases survived ingestion of 1.1, 1.9 and 3.3 g/kg. In the study of Naraqi et al. (1979), the lowest blood methanol concentration associated with fatal outcome was 730 mg/l measured about 24 hours after uptake. Erlanson et al. (1965) reported a lowest blood methanol concentration of 275 mg/l in a fatal case, measured about 52 h after intoxication.

At lower exposure concentrations headache and visual disturbances are the most critical endpoints. In a pharmacokinetic study, 15 subjects were exposed to 800 ppm for 8 hours; the authors made no statement on health effects (Batterman et al., 1998), but in a personal communication a coauthor stated that the subjects did not report any symptoms. Chuwers et al. (1995) found no significant effect on neurobehavioral, neurophysiological and visual performance in an experimental study after a 4-hour exposure to 200 ppm. Similarly, no significant effects on neurobehavioral and neurophysiological test results were observed after a 75-minute exposure to 190 ppm (Cook et al., 1991). After repeated exposure at the workplace to average concentrations of about 1000 ppm headache, dizziness, nausea and blurred vision have been reported (NIOSH, 1980; Frederick et al., 1984). Weak nasal or eye irritation have been

reported after exposure to 7600 ppm for 5 minutes (Flury and Wirth, 1933), 1025 ppm for 25 minutes (NIOSH, 1981) and after repeated occupational exposure to mean concentrations of 459 ppm (Kawai et al., 1991). For the odor threshold, a very wide range of values has been reported in the literature, e.g. the American Industrial Hygiene Association critiqued odor threshold studies and reported a range of 4.2-5960 ppm with a geometric mean of 160 ppm for the odor detection threshold and a range of 53-8940 ppm with a geometric mean of 690 ppm for the odor recognition threshold (AIHA, 1989). In an experimental study, Hellman and Small (1974) determined an odor detection threshold of 4.26 ppm.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

Data on acute lethal concentrations of methanol for single exposure periods and repeated exposures are available for the monkey, cat, rat and mouse. The interpretation of lethality data is difficult, because of the different mechanisms involved in different species: in rodents no accumulation of formate is observed and animals die of central nervous system depression after acute exposure to very high methanol concentrations; in contrast, in humans and non-human primates delayed death at considerable lower concentrations of methanol is seen due to metabolic acidosis caused by formate accumulation (see Section 4.2). In addition, developmental toxicity and fetal death was reported in rodents after subchronic exposure to methanol concentrations well below those causing death in adult animals (see Section 3.3). For this reasons, data from studies on monkeys and developmental toxicity studies on rodents seem relevant for the derivation of AEGL values. The lethality data are summarized in Table 4.

3.1.1. Non-human Primates

McCord (1931) exposed rhesus monkeys to methanol concentrations of 40000, 20000, 10000, 5000 or 1000 ppm. The author reported that exposure at 40000 ppm for 4 hours resulted in prompt death of the monkeys (probably two animals, not exactly stated) and exposure at 40000 ppm for 1 hour (probably of one animal, not exactly stated) resulted in sickness for 2-3 days and delayed death. The authors did not report clinical observations or number of exposed animals for the 20000-ppm and 10000-ppm exposures. 1000 ppm produced death in 1 of 4 animals after an exposure for 18 hours/day for a "total of 41 hours". Another animal "long survive[d] the action of 5000 ppm"; the exact exposure duration and effects were not reported. The author used synthetic methanol from 3 different commercial sources as well as "pure natural", "95% natural" and "crude natural" methanol without specifying which animal was exposed to which type of methanol and whether any differences in toxicity were observed. The monkeys were from a group of 31 rhesus monkeys taken from the wildlife and brought to the USA only shortly before the experiments. One of the monkeys died of pneumonia within 24 hours of arrival and another one was killed due to "low-grade inflammation of the face". The group comprised male and female monkeys, but the gender of the exposed animals was not indicated. The exact duration and frequency of exposure as well as detailed effects were not reported.

Studies with repeated inhalation exposure

NEDO (1987) exposed monkeys (Macaca fascicularis) (number of animals given in brackets) at 3000 (4), 5000 (3), 7000 (1) or 10000 (2) ppm methanol for 21 hours/day for different exposure periods; the control group comprised 6 animals. Continuous monitoring of the exposure concentration revealed mean concentrations of 3053±61, 5071±22 and 5018±34, 7079±37 and 10441±402 ppm, respectively.

One animal exposed at 10000 ppm showed lethargy and after the third exposure (i.e. the third day) was comatose and died. Another animal exposed to 6000-10000 ppm (duration for different exposure concentrations not clearly stated) died after 6 days. One animal exposed to 7000 ppm had to be killed after 6 days. Of three animals exposed to 5000 ppm, two died on the 5th day and the third on the 14th day. No lethality was observed in 4 animals exposed at 3000 ppm for 20 days. Nonlethal effects observed in this experiment are reported in Section 3.2.1.

Andrews et al. (1987) exposed groups of 3 male and 3 female cynomolgus monkeys (Macaca fascicularis) to 0, 500, 2000 or 5000 ppm methanol for 6 hours/day, 5 days/week for 4 weeks. The air exchange rate of the exposure chamber was 0.33 min^{-1}. Methanol exposure levels were monitored with a Wilkes Miran 1A-CVF ® infrared analyzer and measured values were within ±10 % of the nominal concentrations. Animals were observed for signs of toxicity twice each day and given a detailed physical assessment each week without observing any exposure-related effect. No deaths were reported after repeated exposure to methanol concentrations of up to 5000 ppm. See Section 3.2.1 for nonlethal effects.

Studies with non-inhalation exposure

Gilger and Potts (1955) gave single oral doses of 1, 2, 3, 4, 6 or 8 g/kg to rhesus monkeys (one animal/dose). Death was observed at 3 g/kg or higher with the time to death decreasing with increasing concentrations: death occurred after 32-38 h, 29-36 h, 29 h and 6-23 h at 3, 4, 6 and 8 g/kg, respectively. After lethal doses signs of inebriation were observed; semicoma was seen only shortly before death. Deaths occurred from respiratory failure. At doses of 1 and 2 g/kg, animals did not show any symptoms.

3.1.2. Cats

Flury and Wirth (1933) exposed groups of 2 cats to methanol concentrations of 141, 113, 86, 59, 44 or 24 mg/l (107200, 85900, 65400, 44800, 33400 or 18200 ppm) for 6 hours. Somnolence occurred at conclusion of exposure time at 33400 ppm or higher. Prostration was seen at 65400 ppm for 4.4 hours, 85900 ppm for 4.1 hours or 107200 ppm for 4.0 hours. Delayed deaths were observed for one of two animals exposed at 33400, 65400 or 107200 ppm and for both animals exposed at 85900 ppm methanol during the 14-day postexposure observation time.

3.1.3. Rats

LC_{50} values for adult rats reported in industry studies include: 145000 ppm for 1 hour (DuPont Co., Haskell Laboratory, 1974), 97900 ppm for 4 hours (BASF, 1980a) and 66900 ppm for 6 hours (BASF, 1980b). NIPRI (1974) reported an LC_{50} of 64000 ppm for 4 hours.

Loewy and Von der Heide (1914) exposed rats to different concentration-time combinations. 31600 ppm for 18-20 hours resulted in death. 22500 ppm for 8 hours and 50000 ppm for 2.5 hours caused narcosis and 13000 ppm for 20 hours prostration. 8800 ppm for 8 hours led to lethargy and 2000 ppm for 8 hours had no effect.

3.1.4. Mice

Scott et al. (1979) reported that the LC_{50} for male mice was 41000 ppm for 6 hours. The observation period was 24 hours. Izmerov et al. (1982) reported an LC_{Lo} of 37594 ppm for 2 hours in

mice. Pavlenko (1972) reported coma, but no deaths, after exposure of mice to 71000 mg/m³ (54000 ppm) for 3.5-4 hours/day up to a cumulative total of 54 hours (corresponding to about 14 exposure days; no details reported).

Several older studies report effects on mice: Weese (1928) observed that exposure at 53500 ppm for 54 hours or 71800 ppm for 54 or 28 hours led to narcosis and death. Mice exposed at 48000 ppm for 24 hours showed narcosis and those exposed to 10000 ppm for 230 hours showed ataxia. Lehmann and Flury (1943) reported narcosis in mice exposed at 42000 ppm for 7 hours. Marshbitz et al. (1936) exposed white mice to methanol concentrations of 40, 60, 80, 100,120, 133 or 200 mg/l (30560, 45480, 61120, 76400, 91680, 101610 or 152800 ppm) for up to 4 hours. During exposure mice first showed a state of drowsiness, then an excited state, followed by an impairment of coordination and finally narcosis. Narcosis developed after 190, 153, 134, 89, 95, 91 and 94 minutes, respectively. The overall mortality within one month after exposure was 45 % (mortality information for individual groups was not provided).

TABLE 4: SUMMARY OF ACUTE LETHAL INHALATION DATA IN LABORATORY ANIMALS

Species	Concentration (ppm)	Exposure Time	Effect	Reference
Monkey	40000	1 h	sickness in 2-3 days, delayed death	McCord, 1931
Monkey	40000	4 h	death	McCord, 1931
Monkey	10000	21 h/d, 3 d	lethargy, after 3 exposures comatose and died	NEDO, 1987
Monkey	7000	21 h/d, 6 d	animals had to be killed after 6 days	NEDO, 1987
Monkey	5000	21 h/d, 5 d	of 3 animals, 2 died on day 5 and one on day 14	NEDO, 1987
Monkey	5000	6 h/d, 5 d/w, 4 w	no mortality	Andrews et al., 1987
Monkey	3000	21 h/d, 20 d	no mortality	NEDO, 1987
Monkey	1000	18 h/d, 41 h total	shortest exposure resulting in death	McCord, 1931
Cat	33400	6 h	1of 2 animals died	Flury and Wirth, 1933
Rat	145000	1 h	LC_{50}	DuPont Co., Haskell Laboratory, 1974
Rat	97900	4 h	LC_{50}	BASF, 1980a
Rat	64000	4 h	LC_{50}	NPIRI, 1974
Rat	66900	6 h	LC_{50}	BASF, 1980b

Species	Concentration (ppm)	Exposure Time	Effect	Reference
Rat	50000	2,5 h	no mortality, narcosis	Loewy and Von der Heide, 1914
Rat	31600	18-20 h	lethal	Loewy and Von der Heide, 1914
Rat	22500	8 h	narcosis	Loewy and Von der Heide, 1914
Rat	5000	24 h/d, gd 7-17	fetal death in late pregnancy (see Section 3.3.2)	NEDO, 1986
Rat	5000	7 h/d, gd 1-19	no fetal death (see Section 3.3.2)	Nelson et al., 1985
Mouse	71800	54 h	narcosis, death	Weese, 1928
Mouse	71800	28 h	narcosis, death	Weese, 1928
Mouse	53500	54 h	narcosis, death	Weese, 1928
Mouse	54000	3.5-4 h/d, total 24 h	comatose, survived	Pavlenko, 1972
Mouse	48000	24 h	narcosis, survived	Weese, 1928
Mouse	30560-152800	≤ 4 h	narcosis after 190-94 min, overall mortality 45 %	Marshbitz et al., 1936
Mouse	42000	7 h	narcosis	Lehmann und Flury, 1943
Mouse	41000	6 h	LC_{50}	Scott et al., 1979
Mouse	37594	2 h	LCL_{Lo}	Izmerov et al., 1982
Mouse	10000	7 h, gd 7	fetal death (see Section 3.3.3)	Rogers et al., 1995
Mouse	7500	7 h/d, gd 6-15	fetal death; NOEL 5000 ppm (see Section 3.3.3)	Rogers et al., 1993

3.2. Nonlethal Toxicity

Studies reporting nonlethal effects after a single acute exposure to methanol and relevant for derivation of AEGL values are lacking. Several studies report nonlethal effects, affecting mainly liver, the nervous system and kidney, and developmental toxic effects (see Section 3.3). These data are summarized in Table 5.

3.2.1 Non-human Primates

Studies with repeated inhalation exposure

NEDO (1987) exposed monkeys (Macaca fascicularis) (number of animals given in brackets) at 3000 (4), 5000 (3), 7000 (1) or 10000 (2) ppm methanol for 21 hours/day for up to 20 days. As reported in Section 3.1.1, delayed mortality occurred in animals exposed to 5000 ppm or higher. At cassation of exposure to 3000 ppm or higher, animals were restless, moving around the cage and had frequent blinking and yawning, which can be interpreted as signs of eye and respiratory tract irritation. Animals exposed to 3000 ppm became used to methanol exposure after approximately 4 days and recovered activity, movement and appetite. At 5000 ppm or higher, animals showed reduced movement, crouched for a longer time, had difficulty in standing up, showed involuntary hand movements, vomiting and dyspnea. Exposure at 5000 ppm or higher for 5 days or longer induced necrosis of the basal ganglia of the cerebrum, severe cerebral edema, severe liver necrosis and vacuolar degeneration of the kidneys. After exposure at 3000 ppm for 20 days, mild alterations in the cerebral tissue around ventricles without edema or necrosis and a slight fatty degeneration of the liver without necrosis were observed.

In another experiment of this series (NEDO, 1987), monkeys (number indicated in brackets) were exposed at 1000 (5), 2000 (3) or 3000 (4) ppm methanol for 21 hours/day for 7 months, and killed for pathological analysis after recovery periods of 0, 1, 6 or 10 months. Continuous monitoring of the exposure concentration revealed mean exposure levels of 1013±64, 2095±73 and 3089±58 ppm, respectively. During the course of the exposure period, scratching of the body, frequent yawning and runny noses were observed at all concentrations. Slight necrotic changes of basal ganglia nerve cells were found after exposure to 3000 ppm and a recovery period of one month; these alterations were not found after the animals had recovered for periods of 6 or 10 months. Groups exposed to 1000 or 2000 ppm showed the presence of responsive stellate cells in the frontal and parietal lobes, but no necrosis of basal ganglia. These stellate cells disappeared after a recovery period of 6 months. In contrast, the presence of stellate cells persisted throughout the recovery period after exposure at 3000 ppm. A slight increase of glial cells in the optic nerve and a slight degeneration of peripheral nerves was observed in the 1000-ppm group after 6 months recovery, but not in animals examined immediately after the end of the 7-month exposure period. Similar observations were obtained in animals exposed at 2000 ppm and examined after 6 or 10 months of recovery. Monkeys exposed at 3000 ppm showed a slight optic nerve atrophy and a reduction of myelinated nerve fibers. In all groups a concentration-dependent round cell infiltration and slight fibrotic alterations of the liver was found. The liver changes were unrelated to the recovery period, but their strength did correlate with the exposure concentration and exposure period.

In another experiment of this series (NEDO, 1987) monkeys were exposed for 21 hours/day at 10, 100 or 1000 ppm methanol for 7, 19 and 29 months (groups of 2, 3 and 3 animals, respectively). Concentrations measured in the exposure chambers were 9.9±1.3, 101.0±8.2 and 1016±83 ppm, respectively. Runny noses were seen in animals exposed at 100 or 1000 ppm. In the high exposure group animals scratched over the whole body and crouched for long periods of time. No differences in food and water intake and in body weight gain were seen. No signs of degeneration of the basal ganglions of the cerebrum were found in histopathological analysis. A diffuse increase of responsive stellate cells, centered in the subcortical white substance, was evident in a high proportion of cases. Histologically, these cells are not characteristic of degeneration, but they were nearly absent in normal monkeys in the control group. These responsive stellate cells were not correlated with methanol concentration or period of exposure. In the reparatory test, these cells were no longer observed after exposure was ended, so their occurrence is thought to be a reversible transient histological reaction to methanol inhalation. In the visual system no abnormal symptoms were observed that correlated with the exposure concentration. In the groups exposed

to 1000 ppm, round-cell infiltration in the liver was seen after all periods of exposure, but only after exposure for 29 months a fibrosis was seen in 2 of 3 monkeys. This fibrosis was strictly limited and the histopathological effect was considered small. No fibrotic reactions were found in the groups exposed to 10 or 100 ppm.

Andrews et al. (1987) exposed groups of 3 male and 3 female cynomolgus monkeys (Macaca fascicularis) at 0, 500, 2000 or 5000 ppm methanol for 6 hours/day, 5 days/week for 4 weeks. As described in Section 3.1.1, no deaths were observed. Body weights were recorded prior to study initiation and weekly during thereafter. No effects on body weights or organ weights compared to controls were observed except that female monkeys exposed at 5000 ppm had significantly lower absolute adrenal weights (the authors considered this difference as not having any apparent biological significance). Animals showed no upper respiratory tract irritation, gross and histological examination of 35 different tissues of control and high-dose monkeys revealed no effects. No details were given on which tissues were studied and, thus, it is unclear whether histopathology included the optic nerve and peripheral nerves, for which effects were reported in the study by NEDO (1987). No ocular toxic effects were observed after gross, microscopic and ophthalmoscopic examinations.

3.2.2. Dogs

Loewy and Von der Heide (1914) exposed dogs to methanol vapor. They observed no effects at 2000 ppm for 24 hours or 13700 ppm for 4 hours. At 36700 ppm for 8 hours or 50000 ppm for 1 hour, dogs showed prostration and incoordination. The postexposure observation period and technical details were not reported.

3.2.3. Cats

Flury and Wirth (1933) exposed groups of 2 cats to different methanol concentrations (see Section 3.1.2). During exposure of animals at 18200 ppm, increased salivation and disturbance of balance was observed. Delayed deaths were observed after exposure at 33400 ppm) or higher (see Section 3.1.2).

3.2.4. Rats

Studies with repeated inhalation exposure

White et al. (1983) reported no signs of pulmonary toxicity in male Sprague-Dawley rats exposed to 0, 260, 2600 or 13000 mg/m³ (0, 200, 2000 or 10000 ppm) methanol for 6 hours/day, 5 days/week for 6 weeks. Biochemical and cytological parameters of the lung, such as lung weight, DNA content, protein content, ribonuclease and protease activity were evaluated. No lung irritation was observed.

Andrews et al. (1987) exposed male and female Sprague-Dawley rats at 500, 2000 or 5000 ppm methanol for 6 hours/day, 5 days/week for 4 weeks. No effects on body or organ weights were found, except that female rats exposed to 2000 ppm had significantly higher relative spleen weights than controls. The authors considered this difference as not having any apparent biological significance. In all methanol-treated groups increased discharges around the nose and eyes, lacrimation, mucoid nasal discharges, red nasal discharge, dried red nasal discharge were observed. The frequency of these symptoms was increased in the treated groups, but only the incidence of mucoid nasal discharges appeared to be concentration-related. Gross and histological examination of 35 different tissues of control and high-dose rats revealed

no effects. No ocular abnormalities were observed. The red nasal discharge was most likely caused by extravasation of red blood cells (chromadacryorrhea), which is caused easily in the rat not only by locally acting chemicals, but also by stress, dry air or upper respiratory tract infections.

NEDO (1987) exposed groups of 20 male and 20 female Fischer 344 rats continuously for 12 months at 0, 10, 100 or 1000 ppm. During the treatment period, 1 female rat of the 10-ppm group died on day 340, and one female rat of the 1000-ppm group had to be killed on day 337. No alterations in general conditions and behavior were observed. The highest exposure group showed a slightly reduced body weight increase. In clinical, hematological and biochemical examinations, no significant alterations compared to controls were observed. Pathological analysis revealed a slight, dose-dependent increase in liver and spleen weights. No neoplastic alterations were found.

3.2.5. Mice

Studies with repeated inhalation exposure

NEDO (1987) studied groups of 30 male and 30 female B6C3F$_1$ mice continuously exposed for 12 months at 0, 10, 100 or 1000 ppm. Groups of 10 animals were killed for analysis after 6 months. During the treatment period, one female mouse of the 100-ppm group died and another one had to be killed. No alterations in general conditions and behavior were observed. The body weights of male mice and female mice were increased after 6 and 9 months, respectively. This difference (4 % and 6 % relative to controls) was significant only in the groups exposed to 1000 ppm. A significantly reduced food uptake without any effect on body weight was found for the female mice of the 1000-ppm group during the first two months and after 7 months; no correlation with body weight changes was found. In male mice exposed at 1000 ppm an increase liver weight was observed after 6 months and increased kidney and spleen weights were found after 12 months, but the dose-dependency of these effects showed was unclear. After 12 months a fatty degeneration of hepatocytes was observed in higher frequency in male mice of the high exposure group, but was also reported in lower frequency in the control group.

TABLE 5: SUMMARY OF NON-LETHAL EFFECTS IN LABORATORY ANIMALS

Species	Concentration (ppm)	Exposure Time	Effect	Reference
Monkey	5000	6 h/d, 5d/w, 4w	no effects on respiratory tract or eyes, no histopathological alterations	Andrews et al., 1987
Monkey	3000	21h/d, 20 d	weakness and loss of motion during exposure; mild fatty liver degeneration and cerebral tissue alterations, no NOEL reported	NEDO, 1987
Monkey	1000	21h/d, 7 m	mild peripheral nerve degeneration, round cell infiltration and fibrotic alterations of in the liver	NEDO, 1987
Dog	50000	1 h	prostration, incoordination	Loewy and Von der Heide, 1914

Species	Concentration (ppm)	Exposure Time	Effect	Reference
Dog	36700	8 h	prostration, incoordination	Loewy and Von der Heide, 1914
Dog	13700	4 h	none	Loewy and Von der Heide, 1914
Dog	2000	24 h	none	Loewy and Von der Heide, 1914
Cat	18200	6 h	increased salivation, disturbance of balance	Flury and Wirth, 1933
Rat	20000	7 h/d, 19 d	maternal toxic effects in pregnant rats; unsteady gait during exposure; NOEL 10000 ppm (see Section 3.3.2)	Nelson et al., 1985
Rat	13000	20 h	prostration	Loewy and Von der Heide, 1914
Rat	8800	8 h	lethargy	Loewy and Von der Heide, 1914
Rat	2000	8 h	none	Loewy and Von der Heide, 1914
Rat	10000	7 h/d, gd 1-19	fetal malformations; NOEL 5000 ppm (see Section 3.3.2)	Nelson et al., 1985
Rat	10000	6 h/d, 5 d/w, 6 w	no pulmonary toxicity	White et al., 1983
Rat	500; 2000; 5000	6 h/d, 5 d/wk, 4 wk	increased discharges around the nose and eyes at all concentrations	Andrews et al., 1987
Mouse	15000	6 h	maternal toxic effects in pregnant mice; ataxia, circling, tilting heads and depressed motor activity during exposure; NOEL 10000 ppm (see Section 3.3.3)	Bolon et al., 1993
Mouse	5000	7 h	fetal malformations; NOEL 2000 ppm (see Section 3.3.3)	Rogers et al., 1995
Mouse	2000	7 h/d, gd 6-15	fetal malformations; NOEL 1000 ppm (see Section 3.3.3)	Rogers et al., 1993
Mouse	1000	24 h/d, 7 d/w, 12 m	reduced body weights, increased kidney / spleen weights, higher incidence of fatty liver degeneration; not seen at 100 ppm	NEDO, 1987

3.3. Developmental/Reproductive Toxicity

Several studies on the developmental and reproductive toxicity of methanol were carried out. Single and repeated inhalation exposures during the period of embryogenesis induced a wide range of concentration-dependent teratogenic and embryolethal effects in rats and mice. The developmental toxicity data have been reviewed by NTP-CEHRH (2003) and US-EPA (2001) and these panels concluded that despite of toxicokinetic differences between rodents and humans, the available rodent data was relevant for humans.

3.3.1. Nonhuman Primates

Studies with repeated inhalation exposure

Burbacher et al. (1999a; 1999b; 2004a; 2004b) exposed groups of 11-12 female Macaca fascicularis in a two-cohort study at 0, 200, 600 or 1800 ppm for 2 hours/day, 7 days/week, 4 months prior to and throughout pregnancy. During each exposure the methanol delivery to the exposure chamber was stopped after 2 hours, while animals remained in the chamber for another 30 minutes with fast declining methanol concentrations (1/6th of exposure concentration at 124 minutes and 0 ppm at 135 minutes). Animals were exposed individually in an exposure chamber; methanol concentration was measured every 10 minutes by an infrared analyzer and mean concentrations (± SE) during pregnancy were 0±0, 206±0, 610±1 and 1822±1 ppm, respectively. Blood methanol concentrations, determined after the first and the 87th exposure as well as two times during pregnancy, were 4.3-5.5 mg/l at 200 ppm (roughly two-fold higher than background values), 9.5-12.1 mg/l at 600 ppm and 33.2-40.4 mg/l at 1800 ppm. The mean plasma formate concentrations did not show consistent rises following methanol exposure. The chronic methanol exposure did not result overt signs of toxicity, such as lethargy, uncoordinated movements and labored or irregular respiration. No effects were found on maternal weight gain during pregnancy and simple tests for visual problems and fine-motor incoordination (performed after each exposure). The length of the menstrual cycle and the frequencies of conception and live births in the methanol-exposed and control females were very similar. However, all methanol-exposed groups showed a decrease in pregnancy duration of about 8 days (no dose-response relationship). Cesarian section was done in 2 monkeys exposed at 200 ppm and another 2 exposed at 600 ppm because of uterine bleedings (no bleedings were observed in the high exposure group or in control animals). Two cesarian sections were performed on monkeys exposed at 1800 ppm, one for unproductive labor and another because of intrauterine death of a hydrocephalic fetus. The average pregnancy durations of all groups were still within the range of pregnancy duration of 160-169 days reported in the literature for this species. There were no effects on size or body weight of the offspring (8-9 infants per dose group), neither did methanol-exposed infants display a higher incidence of signs of prematurity. Results of behavioral assessments did not indicate significant methanol exposure effects on early reflex responses, gross motor development, spatial and concept learning or memory and social behavior. Exposure was associated with a delay in early sensorimotor development for male, but not female infants: In the Visually Directed Reaching Test (ability to grasp and retrieve a small object) a delay of about 9 days for the 200-ppm group and of about 2 weeks in the 600-ppm and 1800-ppm groups in reaching the testing criterion (8/10 consecutive trials successful) was found. The HEI Institute‘s Health Review Committee recommended to interpret these results cautiously because they are based on 3 males in the 600-ppm and 2 males in the 1800-ppm groups and may have been influenced by the low mean age reported for male control monkeys to reach the test criterion. Visual recognition memory was also affected according to the Fagan Test of Infant Intelligence (the test makes use of the infant‘s proclivity to direct more visual attention to novel rather than to familiar abstract or social stimuli). While the control infants exhibited a significant novelty response for both the abstract patterns and social stimuli (monkey faces), all infants of the methanol-exposed groups failed to

show a significant preference for novel social stimuli (results with the abstract stimuli varied greatly by cohort and no consistent pattern was observed); there were no mean group differences across the 4 groups. However, the Nonmatch-to-Sample Test, used to evaluate the same cognitive function, revealed no significant effects. A severe wasting syndrome (resulting in euthanasia) was observed in 2 of 4 females of cohort 1 and 0 of 3 females of cohort 2 after approximately 1 year of age; the etiology of the syndrome (e.g. a retroviral infection) could not be elucidated.

3.3.2. Rats

Studies with repeated inhalation exposure

NEDO (1987) exposed groups of 36 pregnant Sprague-Dawley rats continuously at 0, 200, 1000 or 5000 ppm during gestational days (gd) 7-17. Maternal toxicity was observed at 5000 ppm: one animal died and another had to be killed; body weight was significantly reduced compared to controls; uptake of food and water was reduced during gestational days 7-12 and even one week after delivery. At 5000 ppm, an increased embryo lethality in the later period of pregnancy and a reduced birth weight was found. The F1 generation showed an increased incidence of deaths, which occurred during the first 4 days, and body weights of females were still reduced at the end of the nursing period. Morphological changes included earlier dentition, eye lid opening and testes descent. At 8 weeks of age, reduced relative weights of brain, thyroid, thymus and testes as well as an increased relative weight of the pituitary gland were found. No histopathological changes were recorded. No effects on the reproduction of the F1 generation were found. In groups exposed at 1000 or 200 ppm, no developmental toxic effects were observed.

Nelson et al. (1985) exposed groups of about 15 pregnant Sprague-Dawley rats for 7 hours/day at 0, 5000 or 10000 ppm on gd 1-19 or to 20000 ppm on gd 7-15. The exposure atmosphere was monitored continuously using a Miran 1A infrared analyzer®. At 20000 ppm dams showed unsteady gait during the first days of exposure and a significantly reduced food uptake, however without any effect on body weight. No signs of maternal toxicity were reported at 5000 or 10000 ppm. On gd 20, dams were killed and half of the fetuses were examined for visceral and the other half for skeletal defects. No effects of methanol was found on the number of yellow bodies, implantations, resorptions or fetal deaths. At 20000 ppm a significantly increased number of litters with malformations and a significantly reduced number of fetuses without malformations was found. Methanol induced a concentration-related decrease in fetal weights at 10000 and 20000 ppm. Skeletal and visceral malformations were significantly increased at 20000 ppm. Malformations predominantly comprised extra or rudimental cervical ribs and urinary or cardiovascular defects. Similar malformations were found at 10000 ppm, but the incidence was not significantly different from that in the control group. Blood methanol concentrations were measured in non-pregnant rats using gas chromatography (see Table 8 for results). Exposure at 5000 ppm did not cause any malformations.

Stern et al. (1996; 1997) exposed 4 cohorts of about 30 (number estimated, not explicitly stated by the authors) pregnant Long-Evans rats at 0 or 4500 ppm methanol for 6 hours/day beginning on gd 6. After birth, both dams and pups were exposed through postnatal day 21. Maternal blood methanol concentrations were constant during gestation (mean 0.55±0.07 (SD) mg/ml) and lactation (mean 0.56±0.09 (SD) mg/ml). Before weaning, pups exhibited blood concentrations approximately twice those attained by their dams (mean 1.26±0.23 (SD) mg/ml). When exposure was continued after weaning on postnatal day 21, blood concentration in pups slowly declined and reached the level of the dams about 48 days after birth. A panel of neurobehavioral tests was performed on the pups. No effects of methanol

exposure on suckling and olfactory conditioned behavior were found. In motor activity tests, methanol-exposed neonates were less active on postnatal day 18, but more active on postnatal day 25 than the equivalent control group pups. Very subtle effects were also seen in two operant behavior tests.

3.3.3. Mice

Rogers et al. (1995, abstract) and Rogers (1999, personal communication) exposed groups of pregnant CD-1 mice on gd 7 to the following concentration-time combinations (CxT) (exposure periods indicated in brackets): 2000 ppm (5 and 7 hours), 5000 ppm (2, 3, 5 and 7 hours), 10000 ppm (2, 3, 5 and 7 hours) or 15000 ppm (1, 2, 3, 5 and 7 hours). The number of litters ranged from 5-39 for CxT combinations and was 106 in control groups. Maternal blood methanol levels determined at the end of the exposure time increased with the CxT to a maximum mean of 4966 mg/l at 15000 ppm for 7 hours. For exposures with the same CxT, blood methanol levels were higher with shorter duration, higher concentration exposures, i.e., 1200 mg/l at 5000 ppm for 7 hours, 1500 mg/l at 10000 ppm for 3 hours, and 2300 mg/l at 15000 ppm for 2 hours were measured. Dams were killed on gd 17 for assessment of teratogenic effects. Fetal death, cleft palate and multiple skeletal defects were significantly increased at CxT combinations of 70000 ppm · h or higher (i.e., no fetal death was found at 5000 ppm for 7 hours; authors expressed data only as CxT values). The most sensitive endpoint was cervical rib induction, which occurred at CxT of 15000 ppm · h or higher (i.e., no effects were observed at 2000 ppm for 5 or 7 hours). Incidences for fetal effects increased with higher exposure concentrations for similar CxT, e.g. percentages of fetuses with C7 cervical rib were about 40 % at 5000 ppm for 7 h and at 10000 ppm for 3 h and about 63 % at 15000 ppm for 2 h (this result also corresponds with the higher blood methanol concentration for the latter concentration-time combination). This study has only been published as an abstract up until now.

In the study of Rogers et al. (1997), groups of 12-19 pregnant CD-1 mice were exposed at 10000 ppm methanol or filtered air for 7 hours/day on 2 consecutive days during gestation, either gd 6-7, 7-8, 8-9, 9-10, 10-11, 11-12 or 12-13, or for 7 hours on a single day of gestation, either on gd 5, 6, 7, 8 or 9. Mice received water but not food during exposure. On analysis on gd 17, a significant effect on maternal body weights was evident only after exposure on gd 7-8. Significantly more dead/resorbed fetuses per litter were found after exposures on gd 6-7 or 7-8 or after single exposure on gd 7. After gd-7 exposure, the number of live fetuses was lower than on any other day. Cleft palate occurred significantly more frequently in groups exposed on gd 6-7, 7-8 or 8-9 and in those exposed on gd 5, 6, 7, 8 or 9 (peak on gd 7). Exencephaly occurred significantly more frequently after exposure on gd 6-7 or 8-9 and in those exposed on gd 5, 6, 7 or 8 (peak on gd 7). The following significantly higher incidences of skeletal malformations were observed: defects of exoccipital (peak gd 6-7, gd 5), atlas (peak gd 6-7, gd 5,6), axis (peak gd 6-7, gd 7), rib on cervical vertebra seven (peak gd 6-7, gd 7), and rib on lumbar vertebra one (peak gd 7-8, gd 7). Maternal blood methanol concentrations were determined at times during, at the end of, and subsequent to a single 7-hour exposure on gd 7 (see Table 8).

Studies with repeated inhalation exposure

Rogers et al. (1993) exposed pregnant CD-1 mice (number of dams examined indicated in brackets) at 1000 (31), 2000 (61), 5000 (61), 7500 (20), 10000 (20) or 15000 (34) ppm for 7 hours/day on gd 6-15. Controls comprised groups that were sham-exposed to filtered air, left untreated in their home cages or left in their home cages and food-deprived for 7 hours/day to match the food deprivation of methanol-exposed mice. The methanol concentration in the exposure chamber (15 air changes per hour) was monitored continuously with a Foxboro Miran 1A Infrared Analyzer®. One dam each died at 7500,

10000 and 15000 ppm. The sham-exposed and food-deprived controls as well as all methanol-exposed dams gained less weight than did unexposed dams fed ad libitum, but methanol did not exacerbate this effect. On gd 17, mice were killed and implantation sites, live and dead fetuses and resorptions were counted. Fetuses were examined externally and weighed as a litter. Half of each litter was examined for soft tissue anomalies, the other half for skeletal morphology. Significant increases were observed in the incidence of exencephaly and cleft palate at 5000 ppm or higher. At 7500 ppm or higher significantly increased number of dead fetuses/litter were found and full-litter resorptions were increased at 10000 and 15000 ppm. A concentration-related increase in cervical ribs was significant at 2000 ppm or higher. Using a log-logistic dose response model, the authors calculated maximum likelihood estimates (MLE_{05}) corresponding to 5% added risk above background (BMD_{05} given in parenthesis). MLE_{05} was 4314 (3398) ppm for cleft palate, 5169 (3760) ppm for exencephaly, 3713 (3142) ppm for cleft palate or exencephaly, 5650 (4865) ppm for resorptions and 824 (305) ppm for cervical rib. Blood methanol levels in dams were measured 15 minutes after cessation of the first exposure (see Table 8).

Bolon et al. (1993) investigated the phase-specific developmental toxicity of methanol in pregnant CD-1 mice. In pilot experiments, mice (5-12 animals/group) were exposed for 6 hours/day at 0 or 10000 ppm on gd 6-15 (i.e. organogenesis), 7-9 (i.e. period of murine neurulation) or 9-11 (i.e. period of potential neural tube reopening). The concentration-response relationship for neural tube defects was determined in a subsequent experiment by exposing dams (20-27 animals/group) at 0, 5000 (gd 7-9), 10000 (gd 6-15, 7-9 or 9-11) or 15000 ppm (gd 7-9 or 9-11). The critical periods of susceptibility to neural tube defects was further narrowed by exposing mice (8-15 animals/group) for 1 (gd 7, 8 or 9) or 2 days (gd 7-8 or 8-9) at 15000 ppm for 8 hours/day. Transient maternal neuronal toxicity was observed at 15000 ppm after the first exposure in 20 % of dams, after the second exposure in 10% and after the third exposure in 5 %. Signs included ataxia, circling, tilting heads and depressed motor activity were observed. Three dams were removed from the study on gd 7 due to the severity of clinical signs, but had no visible lesions. The other affected dams recovered within 12 hours. Clinical signs were not apparent at 5000 or 10000 ppm. Dams were killed at gd 17. In the pilot study in which a single exposure concentration of 10000 ppm was used, significantly reduced fetal weight was observed after gd-6-15 exposure, but not after exposure on gd 7-9 or 9-11. An significantly increased percentage of resorptions/litter was found after exposure on gd 6-15 and 7-9, but not gd 9-11. Neural tube defects, cleft palate and digit malformations were found in significantly higher incidence after exposure on gd 6-15, cleft palate after exposure on gd 9-11. In the dose-response experiments significantly increased percentages were found for resorptions/litter after 15000 ppm on gd 7-9 and for the number of litters with ≥ 1 resorption after 5000 ppm or higher on gd 7-9. Exposure to 5000 ppm or higher on gd 7-9 significantly induced in renal pelvic cavitation. Exposure at 10000 ppm or higher additionally resulted in significantly increased percentages of ocular defects, cleft palate, hydronephrosis and deformed tails, and exposure at 15000 ppm in neural tube defects. Neural tube defects and ocular lesions occurred after methanol inhalation between gd 7 and 9, while limb anomalies only occurred after exposure during gd 9 and 11. In the window-of-susceptibility experiment, significantly increased percentages of resorptions/litter and of litters with ≥ 1 resorption were observed after exposure at 15000 ppm only for the treatment periods gd 7, 7-8 and 7-9. A significant increase in neural tube defects was observed only after exposure on gd 7-8 or 7-9. The authors did not report, whether fetal death was observed.

3.4. Genotoxicity

Simmon et al. (1977) found methanol to give negative results when tested in Salmonella

typhimurium plate incorporation assays with or without metabolic activation using strains TA98, TA100, TA1535, TA1537 and TA1538. De Flora et al. (1984) observed no effect of methanol in Salmonella typhimurium plate incorporation assays with or without metabolic activation using strains TA1535, TA100, TA1538, TA98 and TA1537 and in a DNA repair test using Escherichia coli strains WP2, WP67 and CM871 in the presence or absence of metabolic activation.

Crebelli et al. (1989) reported that methanol (6.0 % (v/v)) induced dose-dependently a statistically significant increased frequency of chromosomal malsegregations in Aspergillus nidulans diploid strain P1. Obe and Ristow (1977) did not observe sister chromatid exchanges in Chinese hamster ovary cells in vitro during treatment for 8 days to a final concentration of 0.1 % (v/v). McGregor et al. (1985) reported an increase in mutation frequency in L5178Y mouse lymphoma cells treated with 7.9 mg/ml methanol, if S-9 mix was present (it should be noted that this concentration was higher than the maximum concentration proposed by the 1997 OECD guideline).

Campbell et al. (1991) found no increased frequencies of micronuclei in blood cells, of sister chromatic exchanges, chromosome aberrations or micronuclei in lung cells in mice exposed by inhalation to 800 or 4000 ppm methanol 6 hours/day for 5 days.

3.5. Carcinogenicity

In a carcinogenicity study (NEDO, 1987; Katoh, 1989), Fischer-344 rats and $B6C3F_1$ mice were exposed at 10, 100 or 1000 ppm for 20 hours/day for 24 and 18 months, respectively. Compared to control groups, no increased mortality in the treated groups was observed. A non-significant reduction of body weight was observed in methanol-treated female rats between weeks 51 and 71, while in male and female mice an increased body weight was found between months 6 and 12 and months 9 and 12, respectively. The increase was significant in female mice exposed at 1000 ppm. No evidence of carcinogenicity was found in either species. Male rats exposed at 1000 ppm showed a higher frequency of papillary adenomas than controls, which, however, was not significantly different from controls. Female rats exposed at 1000 ppm methanol showed a higher number of adrenal pheochromocytoma, which, however, was not significantly different from controls.

3.6. Summary

With regard to lethal effects in animals, three points are important. First, very high methanol concentrations can lead to death by central nervous depression, e.g. 6-hour LC_{50} values of 41000 and 66900 ppm have been reported for mice and rats, respectively (Scott et al, 1979; BASF, 1980b). Second, high methanol concentrations can lead to fetal death in mice, e.g. fetal death was observed after exposure at 7500 ppm or higher for 7 hours/day on gestational days (gd) 6-15 and also after a single 7-hour exposure at 10000 ppm on gd 7, while no fetal death occurred after single or repeated exposure to 5000 ppm (Rogers et al., 1993; 1995, abstract). Third, in monkeys, but not in rodents, delayed deaths can result from metabolic acidosis caused by accumulation of the methanol metabolite formate, e.g. delayed deaths occurred after repeated exposure to 10000 ppm for 21 hours/day (after 3 exposures) and 5000 ppm for 21 hours/day (after 5 exposures), but not after repeated exposure to 5000 ppm for 6 hours/day, 5 days/week, 4 weeks (NEDO, 1987; Andrews et al., 1987).

Severe histopathological effects on central nervous system, liver and kidneys of monkeys have

been reported after exposure at 5000 ppm for 21 hours/day for 20 days (NEDO, 1987), while no histopathological effects were reported at 5000 ppm for 6 hours/day, 5 days/week for 4 weeks (Andrews et al., 1987). While in the first study irritation was observed in monkeys at concentrations of 1000 ppm or higher, no irritation was found in the latter study at 5000 ppm.

Methanol causes developmental toxic effects. In mice, fetal malformations were found a) after single exposure at 5000 ppm (3, 5 or 7 hours), but not at 5000 ppm (2 hours) or 2000 ppm (up to 7 hours), and b) after repeated exposure at 2000 ppm or higher, but not at 1000 ppm, for 7 hours/day (Rogers et al., 1993; 1995, abstract; Rogers, 1999, personal communication). In rats, fetal malformations were found after exposure a) at 10000 ppm or higher, but not 5000 ppm, for 7 hours/day on gd 1-19 and b) at 5000 ppm, but not 1000 ppm, for 24 hours/day on gd 7-17 (Nelson et al., 1985; NEDO, 1987). After exposure of monkeys (Macaca fascicularis) at 200, 600 or 1800 ppm for 2 hours/day, 7 days/week 4 months prior to and throughout pregnancy, some effects indicating developmental effects were observed (shorter pregnancy lengths, a severe wasting syndrome in some of the offspring (of unknown etiology), and a concentration-related delay in sensorimotor development in male offspring) (Burbacher et al., 1999a; 1999b; 2004a; 2004b). After exposure of rats at 4500 ppm for 6 hours/day from gestational day 6 to postnatal day 21, very subtle effects were seen in operant behavior tests, but not in conditioned behavior and motor activity tests (Stern et al., 1996; 1997).

There was no evidence of carcinogenic effects in a lifetime bioassay in rats and mice exposed at 1000 ppm for 20 hours/day, 7 days/week (NEDO, 1987). Methanol showed no mutagenicity in bacterial mutagenicity tests, sister chromatid exchange assay in Chinese hamster ovary cells or the micronucleus test in mice exposed at 4000 ppm for 6 hours/day for 5 days; it increased the mutation frequency in mouse lymphoma cells at very high concentrations (WHO, 1997).

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

4.1.1. Absorption, Distribution and Elimination

The background blood concentrations in humans ranges from 0.32 to 2.61 mg/l (mean 0.73 mg/l) for methanol and from 3 to 19 mg/l (0.07-0.4 mmol/l) for formate. Both substances are taken up from the normal diet and generated in metabolic processes (Kavet and Nauss, 1990).

Methanol is rapidly absorbed after inhalation, the absorption percentage being around 53-85 % (Leaf and Zatman, 1952; Sedivec et al., 1981). After ingestion, it is rapidly absorbed from the gastrointestinal tract with peak absorption occurring after 30-60 minutes (Becker, 1983, Leaf and Zatman, 1952). Liquid methanol shows a very high skin absorption rate with an average of 0.192 mg methanol/cm^2 per minute (Dutkiewicz et al., 1980).

Pollack and Brouwer (1996) studied the disposition of methanol in pregnant rats on gestation days (gd) 7, 14 and 20 and in pregnant CD-1 mice on gd 9 and 18. In these studies, exposure was by the oral, intravenous and inhalation routes (1000-20000 ppm for 8 hours). Saline was the vehicle for oral and intravenous exposure. Three to five animals were examined per dose and exposure condition. Methanol concentrations were measured in blood, urine, and amniotic fluid by gas chromatography (GC). The disposition of methanol after oral or intravenous administration was similar in pregnant and nonpregnant

female rats, regardless of the gestational stage (day 7, 14 or 20 after conception) at which the toxicokinetics of methanol were examined. Parallel experiments in female mice indicated that methanol elimination was approximately twice as rapid in mice as in rats due to a significantly higher maximal velocity for methanol metabolism in the smaller rodent species. As was the case in the rat, relatively small changes in methanol elimination were observed during the course of gestation in pregnant mice. In both species, the rate of methanol metabolism by fetal liver in vitro was less than 10 % that of the metabolic rate in adult liver.

Methanol distributes readily and uniformly to organs and tissues in direct correlation to their water content; its apparent volume of distribution is 0.6-0.7 l/kg (Yant and Schrenk, 1937). In humans, clearance of methanol from the body proceeds with a half-life of 1 day or more for high doses exceeding 1 g/kg and about 3 hours for low doses, i.e., less than 0.1 g/kg (Leaf and Zatman, 1952). From volunteers breathing methanol concentrations between 50 and 300 mg/m³ (38-229 ppm) for 8 hours, Sedivec et al. (1981) estimated a half-life of 1.5-2 hours. From volunteer exposures at up to 800 ppm for 8 hours and using blood and urine sampling, Batterman et al. (1998) calculated a half-lifes of 1.44 and 1.55 hours, respectively.

4.1.2. Metabolism

During metabolic degradation, methanol is initially oxidized to formaldehyde. The enzymes mainly catalyzing this reaction are alcohol dehydrogenase in humans and non-human primates and catalase in rats and other non-primate species (see Table 6); in addition microsomal oxidation by cytochrome P450 2E1 may contribute to methanol transformation (WHO, 1997). Formaldehyde is very rapidly oxidized to formate by several enzymes including a specific formaldehyde dehydrogenase. Formate has to combine with tetrahydrofolate to form 10-formyl-tetrahydrofolate in order to be further oxidized to CO_2. Tetrahydrofolate is derived from folic acid (folate) in the diet and is the major determinant of the rate of formate metabolism (McMartin, 1975). The enzymes involved in the metabolism of methanol in primates respectively rodents are listed in Table 6.

In humans, methanol is primarily eliminated by metabolism to formaldehyde and further to formate, which may be excreted in the urine or further oxidized to carbon dioxide. Of a 50-mg/kg dose of methanol, only 2 % is excreted unchanged by the lungs and kidney (Leaf and Zatman, 1952). Likewise, studies on rats and monkeys have shown that about 80 % of administered methanol is oxidized to CO_2 (WHO, 1997).

With regard to the methanol concentrations in blood resulting from inhalation exposure, species differences occur: on the one hand side, the increased ventilation per unit body weight associated with the smaller species (about 10-fold higher in mice and 3.5-fold higher in rats compared to humans) leads to higher blood concentrations in rodents. On the other hand side, K_m values are lower in rodents than in primates and thus enzymatic methanol oxidation in rodents is faster at low methanol exposure concentrations (enzymatic rate determined by K_m), while it is about equal at high concentrations (enzymatic rate determined by $V_{max,}$ with similar V_{max} values in rodents and primates; cf. Table 7). The opposing effects on blood methanol concentration of higher specific ventilation rate and lower K_m in rodents, are responsible for the finding that the differences in blood methanol concentrations between rodents and humans are small at concentrations of up to 1000 ppm, but become increasingly larger at higher concentrations (see Table 8 and Figure 1) (Perkins et al., 1995a).

The metabolic detoxification of formate in rodents occurs with a higher v_{max} (about 2-3-fold higher in rats and 8-10-fold higher in mice compared to primates) and a lower K_m, which results in a much faster elimination of formate in rodents. In contrast to rodents, formate accumulates in primates during exposure to high methanol concentrations, since formate is formed faster than it is metabolized.

TABLE 6: METABOLISM OF METHANOL AND ENZYMES INVOLVED; adopted from WHO (1997) and Watkins et al. (1970)

Metabolic step	Humans and non-human primates	Rodents
Methanol CH_3OH ↓ Formaldehyde HCHO	alcohol dehydrogenase (about 80-90% in monkey; Watkins et al., 1970) cytochrome P450 monooxygenase	catalase (peroxidase activity) alcohol dehydrogenase (about 40-45%; Watkins et al., 1970)
Formaldehyde HCHO ↓ Formic acid HCOOH	formaldehyde dehydrogenase	formaldehyde dehydrogenase
Formic acid HCOOH ↓ Carbon dioxide CO_2	10-formyl-THF-synthetase * 10-formyl-THF-dehydrogenase	10-formyl-THF-synthetase 10-formyl-THF-dehydrogenase

* THF, tetrahydrofolate

TABLE 7: KINETIC PARAMETERS OF METHANOL METABOLISM

Metabolic step	Species	V_{max} [a]	K_m	Reference
Methanol CH_3OH ↓ Formaldehyde HCHO	monkey (Mac. mulata)		360 mg/l [b]	Dafeldecker et al., 1981
	monkey (Mac. fascicularis)	70 mg/l h		Noker et al., 1980
	monkey (Mac. fascicularis)	171 mg/h	63 ±11 mg/l	Burbacher et al., 1999a; Burbacher et al., 2004a
	monkey (Mac. nemestrina)	75 mg/l h	278 mg/l	Makar et al., 1975
	monkey	27.5 mg/kg h	44.8±19.0 mg/l	Watkins et al., 1970
	monkey (Mac. fascicularis)	44 mg/l h	33.9±15.4 mg/l	Eells et al., 1983
	monkey (Mac. mulata)	48 mg/l h	52.9±14.5 mg/l	Makar et al., 1968
	rat, non-pregnant	63.2±6.3 mg/kg h	48.7 mg/l	Ward et al., 1997
	rat, pregnant gd 14	60.5±6.4 mg/kg h	48.7 mg/l	Ward et al., 1997
	rat, pregnant gd 20	50.6±2.5 mg/kg h	48.7 mg/l	Ward et al., 1997
	mouse, non-pregnant	134±6 mg/kg h		Ward et al., 1997
	mouse, pregnant gd 8	131±3 mg/kg h		Ward et al., 1997
	mouse, pregnant gd 18	96.8±6.2 mg/kg h		Ward et al., 1997
Formaldehyde HCHO ↓ Formic acid HCOOH	human	75 mg/kg h	3.8 mg/l	Horton et al., 1992
	monkey	144 mg/kg h	3.8 mg/l	Horton et al., 1992
	rat	300 mg/kg h	3.8 mg/l	Horton et al., 1992
Formic acid HCOOH ↓ Carbon dioxide CO_2	monkey (Mac. fascicularis)	19.9±0.5 mg/kg h		Eells et al., 1983
	monkey (Mac.)	35 mg/kg h	175 mg/kg	McMartin et al., 1977
	primates	34 mg/kg h		Greim, 1995
	rat (Sprague-Dawley)	85 mg/kg h	100 mg/kg	Palese and Tephly, 1975
	rat	75 mg/kg h	60 mg/kg	McMartin et al., 1977
	rat	78 mg/kg h		Johlin et al., 1987 (Ward et al., 1995)
	mouse	300 mg/kg h		Johlin et al., 1987 (Ward et al., 1995)

[a] values of V_{max} are given for substrate concentrations
[b] values in mg/l refer to methanol concentrations in blood

4.1.3. Pharmacokinetic Models

Bouchard et al. (2001) developed a multicompartment biologically based dynamic model to describe the time evolution of methanol and its metabolites in rats, monkeys and humans following oral uptake or inhalation exposure. The dynamic of intercompartment exchanges was described mathematically by a mass balance differential equation system. The model's conceptual and functional representation was the same for rats, monkeys, and humans, but relevant published data specific to the species of interest served to determine the critical parameters of the kinetics. For model development, the kinetic data of Horton et al. (1992) for rat (intravenous route), Dorman et al. (1994) for monkey and Osterloh et al. (1996) and Sedivec et al. (1981) for humans were used. The model was validated using inhalation data for

rat and monkey (Horton et al., 1992) and humans (Batterman et al., 1998). Simulations provided a good agreement between measured data and model calculations.

Perkins et al. (1995a) established a pharmacokinetic model allowing calculation of blood methanol concentrations in humans, rats and mice after inhalation exposure (see Appendix B). The authors calculated that an 8-hour exposure at 5000 ppm methanol would result in blood methanol concentrations of 2976-4188 mg/l in mice, 1018 mg/l in rats and 224 mg/l in humans, while exposure at 1000 ppm would result in 132-268, 93.5, and 38.5 mg/l, respectively, and exposure at 200 ppm in 9-12, 11, and 7.5 mg/l, respectively.

Horton et al. (1992) developed a pharmacokinetic model of inhaled methanol based on data from Fischer-344 rats and rhesus monkeys. The blood methanol concentrations after a 6-hour inhalation exposure predicted for humans, monkeys, and rats were 140, 230 and 400 mg/l at 5000 ppm, 50, 70 and 90 mg/l at 2000 ppm and 30, 30 and 40 mg/l, respectively, at 1200 ppm.

The models are in agreement with experimental data for exposure periods of up to 8 hours, which are summarized in Table 8 and in Figure 1. For 5 individuals exposed to methanol concentrations between 3000 and 5500 ppm during an 8-hour-work shift (Kawai et al., 1991) blood methanol concentrations were calculated from the reported urine concentrations and the relationship between methanol concentrations in urine and blood:

(mg/l (urine) = 0.867 x mg/l (blood) + 0.687) (Kawai et al., 1992).

The calculated mean blood concentration of 442 mg/l at an exposure concentration of 3936 ppm was almost a factor 2 higher than expected from the pharmacokinetic models. It remains unclear whether this difference was caused by the use of values of V_{max} and K_m estimated from monkey data, a concomitant ethanol consumption of the workers, higher actual ventilation rates than assumed in the model or genetic polymorphisms of involved enzymes present in Japanese. In summary, blood concentrations are similar between different species up to exposure concentrations of about 1000 ppm. At higher concentrations, resulting blood concentrations in rats and mice are about 3-fold and 10-fold, respectively, higher than in humans.

Fisher et al. (2000) described a physiologically based pharmacokinetic (PBPK) model for the monkey, to account for fractional systemic uptake of inhaled methanol vapors in the lung.

TABLE 8: BLOOD METHANOL CONCENTRATIONS IN HUMANS AND ANIMALS AFTER A SINGLE EXPOSURE TO METHANOL

Species	Exposure time (h)	Exposure concentration (ppm)	Blood methanol concentration at end of exposure (mg/l)	Remarks	Reference
human	8	3936	442	occupational; n=5	Kawai et al., 1991
human	8	800	30.7 ± 6.9 (SD)	experimental; n=15; 0.6 ± 0.5 in controls	Batterman et al., 1998

Species	Exposure time (h)	Exposure concentration (ppm)	Blood methanol concentration at end of exposure (mg/l)	Remarks	Reference
human	6	200	7.0 ± 1.2 (SD)	experimental; n=6; subjects resting, 1.8±1.2 before exposure	Lee et al., 1992
human	6	200	8.1 ± 1.5 (SD)	experimental; n=6; exercising subjects	Lee et al., 1992
human	4	200	6.5 ± 2.7 (SD)	experimental; n=20; 1.8 ± 2.6 before exposure	Chuwers et al., 1995
human	1,25	190	1.9 ± 0.5	experimental; n=24; 0.6 ± 0.3 after sham exposure	Cook et al., 1991
human	8	111 ± 68 (SD)	8.9 ± 14.7 (SD)	occupational; n=16	Heinrich and Angerer, 1982
monkey	6	2000	64.4 ± 10.7 (SEM)	n=3	Horton et al., 1992
monkey	2	1800	33.2-40.4	pregnant animals	Burbacher et al., 1999a; 2004a
monkey	6	1200	37.6 ± 8.5 (SEM)	n=3	Horton et al., 1992
monkey	2	600	9.5-12.1	pregnant animals	Burbacher et al., 1999a; 2004a
monkey	6	200	3.9 ± 1.0 (SEM)	n=3	Horton et al., 1992
monkey	2	200	4.3-5.5	pregnant animals	Burbacher et al., 1999a; 2004a
rat	8	20000	3916 ± 907 (SD)		Perkins et al., 1995b
rat	7	20000	8650 ± 400 (SD)	n=3	Nelson et al., 1985
rat	8	15000	2667 ± 372 (SD)		Perkins et al., 1995b
rat	7	15000	3826 ± 162 (SE)	pregnant rats; n=13; 2.7 ± 0.8 in controls	Stanton et al., 1995
rat	8	10000	1656 ± 330 (SD)		Perkins et al., 1995b
rat	7	10000	2240 ± 200 (SD)	n=3	Nelson et al., 1985
rat	8	5000	1047 ± 298 (SD)		Perkins et al., 1995b

Species	Exposure time (h)	Exposure concentration (ppm)	Blood methanol concentration at end of exposure (mg/l)	Remarks	Reference
rat	7	5000	1000 ± 210 (SD)	n=3	Nelson et al., 1985
rat	6	4500	550 ± 70 (SD)	pregnant rat; n not state, about 60	Stern et al., 1996
rat	6	2000	79.7 ± 6.1 (SEM)	n=4	Horton et al., 1992
rat	6	1200	26.6 ± 2.0 (SEM)	n=4	Horton et al., 1992
rat	8	1000	83 ± 15 (SD)		Perkins et al., 1995b
rat	6	200	3.1 ± 0.4 (SEM)	n=4	Horton et al., 1992
mouse	8	15000	11165 ± 3290 (SD)	n=2-4; individual exposure; high activity	Perkins et al., 1995b
mouse	7	15000	7720 ± 581 (SEM)	pregnant mice; n=3; 1.6 ± 0.4 in controls	Rogers et al., 1993
mouse	2	15000	2300	pregnant animals	Rogers, 1999
mouse	8	10000	6028 ± 506 (SD)	n=2-4; individual exposure; high activity	Perkins et al., 1995b
mouse	8	10000	3348 ± 36 (SD)	n=3-4; group exposure; moderate activity	Perkins et al., 1995b
mouse	7	10000	4653 ± 552 (SEM)	see above	Rogers et al., 1993
mouse	3	10000	1500	pregnant animals	Rogers, 1999
mouse	7	7500	2801 ± 35 (SEM)	see above	Rogers et al., 1993
mouse	8	5000	3580 ± 599 (SD)	n=2-4; individual exposure; high activity	Perkins et al., 1995b
mouse	8	5000	2313 ± 338 (SD)	n=3-4; group exposure; moderate activity	Perkins et al., 1995b
mouse	7	5000	2126 ± 157 (SEM)	see above	Rogers et al., 1993
mouse	7	5000	1200	pregnant animals	Rogers, 1999

Species	Exposure time (h)	Exposure concentration (ppm)	Blood methanol concentration at end of exposure (mg/l)	Remarks	Reference
mouse	8	2500	1883 ± 1278 (SD)	n=2-4; individual exposure; high activity	Perkins et al., 1995b
mouse	8	2500	718 ± 57 (SD)	n=3-4; group exposure; moderate activity	Perkins et al., 1995b
mouse	7	2000	487 ± 125 (SEM)	see above	Rogers et al., 1993
mouse	7	1000	63 ± 4 (SEM)	see above	Rogers et al., 1993

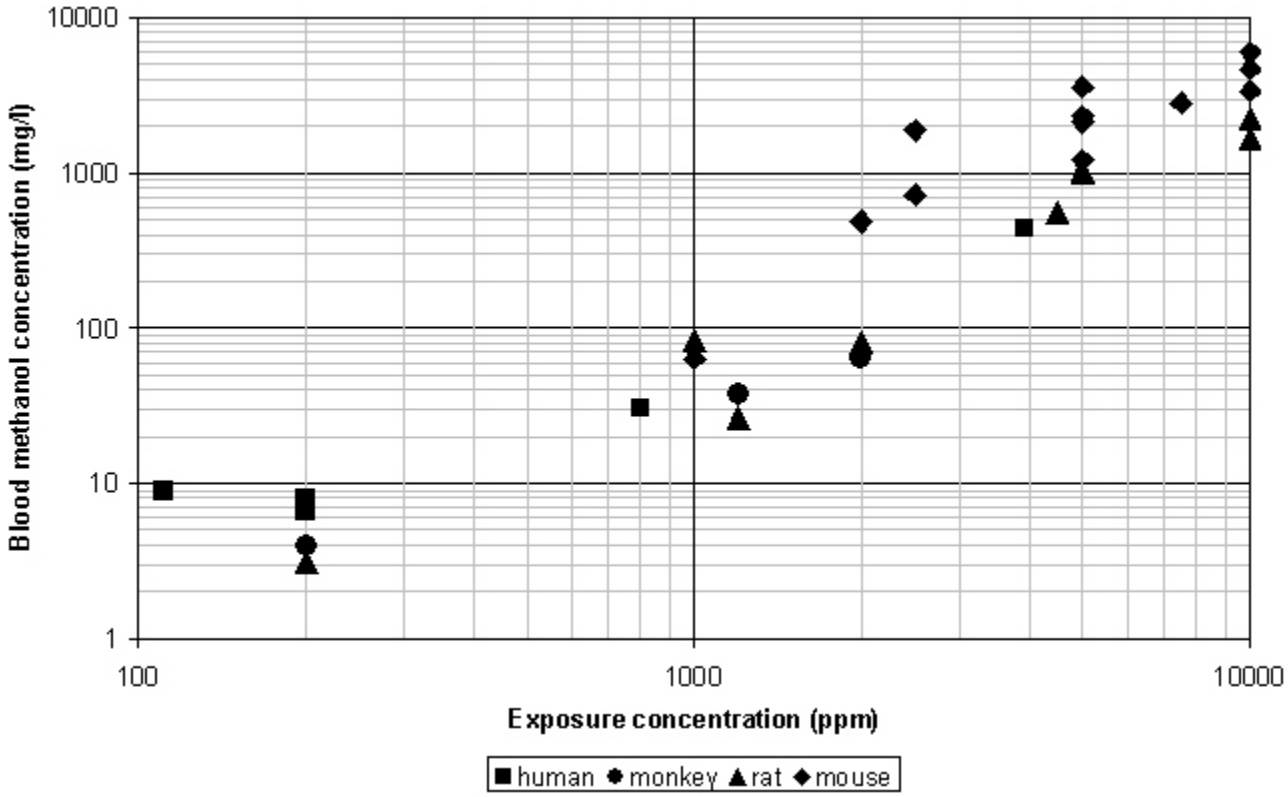


FIGURE 1: BLOOD CONCENTRATIONS OF METHANOL IN DIFFERENT SPECIES
Data for actual exposure concentrations up to 10000 ppm and exposure periods between 6 and 8 hours were taken from Table 8.

4.2. Mechanism of Toxicity

The first effects on humans caused by methanol exposure are central nervous system effects, such as headache, dizziness and nausea, weakness, peripheral nervous effects, such as shooting pains, paresthesia, prickling and numbness in the extremities, and ocular effects, such as changes in color perception and, blurred vision (NIOSH, 1976; Kavet and Nauss, 1990; ACCT, 2002). Due to their fast appearance after exposure these effects are probably caused by methanol itself and not by a metabolite. More marked effects on the central nervous system, such as ataxia, incoordination, lethargy, prostration, narcosis and coma, are seen in rodents.

After occurrence of the immediate symptoms mentioned above, which can be rather weak, an asymptomatic latent period follows and may last from several hours to a few days, although 12 to 24 hours is most common. The latent period gives way to the onset of a syndrome that consists of an uncompensated metabolic acidosis with superimposed toxicity to the visual system (Kavet and Nauss, 1990; AACT, 2002). There is substantial clinical and experimental evidence that formic acid is the toxic metabolite responsible for metabolic acidosis (Jacobsen and McMartin, 1986) and ocular toxicity (Lee et al., 1994a; 1994b).

Rats rendered folate-deficient by either feeding a folate-deficient diet (Lee et al. 1994a; 1994b) or chemical treatment (Eells, 1991), developed metabolic acidosis, ocular toxicity and retinal histopathological changes analogous to the human methanol-poisoning syndrome. A reduced folate level leads to a shortage of tetrahydrofolate, the cofactor required for metabolic oxidation of formate, and thus causes accumulation of formate in these animals. Martinasevic et al. (1996) found that total folate levels in human and rat retinal tissues were much lower than the respective levels in liver. Absolute folate concentrations in human retinal tissue were only 14 % of those found in rat retina. The levels of 10-formyl tetrahydrofolate dehydrogenase were three times higher in human retina compared with rat retina. Taking into account the lower detoxification capacity of human retina, it seems probable that the ocular toxic effects of methanol are also caused by the metabolite formate.

In experiments in vitro (Nicholls, 1975), formate has been shown to inhibit cytochrome c oxidase, a component of the mitochondrial electron transport chain, through binding to the ferric heme iron. Wallace et al. (1997) report that in vitro studies using isolated retinal and cardiac mitochondria revealed that formate selectively inhibited retinal mitochondrial ATP synthesis. Hayreh et al. (1977) postulated that formate interferes with ATP production in the retina and optic nerve, which could result in retinal dysfunction, axoplasmic flow stasis in the optic nerve, optic disc edema, interference with the neural conduction process, ultimately resulting in blindness.

The developmental toxicity of methanol in rodents may be caused by methanol itself. Dorman et al. (1995) exposed pregnant CD-1 mice on day 8 of gestation at 10000 or 15000 ppm methanol for 6 hours by inhalation. Other groups were treated by gavage with 1.5 g/kg methanol or 750 mg/kg sodium formate. Peak formate levels in maternal plasma and decidual swelling from pregnant mice given sodium formate were similar to those observed following a 6-hour methanol inhalation at 15000 ppm. No significant effect on folate concentrations in red blood cells and the decidual swelling was found during and up to 16 hours after the exposure. Exencephaly was only observed after exposure to methanol, but not sodium formate. Sakanashi et al. (1996) and Fu et al. (1996) observed that a low dietary folate level, that led to a liver folate level of about half the normal value and that did not affect maternal hematocrit levels, led to a 4-fold increase in methanol-induced incidences of cleft palate. Increased exencephaly was found in the low folate group treated with methanol, but was not increased by low dietary folate alone. The

methanol treatment did not influence folate levels in liver and plasma, as measured on gestational day 18, i.e., three days (Sakanashi et al., 1996) or 10 days (Fu et al., 1996) after the last methanol dosing. These results to not suggest that methanol exerts its developmental toxic effect by decreasing folate concentrations in the body; rather it seems to exert developmental toxic effects in parallel to a suboptimal dietary folate concentration.

This conclusion is supported by the results of Andrews et al. (1998) who conducted in vitro studies with rat embryos to compare toxicities of methanol and formate alone and in combination. Treatment with individual compounds produced significant decreases in development score, somite number, crown-rump length, and head length in Simplex 1 and Simplex 2. In Simplex 2, the methanol/formate mixtures also produced significant decreases in those parameters. However, in all cases, the reductions following exposure to either methanol or formate alone were greater than reductions observed with methanol/formate mixtures. The observation led Andrews and colleagues to conclude that methanol and formate have an infra-additive (less than additive) interaction and produce effects through different mechanisms of toxicity.

4.3. Pharmacokinetics and Toxic Effects in Normal and Folate-Deficient Animals

In animals rendered folate-deficient through a folate-reduced or folate-deficient diet, higher formate concentrations, but not higher methanol concentrations, are found in the blood.

Lee et al. (1994b) exposed a group of 10 folate-reduced Long-Evans rats at 2000 ppm methanol for 20 hours/day for 3 days. Rats had been on a folate-deficient diet for at least 18 weeks. Their liver folate levels were between 10-30 % of animals fed a normal standard diet. Blood methanol concentrations measured after 24, 48 and 72 hours revealed a plateau and were between 9 and 13 mmol/l (290 to 420 mg/l). Values of folate-sufficient and folate-reduced rats were not statistically different. The blood formate concentrations during the exposure period showed a linear increase in folate-reduced animals to about 8 mmol/l at 72 hours. Folate concentrations in folate-sufficient control animals were always <0.5 mmol/l and not different from pretreatment values. Lee et al. (1994a) exposed a group of 11 folate-reduced Long-Evans rats at 3000 ppm methanol for 20 hours/day for up to 14 days. One animal died after 3 days and another 7 animals died after 4 days. The blood formate levels in the surviving animals were 20.8±1.2 mmol/l. After exposure of folate-reduced rats at 1200 ppm for 6 hours, blood formate concentrations increased to 370 % of that of unexposed controls. An additional 72 % increase was observed after exposure at 2000 ppm. In folate-sufficient rats, formate levels were not increased over the endogenous levels after a 6-hour exposure at 1200 or 2000 ppm. Horton et al. (1992) reported that an oral methanol dose of 2 g/kg resulted in a maximum blood formate concentration of 11.7 mmol/l at 48 hours post administration in folate-reduced rats. A formate concentration of 8.1 mmol/l was found after 24 hours.

No increased formate blood levels were found in rhesus monkeys after exposure at 2000 ppm methanol for 6 hours (Horton et al., 1992). In another study (Dorman et al., 1994; Medinsky et al. 1997) monkeys were rendered folate deficient by feeding a folate-deficient diet for 6 weeks before methanol exposure. At that time, serum folate levels ranged from 0.5-2.4 ng/ml and thus were below the level of 3 ng/ml, which is considered indicative of folate deficiency in humans. After exposure for 2 hours at 10, 200 or 900 ppm methanol, blood methanol concentrations in folate-sufficient monkeys were 0.2-0.8, 10-30 and 30-200 µmol/l (0.006-0.025, 0.32-0.96, 0.96-6.4 mg/l), respectively. In folate-deficient animals exposed at 900 ppm, 100-300 µmol/l (0.32-9.6 mg/l) were found. Twentyfour hours after an oral dose of 2 g/kg, a peak formate level of 6.5 mmol/l was found in monkeys (Noker et al., 1980).

In contrast to folate-sufficient rats, folate-deficient rats show metabolic acidosis and delayed deaths and are more susceptible to neurotoxic effects of methanol: In the study of Lee et al. (1994a), one animal died after 3 days and another 7 animals died after 4 days from exposure at 3000 ppm for 20 hours/day for up to 14 days, while none of 11 folate-sufficient rats died. The surviving animals were lethargic and their blood pH values were 6.9 ± 0.04. Immediately cessation of exposure to 2000 ppm for 20 hours/day for 3 days, Lee et al. (1994b) recorded flash-evoked potentials in anesthetized rats. In all folate-reduced and methanol-exposed animals, a reduction of the b-wave amplitude in the electroretinogram by an average of 67 % was observed, indicating an effect on the retinogeniculocortical visual pathway. After oral administration of 2.0 g/kg, a b-wave amplitude reduction of 61 % was obtained. The reversibility and persistence of the effect was not investigated.

In rats, methanol treatment did not affect liver and plasma folate concentrations. In folate-deficient rats higher incidences of malformations are found than in folate-sufficient rats and these incidences are increased by methanol treatment.

Sakanashi et al. (1996) assessed the influence of the maternal folate status on the developmental toxicity of methanol. CD-1 mice were fed a folic acid-free diet supplemented with 400 (low), 600 (marginal) or 1200 (adequate) nmol folic acid/kg for 5 weeks prior to breeding. All diets contained 1 % (w/w) succinylsulfathiazole to inhibit endogenous folate production by the intestinal microflora. There were no effects of the dietary treatment on body weights before breeding. Pregnant animals of each group were exposed by gavage to 0, 2.0 or 2.5 g methanol/kg twice daily on gestational days (gd) 6-15. Dams receiving the lowest folate supplementation had significantly lower body weights at gd 12 and 18. Methanol significantly reduced the gestational weight gain in dams fed the 600 or 1200 nmol folate/kg diet. Mice were killed and fetuses analyzed on gd 18. In non-methanol exposed animals, maternal folate concentrations were 4.9±0.7, 14.5±0.8 and 13.0±1.7 nmol/g in the liver and 5.1±0.2, 6.3±0.6 and 9.2±3.6 nmol/l plasma in groups receiving 400, 600 and 1200 nmol folate/kg diet, respectively. Methanol treatment did not significantly influence these folate concentrations. The reduced folate levels did not cause any effect on hematocrit. Fetal body weights were marginally affected by the diet alone, but significantly lowered by methanol treatment compared to the respective vehicle-treated groups in the low and marginal folate groups. The percent of litters affected by cleft palate was increased by methanol treatment and this effect was exacerbated by low dietary folate. In the adequate, marginal and low groups, percentages of affected litters were 7.4, 0.0 and 18.5 % without methanol treatment, 30.8, 6.7 and 100 % at 4.0 g/kg and 34.5, 66.7 and 86.2 % at 5.0 g/kg, respectively. The percentage of litters affected with exencephaly were 0.0, 0.0 and 3.7 % without methanol treatment, 7.7, 0.0 and 0.0 % at 4.0 g/kg and 3.4, 13.3 and 34.5 % at 5.0 g/kg.

The same investigators performed similar experiments with a reduced exposure period (Fu et al., 1996): CD-1 mice were fed a folic acid-free diet supplemented with 400 (low) or 1200 (adequate) nmol folic acid/kg for 5 weeks prior to breeding, as described by Sakanashi et al. (1996). Pregnant animals of each group were exposed by gavage to 0 or 2.5 g methanol/kg twice daily on gd 6-10. Folate concentrations in the low dietary folate group were reduced by 50 % in maternal liver, 30 % in red blood cells and 60-70 % in fetal tissue (low dietary group: 1.86±0.15 nmol/g in controls and 1.69±0.12 nmol/g in methanol-treated group; adequate dietary group: 5.04±0.22 nmol/g in controls and 5.89±0.39 nmol/g in methanol-treated group). Low dietary folate alone resulted in cleft palate in 14 % of the litters, while no litters were affected in the adequate folate group. Methanol treatment increased the incidence of cleft palate to 73 % in the low and 19 % in the adequate group. The incidence of exencephaly was increased by

methanol from 14 to 23 % in the low and from 4 to 19 % in the adequate group; the increase was not statistically significant.

4.4. Structure-Activity Relationships

There are no structure-activity relationships applicable to estimating acute exposure limits for methanol. The nature and delayed onset of its toxicity, which involves metabolism into the toxic metabolite formic acid are notably different from other alcohols.

Youssef et al. (1992) determined the 24-hour oral LD_{50} values of methanol and ethanol in female albino rats. The estimated LD_{50} were 12.25 ml/kg for methanol and 19.00 ml/kg for ethanol, which corresponds to 0.303 mol/kg for methanol and 0.325 mol/kg for ethanol. A very steep dose-response curve was observed for methanol-induced lethality, with 5 % lethality at a dose of about 2.2 mol and 95 % lethality at a dose of about 3.5 mol.

Rogers (1995, abstract) found methanol to be a more potent developmental toxicant than ethanol, when pregnant mice were administered two intraperionteal injections of ethanol (2.45 g/kg each) or methanol (2.45 g/kg or 1.7 g/kg; the latter is the molar equivalent of the ethanol dose used). Unlike methanol, ethanol induced a transient ataxia lasting several hours. While the dose of ethanol used caused only a low incidence of microphthalmia, with no effects on viability or fetal weight, the higher methanol dose resulted in 100 % of live fetuses having holoprosencephaly spectrum malformations including absence of the forebrain, cebocephaly, complete premaxillary agenesis, and micro- or anophthalmia. A mean of 55 % of implants/litter were resorbed, and fetal weight was reduced. The lower methanol dose was still clearly more toxic than the equimolar ethanol dose, producing 30 % resorptions and midfacial deficiencies and micro- or anophthalmia in over 50 % of live fetuses.

Nelson et al. (1985) also found methanol to be a more potent developmental toxicant as ethanol: groups of approximately 15 pregnant Sprague-Dawley rats were exposed for 7 hours/day to methanol concentrations of 20000 ppm (during gestational days (gd) 7-15), 10000 ppm (gd 1-19) or 5000 ppm (gd 1-19) (see Section 3.3.2) or to ethanol concentrations of 20000 ppm (gd 1-19), 16000 ppm (gd 1-19) or 10000 ppm (gd 1-19). For both alcohols, unexposed groups served as controls. Analysis on gd 20 revealed slight maternal toxicity and a high incidence of congenital malformations ($p< 0.001$) (predominantly extra or rudimentary cervical ribs and urinary or cardiovascular defects) in the 20000-ppm-methanol group. Similar, but not significantly increased malformations were seen in the 10000-ppm group. No adverse effects were noted in the 5000-ppm group. Dams exposed to 20000 ppm ethanol were narcotized at the end of exposure, and maternal weight gain and feed intake were decreased during the first week of exposure. The 16000-ppm dams had significantly depressed weight gain during the first week of exposure, but there were no significant effects on feed consumption. There was no definite increase in malformations at any level of ethanol, although the incidence in the 20000-ppm group was of borderline significance.

In humans, fetal alcohol syndrome is the most common preventable cause of mental retardation. Diagnostic criteria for fetal alcohol syndrome include heavy maternal alcohol consumption during gestation, pre- and postnatal growth retardation, craniofacial malformations including microcephaly, and metal retardation. Less complete manifestations of gestational alcohol exposure also occur and are referred to as fetal alcohol effects or alcohol-related neurodevelopmental disorder. Although the total amount of

alcohol consumed and the pattern of drinking are both important factors, peak maternal blood alcohol level is the most important determinant of the likelihood and severity of effects. Overconsumption during all three trimesters of pregnancy can result in certain manifestations, with the particular manifestations dependent upon the period of gestation during which insult occurs. Despite an intensive research effort, the mechanisms underlying fetal alcohol syndrome remain unclear (Bruckner and Warren, 2001).

4.5. Other Relevant Information

4.5.1. Species Variability

The species differences in methanol toxicity result from differences in metabolism of methanol via formaldehyde and formic acid to carbon dioxide. In contrast to rodents, formic acid accumulates in human and non-human primates, which leads to the symptoms of metabolic acidosis and, probably, is also responsible for the ocular toxicity. Rodents develop higher blood methanol levels after inhalation exposure compared to primates, which favors development of methanol-caused central nervous system and developmental toxicity.

The mouse is considerably more susceptible for the developmental toxic effects than the rat: For repeated 7-hours/day exposures the LOEL for malformations was 10000 ppm in rats (corresponding to a blood methanol concentration of 2247 mg/l) (Nelson et al., 1985) and 2000 ppm in mice (corresponding to 487 mg/l) (Rogers et al., 1993) and the NOEL was 5000 ppm in rats (corresponding to 1000 mg/l) and 1000 ppm in mice (corresponding to 63 mg/l). Thus, the blood methanol concentration at the LOEL was about 5fold lower and at the NOEL it was about 16fold lower in mice compared to rats. Similar data for other species are not available.

4.5.2. Intraspecies Variability

Several factors contribute to variability in methanol-induced toxicity between. The rate of methanol metabolism and formate accumulation is influenced by the folate status. Lee et al. (1994a) have shown that Long-Evans rats fed a folate-reduced diet and having only about 10-30 % of the normal folate-level in the liver - unlike normal control animals - developed metabolic acidosis. Thus, folate-deficient individuals, which include pregnant women, the elderly, individuals with poor-quality diet, and alcoholics might develop higher formate concentrations compared to normal individuals (WHO, 1997). For the lack of data, it is very difficult to estimate this variability in quantitative terms.

4.5.3. Combination Effects

Methanol shows a markedly prolonged half-life when exposure is combined with exposure to ethanol (WHO, 1997). This has firmly been established for oral exposure. The slower methanol metabolism due to the higher affinity of alcohol dehydrogenase for ethanol is used therapeutically in methanol poisonings in order to prevent metabolism of methanol to formic acid. A blood ethanol level of about 22 mmol/l (1000 mg/l) has been recommended to block methanol metabolism in poisoned humans (AACT, 2002; Jacobsen and McMartin, 1986; Becker, 1983). In monkeys methanol oxidation was reduced by 90 % when the molar ratio of ethanol to methanol in the orally applied mixture was 1:1 and by 70 % when the ratio was 1:4 (Jacobsen and McMartin, 1986).

4.5.4. Role of Folate in Human Birth Defects

It has been estimated that about half of the neural tube defects in humans are caused by an insufficient intake of folic acid with the normal diet. The folate dose in normal diet is only about half of the value of 0.4 mg/day which is recommended for women capable of becoming pregnant (Butterworth and Bendich, 1996; Forman et al., 1996). A correlation with other congenital birth defects, such as orofacial clefts, has also been found (Tolarova and Harris, 1995). Periconceptional folate supplementation has been shown to give effective protection against the development of neural tube defects (Butterworth and Bendich, 1996; Czeizel, 1996). Folate supplementation is only effective when given before and very early in pregnancy because closure of the neural tube and the palate and upper jaw occurs in week 3-4 and week 3-8 of pregnancy, respectively.

While a suboptimal folate status of pregnant women constitutes itself a significant risk factor, it is unlikely that methanol exposure lowers folate concentrations in the body and thus contributes indirectly to a lower folate status and an increased rate of birth defects. There are no experimental findings that would support the possibility that a single methanol exposure decreases body folate concentrations. In mice, a 6-hour exposure at 15000 ppm methanol had no significant effect of on folate concentrations in red blood cells and in the decidual swelling during and up to 16 hours after cessation of the exposure (Dorman et al., 1995). Likewise, oral methanol doses of up to 5 g/kg/day given on gestational days 6-15 (Sakanashi et al., 1996) or on gestational days 6-10 (Fu et al., 1996) did not influence liver and plasma folate concentrations (cf. Section 4.3) when measured 3 days and 8 days, respectively, after the last dosing.

In addition, the folate status is unlikely to influence blood methanol concentrations. As discussed in Section 4.1.4, in folate-deficient monkeys and rats much higher formate concentrations accumulate in the blood, but the effect on the methanol concentration was small (Lee et al., 1994a; 1994b; Dorman et al., 1994; Medinsky et al., 1997).

5. RATIONALE AND PROPOSED AEGL-1

5.1. Human Data Relevant to AEGL-1

Batterman et al. (1998) exposed 15 healthy subjects at 800 ppm for 8 hours in a pharmacokinetic study. In a personal communication, the coauthor Dr. Alfred Franzblau stated that subjects did not report symptoms (Franzblau, 1999; 2000). Chuwers et al. (1995) exposed 26 healthy subjects at 200 ppm for 4 hours. No symptoms were reported and in a number of neurobehavioral, neurophysiological and visual performance tests, no significant effects were found. Likewise, Cook et al. (1991) reported neither symptoms nor effects in neurobehavioral and neurophysiological tests after exposure of 12 subjects at 190 ppm for 75 minutes. Muttray et al. (2001) reported electroencephalogram alterations, which were not considered adverse, in 12 subjects exposed at 200 ppm for 4 hours.

NIOSH (1980) and Frederick et al. (1984) studied the health effects of methanol exposure from spirit duplicators in 66 teacher aides. Measured methanol concentrations ranged from 365 to 3080 ppm (mean concentration 1060 ppm, median concentration 1040 ppm). Exposure durations ranged from 1 hour/day for 1 day/week to 8 hours/day for 5 days/week during about 3 years. Compared to a control group of teachers from the same schools the aides reported significantly higher frequencies of headaches, dizziness, blurred vision and nausea/upset stomach. No information on the exact exposure duration and time between start of exposure and occurrence of symptoms was provided. NIOSH (1981) reported that exposure of one worker at 1025 ppm for 25 minutes resulted in eye irritation at the end of exposure.

Kingsley and Hirsch (1955) reported that repeated exposure at the workplace to methanol concentrations of about 200-375 ppm can lead to headaches. However, information about the exact exposure concentrations and exposure durations is lacking. In addition, simultaneous exposure to other volatile organic compounds cannot be ruled out.

Flury and Wirth (1933) reported weak nasal irritation in volunteers after exposure at 7600 ppm for 5 minutes. No irritation was observed at 760 ppm. Eye irritation was reported at 1025 ppm for 25 minutes in a case study (NIOSH, 1981) and weak nasal irritation was reported after repeated exposure to mean concentrations of 459 at the workplace (Kawai et al., 1991). Considerable uncertainty exists in characterization of the exposure conditions in the latter study and the range of exposure concentrations was large (up to 5500 ppm; the authors did not state the lower exposure concentration limit defining the "high" exposure group).

5.2. Animal Data Relevant to AEGL-1

NEDO (1987) exposed monkeys (Macaca fascicularis) at 1000, 2000 or 3000 ppm for 21 hours/day for 7 months. During the first exposures, frequent yawning and runny noses were observed at all concentrations, which might be indicative of a weak irritative effect. At histopathology, the 1000-ppm group showed a dose-dependent round cell infiltration and slight fibrotic alterations of the liver. Andrews et al. (1987) exposed monkeys (Macaca fascicularis) at 500, 2000 or 5000 ppm methanol for 6 hours/day, 5 days/week for 4 weeks. No irritative effects were observed at exposure concentrations as high as 5000 ppm. The authors did not report on any effects observed in the histopathological analysis.

5.3. Derivation of AEGL-1

Several experimental human studies are available that used methanol concentrations of about 200 ppm. Chuwers et al. (1995) found no significant effects in a panel of neurophysiological and neuropsychological tests after exposure at 200 ppm for 4 hours. Using the same exposure conditions, Muttray et al. (2001) observed electroencephalogram alterations which the authors did not considered adverse; no clinical symptoms were reported by the subjects. Likewise, the NAC/AEGL committee considered these findings as below the threshold for AEGL-1. Batterman et al. (1998) exposed volunteers at a higher level (i.e. 800 ppm for 8 hours). As this was a pharmacokinetic study, health effects were not formally evaluated. In a personal communication the coauthor Dr. Franzblau stated that individual symptoms were asked of some subjects, other subjects were only asked generally if they had symptoms, and that in some exposure sessions subjects might not have been queried. According to Dr. Franzblau, none of the subjects reported symptoms. Since the subjects knew the exposure concentration by means of a meter showing the actual concentration, if might be expected that this would have increased the inclination of subjects to report symptoms.

NIOSH (1980) and Frederick et al. (1984) reported significantly higher frequencies of headaches, dizziness, blurred vision after occupational exposure at 1060 ppm (mean concentration). NIOSH (1981) reported eye irritation in a worker after exposure at 1025 ppm for 25 minutes. Since the 1000-ppm level was considered already a discomfort level, the 800 ppm for 8 hour exposure from the Batterman et al. (1998) study was chosen as a starting point for AEGL-derivation. Since the local irritation effects are determined by the concentration of methanol in air and not to the blood methanol level, calculation of AEGL-1 values was not done using a pharmacokinetic model (as done for AEGL-2 and -3) based on the

end-of-exposure blood methanol level of 30.7 mg/l reported by Batterman et al. (1998). Instead, exposure to 800 ppm for 8 hours was used as the basis for AEGL-1 derivation.

Time scaling using the equation $C^n \times t = k$ was carried out to derive exposure duration-specific values. Due to lack of a definitive data set, a default value for n of 3 was used in the exponential function for extrapolation from the experimental period (8 hours) to shorter exposure periods. For the 10-minute AEGL-1 the 30-minute value was applied because no studies were available that demonstrated the absence of notable discomfort (with respect to irritation) in the general population, including susceptible subpopulations, at 970 ppm (extrapolated value for 10-minute period). The calculations of exposure concentrations scaled to AEGL-1 time periods are shown in Appendix A.

A total uncertainty factor of 3 was used. An uncertainty factor of 3 for intraspecies variability was applied because interindividual variability with regard to slight central nervous system effects (e.g. headache) is likely to exist (although it cannot be quantified exactly from the existing experimental and epidemiological studies) and because subpopulations with a less than optimal folate status may be more susceptible to the health effects of methanol.

The values are listed in Table 9 below.

TABLE 9: AEGL-1 VALUES FOR METHANOL

AEGL Level	10 minutes	30 minutes	1 hour	4 hours	8 hours
AEGL-1	670 ppm (880 mg/m³)	670 ppm (880 mg/m³)	530 ppm (690 mg/m³)	340 ppm (450 mg/m³)	270 ppm (350 mg/m³)

A level of distinct odor awareness (LOA) for methanol of 8.9 ppm was derived on the basis of the odor detection threshold from the study of Hellman and Small (1974) (see Appendix C for LOA derivation). The LOA represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, about 10 % of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odor perception.

6. RATIONALE AND PROPOSED AEGL-2

6.1. Human Data Relevant to AEGL-2

Blindness can result from exposure to methanol. However, no data are available that would allow derivation of a threshold exposure concentration for blindness in humans. Appropriate data from animal models are also lacking for this endpoint. Moreover, reports about acute oral methanol poisoning indicate that blindness results only after live-threatening doses and thus no clear distinction is possible between methanol doses leading to blindness and those causing lethal effects (Naraqi et al., 1979; WHO, 1997; IUCLID, 1996; NIOSH, 1976).

Humperdinck (1941) reported that one of 23 exposed workers became ill, blind in the right eye with marked narrowing of the visual field in the left eye after 4 years at the workplace without any

previous symptoms. Examination of the workplace air revealed methanol concentrations ranging from 1200 to 8300 ppm. Effects on vision were not reported in another 22 workers exposed to methanol, however, no statement was made on whether these workers experienced any other symptoms.

NIOSH (1980) and Frederick et al. (1984) studied the health effects of methanol exposure from spirit duplicators in 66 teacher aides. Measured methanol concentrations ranged from 365 to 3080 ppm (mean concentration 1060 ppm, median concentration 1040 ppm). Exposure times ranged from 1 hour/day for 1 day/week to 8 hours/day for 5 days/week during about 3 years. Compared to a control group of teachers from the same schools the aides reported significantly higher frequencies of headaches, dizziness, blurred vision and nausea/upset stomach. No information on the exact exposure duration, time between start of exposure and occurrence of symptoms, and relationship between symptom severity and exposure time was provided.

NIOSH (1981) reported that exposure of one worker to 1025 ppm for 25 minutes resulted in eye irritation..

Kawai et al. (1991) reported that workers exposed to higher methanol concentrations complained significantly more often of dimmed vision (the authors suggested that visibility was temporarily reduced by fog in the workroom) and nasal irritation than workers exposed to lower methanol concentrations. Measurement of breathing-zone air for 31 subjects revealed time-weighted average methanol concentrations during an 8-hour work shift of 3000-5500 ppm for 5 samples, 1000-2000 ppm for 10 samples, 500-1000 ppm for 4 samples and <500 ppm for 19 samples. The authors did not try to correlate incidence or severity of symptoms with measured breathing-air concentrations.

6.2. Animal Data Relevant to AEGL-2

Rogers et al. (1995, abstract) and Rogers (1999, personal communication) performed single-exposure experiments with pregnant CD-1 mice, exposing them on day 7 of gestation for 1, 2, 3, 5 or 7 hours at 2000, 5000, 10000 or 15000 ppm (Rogers et al., 1995). Since cervical rib induction occurred at concentration-time products (CxT) greater than or equal to 15000 ppm · h (the authors expressed results only as CxT products), a NOEL for cervical rib induction of 2000 ppm for 7 hours can be derived from this study. This study is supported by another study of the same group that used repeated 7-hour exposures (Rogers et al., 1993) and found a dose-related increase in cervical ribs at exposure concentrations of 2000 or higher. In that study (Rogers et al., 1993), a NOEL of 1000 ppm for developmental toxic effects after repeated exposure was derived.

In pregnant rats, repeated 7-hour exposures at 20000 ppm resulted in significantly increased numbers of litters with malformations, such as extra or rudimental cervical ribs and urinary or cardiovascular defects and 10000 ppm caused increased, but not statistically significant incidences of malformations, while 5000 ppm for 7 hours/day did not lead to an increase in malformations (Nelson et al., 1985). Upon continuous exposure of pregnant rats on days 7-17 of gestation, 5000 ppm led to maternal toxic effects, an increased embryo lethality, reduced birth weight and morphological changes, while 1000 ppm caused no developmental toxic effects (NEDO, 1987).

In monkeys (Macaca fascicularis), exposure at 200, 600 or 1800 ppm for 2 hours/day, 7 days/week 4 months prior to and throughout pregnancy caused effects indicating developmental toxicity.

All methanol-exposed groups had significantly shorter pregnancy lengths. A dose-response relationship was not observed for these effects. A severe wasting syndrome was observed in 2/7 female offspring of the 1800-ppm group; the etiology of the wasting syndrome could not be identified. A concentration-related delay in sensorimotor development was measured in male offspring during the first month of life (Burbacher et al., 1999a; 1999b; 2004a; 2004b).

NEDO (1987) reported on experiments in which monkeys (Macaca fascicularis) were exposed for 21 hours/day a) at 3000, 5000, 7000 or 10000 ppm methanol for 15-20 days, b) at 2000 or 3000 ppm for 7 months and c) at 10, 100 or 1000 ppm for 7, 19 or 29 months. In animals exposed at 5000 ppm or higher, necrosis of the basal ganglia of the cerebrum, cerebral edema, kidney degeneration and necrotic lesions in the liver were described. 3000 ppm induced slight necrotic changes in basal ganglia after exposure for 7 months, while only mild alterations were found after 20 days. A prolonged exposure at 1000 ppm methanol for 7 months or longer resulted in round-cell infiltration and slight necrotic changes in the liver.

6.3. Derivation of AEGL-2

Although methanol intoxication can cause blindness in humans, it is not possible to derive a threshold for this effect from the available data. Moreover, available reports indicate that blindness results only after live-threatening poisoning (Naraqi et al., 1979; WHO, 1997; IUCLID, 1996; NIOSH, 1976).

The epidemiological studies evaluating reversible effects on humans, such as slight neurotoxic and irritative effects at the workplace, though evaluating a relevant toxicological endpoint, will not be used for derivation of AEGL-2 values because data on exposure concentration and duration were considered insufficient. However, these reports provide valuable supporting evidence.

The derivation of AEGL-2 values was based on developmental toxic effects in animals. The available data have been reviewed by US-EPA (2001) and NTP-CEHRH (2003) and both panels considered the developmental toxic effects in rodents as relevant for humans. The NTP-CEHRH panel "recognized the need to consider species differences in methanol metabolism and toxicity in its evaluation of the risk to reproduction posed by methanol exposure in humans. The Expert Panel agreed that blood methanol concentrations provide a useful dosimetric for the comparison of results among various studies. There are sufficient pharmacokinetic data to determine blood methanol concentrations in rodents associated with adverse reproductive and developmental effects. Mean maternal blood methanol concentrations observed in mice following inhalation exposure to 1000 ppm methanol for 7 hour/day on gd 6-15 (i.e., the fetal NOAEL for teratogenicity) was 97 mg/l. Mean maternal blood methanol concentration observed in mice following inhalation exposure to 2000 ppm methanol for 7 hours/day on gd 6-15 (i.e., the fetal LOAEL for teratogenicity) was 537 mg/l. In humans, achievement of such a blood methanol concentration has resulted in formate accumulation, metabolic acidosis, ocular toxicity, and other signs of methanol toxicity. These observations suggest that there may be overlap between exposures resulting in clinical signs of acute toxicity and those that might result in developmental toxicity in humans. The toxicity data available to the Panel that was collected in monkeys provide suggestive but insufficient evidence that adverse developmental effects may occur in primates exposed by inhalation to methanol at maternally non toxic doses. The Panel's confidence in these data may have been strengthened had statistical analyses that adjust for multiple testing been applied to the data. The Expert Panel concludes that there is insufficient evidence to determine if the human fetus is more or less sensitive than the most

sensitive rodent species (i.e., mouse) to methanol teratogenesis. Moreover, other factors (e.g., genetic polymorphisms in key metabolizing enzymes, maternal folate status) that alter methanol metabolism may predispose some humans to developmental toxicity at lower blood methanol concentrations (<100 mg/l). This caveat is especially important since the Expert Panel recognized that there are limited human exposure data for pregnant women and other potentially susceptible subpopulations. The Expert Panel concluded that developmental toxicity was the most sensitive endpoint of concern with respect to evaluating the risk to reproduction posed by methanol exposure in humans. In particular, the data obtained from rodent studies indicate that the gastrulating and early organogenesis stage embryo is particularly sensitive to the adverse developmental effects of methanol. The Panel concluded that methanol is the most likely proximate teratogen; however, the biological basis by which it induces such effects remains unknown. The Panel assumed the available rodent data was relevant for humans." (NTP-CEHRH, 2003).

The study in monkeys by Burbacher et al. (1999a; 1999b; 2004a; 2004b), provides some evidence for neurobehavioral effects (delayed development of visually directed reaching and absence of novelty preference) in monkeys after prenatal exposure at 200, 600 and 1800 ppm for 2 hours/day, 7 days/week throughout pregnancy. It is difficult to decide whether these slight effects would also be seen after reducing the number of exposure days to a single day. It seems reasonable, however, to assume that a single exposure during pregnancy would have a much lesser effect than a daily exposure during the whole intrauterine development. Further research would be necessary to establish a clear causality and dose-response relationship for this and the other effects (vaginal bleeding, shortened pregnancy length, wasting syndrome in offspring). In conclusion, the results of Burbacher et al. (1999a; 1999b; 2004a; 2004b) were not considered a suitable basis for derivation of AEGL-2 values. They are, however, not incompatible with the AEGL-2 values derived below.

In mice, repeated 7-hour/day exposures during gestational days 6 to 15 caused a dose-related, significant increase in cervical ribs at 2000 ppm or higher; other malformations, such as exencephaly and cleft palate occurred concentration-dependently at 5000 ppm or higher (Rogers et al., 1993). The same type of malformations was found after a single 7-hour exposure at 10000 ppm (no other concentrations tested) (Rogers et al., 1997). In another study, which has not been formally published up until know, Rogers and coworkers (Rogers et al. 1995, abstract; Rogers, 1999, personal communication) exposed mice on gestational day 7 to different concentration-time combinations. The most sensitive endpoint was cervical rib induction, which occurred at concentration-time products greater than or equal to 15000 ppm · h, but not at concentration-time products below 15000 ppm · h (i.e. no effects were observed at 2000 ppm for 5 h, 2000 ppm for 7 h or 5000 ppm for 2 h; authors expressed data only as CxT values). Thus, while 2000 ppm for 7 hours was a LOEL in the repeated exposure study (Rogers et al., 1993), it was a NOEL after single exposure. Although the single exposure study had shortcomings in the reporting, it was very consistent with the well-documented repeated exposure study. It was therefore considered adequate to use an exposure at 2000 ppm for 7 hours as a starting point for AEGL-2 derivation.

As discussed in Section 4.2, there is experimental evidence that developmental toxic effects are caused by methanol itself and not by a metabolite, such as formate (Dorman et al., 1995). It is therefore reasonable use blood methanol concentrations as the dose metric. The corresponding end-of-exposure blood concentration in mice after exposure to 2000 ppm for 7 hours was measured as 487 mg/l (Rogers et al., 1993).

A total uncertainty factor of 10 was used. An uncertainty factor of 1 was applied for interspecies variability because a sensitive species was used for derivation of AEGL-2 values and because toxicokinetic differences between species were accounted for by using a pharmacokinetic model for calculating exposure concentrations. An uncertainty factor of 10 was used for intraspecies variability because no information on developmental toxic effects of methanol on humans is available and because also for other chemicals the variability in susceptibility of humans for developmental toxic effects is not well characterized. Moreover, pregnant women are a subpopulation with a less than optimal folate status and, thus, may be more susceptible to the health effects of methanol.

Using a total uncertainty factor of 10, a blood methanol concentration of 48.7 mg/l was derived as the basis for calculation of exposure concentrations. Application of the uncertainty factor to the blood methanol concentration was preferred because the calculated exposure concentrations in air stayed better in the concentration range for which the pharmacokinetic model was validated and the effect of methanol metabolism for longer exposure periods was more adequately taken into account. In contrast, first calculating exposure concentrations that would lead to a blood methanol level of 487 mg/l, and then applying a factor of 10 to the derived exposure concentration would result in calculation of extremely high concentrations in the fist step at which metabolic pathways would be saturated. After application of the uncertainty factor, concentrations would be below saturation level which would mean that the end-of-exposure methanol levels would vary for the AEGL-2 exposure concentration-time combinations.

Using the pharmacokinetic model of Perkins et al. (1995a), inhalation exposure concentrations were calculated for appropriate time periods that would lead to a blood methanol concentration of 48.7 mg/l at the end of the time period (see Appendix C, Table 15). The calculated exposure concentrations were set as AEGL-2 values.

The values are listed in Table 10 below.

TABLE 10: AEGL-2 VALUES FOR METHANOL

AEGL Level	10 minutes	30 minutes	1 hour	4 hours	8 hours
AEGL-2	11000 ppm [a] (14000 mg/m³)	4000 ppm (5200 mg/m³)	2100 ppm (2800 mg/m³)	730 ppm (960 mg/m³)	520 ppm (680 mg/m³)

[a] The 10-minute AEGL-2 value is higher than 1/10 of the lower explosive limit (LEL) of methanol in air (LEL = 55,000; 1/10th LEL = 5500 ppm). Therefore, safety considerations against the hazard of explosion must be taken into consideration.

The derived AEGL-2 values are supported by the occupational exposure study of Kawai et al. (1991), in which 8-hour mean concentrations were 3000-5500 ppm in 5 samples and 1000-2000 ppm in another 10 samples and resulted in dimmed vision (the authors suggested that visibility was temporarily reduced by fog in the workroom) and nasal irritation, but not in severe or irreversible toxicity.

7. RATIONALE AND PROPOSED AEGL-3

7.1. Human Data Relevant to AEGL-3

Although several case reports on lethal methanol poisoning of humans due to exposure by inhalation have been published in the literature, data on exposure concentration and exposure duration are usually lacking. A fatal case after occupational exposure to an estimated concentration of 4000-13000 ppm for 12 hours was reported (Anonymous, 1932).

From a large number of reports on oral methanol poisonings, it was concluded that the minimum lethal oral dose is about 1 g/kg (Buller and Wood, 1904; Röe, 1982) (this value is also supported by monkey data; see below). Using a volume of distribution of 0.65 l/kg (Yant and Schrenk, 1937) a theoretical maximum blood methanol concentration of

1.0 g/kg / 0.65 l/kg = 1540 mg/l

can be calculated.

From the large number of case reports on methanol intoxication, the studies from Naraqi et al. (1979), Erlanson et al. (1965), Bennett et al. (1953), Gonda et al. (1978) and Meyer et al. (2000) are presented in Section 2.1, because theses studies report cases of methanol intoxication without concomitant ethanol uptake and report both blood methanol concentrations and the time between intoxication and measurement. These data are graphically presented in Figure 2.

Kahn and Blum (1979) report the case of a fatal dermal methanol exposure in an 8-month-old boy. The child had been "treated" with methanol-soaked compresses during two nights (about 12 hours each) before he was admitted to hospital. A blood methanol concentration of 400 mg/l was determined in the early afternoon. Due to lack of information on methanol toxicokinetics in small children, a peak blood methanol concentration cannot be estimated in this case.

In an epidemiological study, Kawai et al. (1991) reported symptoms, such as dimmed vision (the authors suggested that visibility was temporarily reduced by fog in the workroom) and nasal irritation during work, in a group of 22 workers exposed to a time-weighted average methanol concentration of 459 ppm during an 8-hour work shift; a group of 5 breathing-zone samples revealed concentrations between 3000 and 5500 ppm.

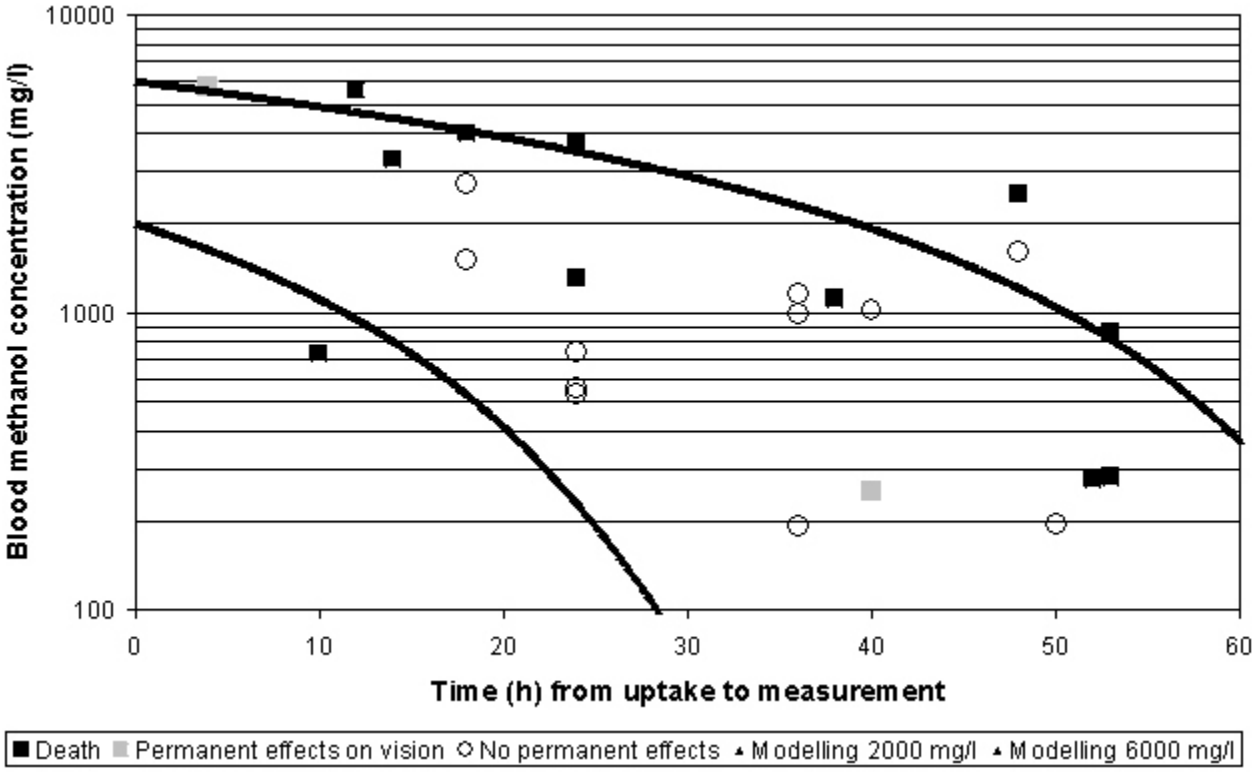


FIGURE 2: MEASURED BLOOD METHANOL CONCENTRATIONS IN HUMAN FATALITIES

Data points are from studies cited in Table 2, Section 2.1. For comparison, concentration-time curves for blood methanol concentrations of 2000 and 6000 mg/l are shown (black lines). Calculations were done using the pharmacokinetic model by Perkins et al. (1995a) (see Appendix B).

7.2. Animal Data Relevant to AEGL-3

Gilger and Potts (1955) observed death of rhesus monkeys after doses of 3 g/kg or higher, while at doses of 1 and 2 g/kg animals did not showed any symptoms. After lethal doses, signs of inebriation were observed; semicoma was seen only shortly before death.

Rogers et al. (1993) exposed pregnant CD-1 mice at 1000, 2000, 5000, 7500, 10000 or 15000 ppm for 7 hours/day on days 6-15 of gestation. 7500 ppm or higher induced a significantly increased number of dead fetuses/litter, while no fetal death occurred at 5000 ppm. When CD-1 mice were exposed for only one time on day 7 of gestation, increased fetal death was observed at 10000 ppm for 7 hours or at 15000 ppm for 5 hours, but not at 5000 ppm for 7 hours, 10000 ppm for 5 hours or 15000 ppm for 3 hours (Rogers et al., abstract, 1995; Rogers, personal communication, 1999). From these studies, a NOEL for fetal death of 5000 ppm for 7 hours can be derived.

NEDO (1987) reported on experiments in which groups of 4 monkeys (Macaca fascicularis) were exposed at 3000, 5000, 7000 or 10000 ppm methanol for 21 hours/day for at least 15 days. Animals exposed at 10000 ppm showed lethargy and after the third exposure were comatose and died. Animals exposed at 7000 ppm had to be killed after 6 days and of three animals exposed at 5000 ppm, two died on day 5 and one on day 14. No deaths occurred at 3000 ppm. Andrews et al. (1987) observed no deaths after exposure of 6 monkeys (Macaca fascicularis) at 5000 ppm for 6 hours/day, 5 days/week for 4 weeks. A NOEL of 5000 ppm for 6 hours could be derived from the latter study.

The reported LC_{50} values for adult rodents are 41000 ppm for 6 hours for mice and for rats 145000 ppm for 1 hour, 97400 ppm for 4 hours, 64000 ppm for 4 hours and 66500 ppm for 6 hours (see Table 4).

7.3. Derivation of AEGL-3

Due to the lack of data on fatalities after inhalation, AEGL-3 values were based on acute oral intoxication data in humans.

The minimum lethal oral dose of about 1 g/kg reported in review articles by Buller and Wood (1904) and Röe (1982) was not used as the basis for AEGL derivation because the value was not sufficiently supported by data in these articles. However, the reported minimum lethal oral dose which corresponds to a peak blood methanol level of about 1540 mg/l is supported by case studies on intoxication with methanol only (i.e. without concomitant ethanol consumption) (Naraqi et al., 1979; Erlanson et al., 1965; Bennett et al., 1955; Gonda et al., 1978; Meyer et al., 2000). These studies reported measured blood methanol concentrations and time periods between intoxication and measurement. Given the time that elapsed until blood sampling, during which part of the methanol was metabolized, it can be concluded that peak blood methanol concentrations have been above 1000 mg/l in all fatal cases (see Figure 2). Based on the extensive clinical experience with methanol intoxications, the American Academy of Clinical Toxicology (AACT, 2002) published clinical practice guidelines on the treatment of methanol poisoning. According to these guidelines, peak blood methanol concentrations >500 mg/l indicate serious poisoning for which hemodialysis is recommended. Based on the human experience, a peak blood methanol concentration of 500 mg/l was chosen as the basis for AEGL-3 derivation.

A total uncertainty factor of 3 was used. An uncertainty factor of 3 was applied for intraspecies variability because clinical experience with methanol intoxications is mainly based on cases involving adult men while much less data is available for women, children or elderly persons, and because subpopulations with a less than optimal folate status may be more susceptible to the health effects of methanol.

Using a total uncertainty factor of 3, a blood methanol concentration of 167 mg/l was derived as the basis for calculation of exposure concentrations. Application of the uncertainty factor to the blood methanol concentration was preferred because the calculated exposure concentrations in air stayed better in the concentration range for which the pharmacokinetic model was validated and the effect of methanol metabolism for longer exposure periods was more adequately taken into account. In contrast, first calculating exposure concentrations that would lead to a blood methanol level of 500 mg/l and then applying a factor of 3 to the derived exposure concentration would result in calculation of extremely high concentrations in the fist step at which metabolic pathways would be saturated.

Using the pharmacokinetic model of Perkins et al. (1995a), inhalation exposure concentrations were calculated for appropriate time periods that would lead to a blood methanol concentration of 167 mg/l at the end of the time period (see Appendix C, Table 16). The calculated exposure concentrations were set as AEGL-3 values.

The values are listed in Table 11 below.

TABLE 11: AEGL-3 VALUES FOR METHANOL

AEGL Level	10 minutes	30 minutes	1 hour	4 hours	8 hours
AEGL-3	#	14000 ppm [a] (18000 mg/m³)	7200 ppm [a] (9400 mg/m³)	2400 ppm (3100 mg/m³)	1600 ppm (2100 mg/m³)

[a] The 1-hour AEGL-3 values are higher than 1/10 of the lower explosive limit (LEL) of methanol in air (LEL = 55,000; 1/10th LEL = 5500 ppm). Therefore, safety considerations against the hazard of explosion must be taken into consideration.

[#] The 10-minute AEGL-3 value of 40,000 ppm is higher than 50% of the lower explosive limit of methanol in air (LEL = 55,000 ppm; 50% of the LEL = 27,500 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

The derived values are supported by the study of Kawai et al. (1991), which reported dimmed vision (the authors suggested that visibility was temporarily reduced by fog in the workroom) and nasal irritation during work, in a group of 22 workers exposed to a mean methanol concentration of 459 ppm for 8 hours; a group of 5 breathing-zone samples revealed concentrations between 3000 and 5500 ppm. The values are also supported by an older study that reported severe nasal and eye irritation in volunteers after exposure at 65400 ppm for 5 minutes (Flury and Wirth, 1933).

With regard to fetal death observed in rodents, the derived AEGL-3 values are supported on basis of the following rationale: the NOEL for fetal death in mice was 5000 ppm for 7 hours after both single and repeated exposure (Rogers et al. 1993; 1995; Rogers, 1999). As pointed out in Section 7.2, methanol itself and not a metabolite is probably responsible for the developmental toxic effects in rodents (Dorman et al., 1995) and, therefore, it seems reasonable to assess the developmental toxicity on the basis of blood methanol concentrations. The corresponding end-of-exposure blood concentration in mice after exposure at 5000 ppm for 7 hours was 2126 mg/l (Rogers et al., 1993). The blood methanol concentration that was used for derivation of AEGL-3 values was 167 mg/l, which is about 13-fold lower than the NOEL blood concentration for fetal death in mice, and thus should provide sufficient protection to humans against this effect.

The derived values are also supported by studies on monkeys: since no toxic effects were observed in monkeys exposed repeatedly at 5000 ppm for 6 hours/day (Andrews et al., 1987) it can be concluded that these exposure conditions are considerably below the lethality threshold. In the study of NEDO (1987) no deaths were observed after repeated exposure at 3000 ppm for 21 hours per day. Since the biological half life of methanol and formate is in the order of a few hours, the short period of 3 hours between exposures in the NEDO study did not allow for complete elimination and, thus, after the first exposure higher blood concentrations of methanol and formate must have been present during subsequent

exposures. This may explain the delayed deaths observed after repeated exposure for 21 hours/day to 10000 ppm (death after 3 days), 7000 ppm (death after 6 days) and 5000 ppm (death after 5 days).

8. SUMMARY OF PROPOSED AEGLS

8.1. AEGL Values and Toxicity Endpoints

The AEGL values for various levels of effects and various time periods are summarized in Table 12. They were derived using the following key studies and methods.

The AEGL-1 was based on a study in which human volunteers were exposed to 800 ppm methanol for 8 hours (Batterman et al., 1998). While the study made no statement on health effects, the coauthor Dr. Franzblau stated in a personal communication that the subjects reported no symptoms (Franzblau, 1999; 2000). A total uncertainty factor of 3 was applied. The other exposure duration-specific values were derived by time scaling according to the dose-response regression equation $C^n \times t = k$, using the default of n=3 for shorter exposure periods. For the 10-minute AEGL-1 the 30-minute value was applied.

The AEGL-2 values were based on developmental toxic effects in mice. After a single exposure to different concentration-time combinations on gestational day 7, the most sensitive endpoint was cervical rib induction, which occurred at concentration-time products greater than or equal to 15000 ppm · h, but not at concentration-time products (CxT) below 15000 ppm · h (i.e. no effects were observed after exposure at 2000 ppm for 5 hours, 2000 ppm for 7 hours and 5000 ppm for 2 hours; authors expressed data only as CxT values) (Rogers et al. 1995, abstract; Rogers, 1999, personal communication). For the NOEL of 2000 ppm for 7 hours (Rogers et al. 1995, abstract; Rogers, 1999, personal), the corresponding end-of-exposure blood concentration was measured as 487 mg/l (Rogers et al., 1993). An interspecies uncertainty factor of 1 and an intraspecies uncertainty factor of 10 were used. The total uncertainty factor was applied to the blood methanol concentration resulting in a concentration of 48.7 mg/l. A pharmacokinetic model was used to calculate inhalation exposure concentrations for appropriate time periods that would lead to a blood methanol concentration of 48.7 mg/l at the end of the time period. These exposure concentrations were set as AEGL-2 values.

The AEGL-3 values were based on acute lethal effects on humans after oral methanol uptake. Case studies (Naraqi et al., 1979; Erlanson et al., 1965; Bennett et al., 1955; Gonda et al., 1978; Meyer et al., 2000) reported measured blood methanol concentrations and time periods between intoxication and measurement. Given the time that elapsed until blood sampling, during which part of the methanol was metabolized, it can be concluded that peak blood methanol concentrations have been above 1000 mg/l in all fatal cases. Based on the extensive clinical experience with methanol intoxications, the American Academy of Clinical Toxicology (AACT, 2002) published clinical practice guidelines on the treatment of methanol poisoning. According to these guidelines, peak blood methanol concentrations >500 mg/l indicate serious poisoning for which hemodialysis is recommended. Based on the human experience, a peak blood methanol concentration of 500 mg/l was chosen as the basis for AEGL-3 derivation. An intraspecies uncertainty factor of 3 was used. The uncertainty factor was applied to the blood methanol concentration resulting in a concentration of 167 mg/l. A pharmacokinetic model was used to calculate inhalation exposure concentrations for appropriate time periods that would lead to a blood methanol

concentration of 167 mg/l at the end of the time period. These exposure concentrations were set as AEGL-3 values.

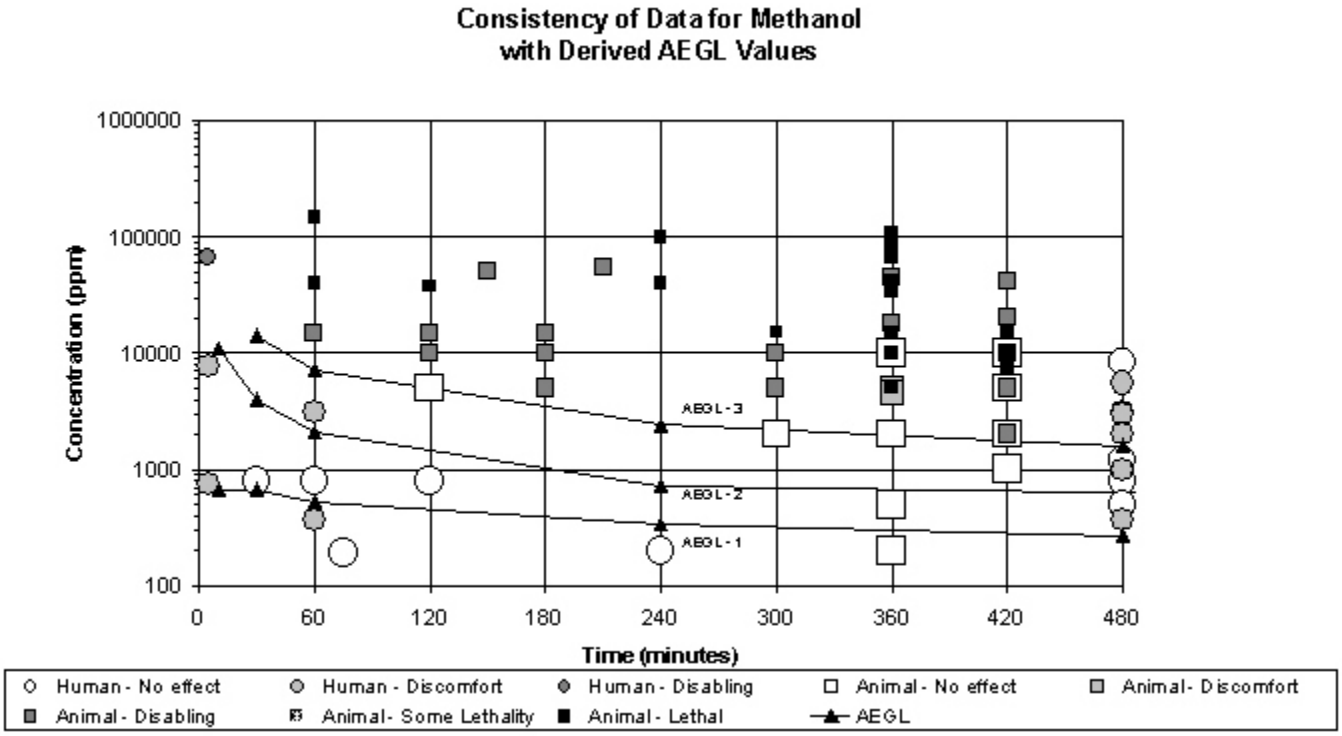
Because liquid methanol is absorbed through the skin, a skin notation was added to the table of values.

TABLE 12: SUMMARY/RELATIONSHIP OF PROPOSED AEGL VALUES FOR METHANOL [a]

Classification	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour
AEGL-1 (Nondisabling)	670 ppm (880 mg/m³)	670 ppm (880 mg/m³)	530 ppm (690 mg/m³)	340 ppm (450 mg/m³)	270 ppm (350 mg/m³)
AEGL-2 (Disabling)	11000 ppm [b] (14000 mg/m³)	4000 ppm (5200 mg/m³)	2100 ppm (2800 mg/m³)	730 ppm (960 mg/m³)	520 ppm (680 mg/m³)
AEGL-3 (Lethal)	#	14000 ppm [b] (18000 mg/m³)	7200 ppm [b] (9400 mg/m³)	2400 ppm (3100 mg/m³)	1600 ppm (2100 mg/m³)

[a] Cutaneous absorption may occur; direct skin contact with the liquid should be avoided.
[b] The 10-minute AEGL-2 value and the 30-minute and 1-hour AEGL-3 values are higher than 1/10 of the lower explosive limit (LEL) of methanol in air (LEL = 55,000; 1/10th LEL = 5500 ppm). Therefore, safety considerations against the hazard of explosion must be taken into consideration.
[#] The 10-minute AEGL-3 value of 40,000 ppm is higher than 50% of the lower explosive limit of methanol in air (LEL = 55,000 ppm; 50% of the LEL = 27,500 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

All inhalation data are summarized in Figure 3 below. The data were classified into severity categories chosen to fit into definitions of the AEGL level health effects. The category severity definitions are "No effect"; "Discomfort"; "Disabling"; "Lethal"; "Partial lethality" (at an experimental concentration in which some of the animals died and some did not, this label refers to the animals which did not die) and "AEGL". Note that the AEGL-2 values are designated as triangles.



2077 **FIGURE 3: CATEGORICAL REPRESENTATION OF ALL METHANOL INHALATION**
2078 **DATA**

2079 **8.2. Comparison with Other Standards and Criteria**

2080 Standards and guidance levels for workplace and community exposures are listed in Table 13. In
2081 addition, biological exposure values exist: the ACGIH BEI (biological exposure index) is 15 mg methanol
2082 per liter urine at the end of shift at the end of workweek (ACGIH, 1999). The German BAT (Biologischer
2083 Arbeitsstoff-Toleranz-Wert; biological tolerance value) is 30 mg methanol per liter urine during the
2084 second half of shift at the end of workweek (Henschler und Lehnert, 1983).

TABLE 13. EXTANT STANDARDS AND GUIDELINES FOR METHANOL

Guideline	Exposure Duration				
	10 minutes	**30 minutes**	**1 hour**	**4 hours**	**8 hours**
AEGL-1	670 ppm	670 ppm	530 ppm	340 ppm	270 ppm
AEGL-2	11000 ppm	4000 ppm	2100 ppm	730 ppm	520 ppm
AEGL-3	#	14000 ppm	7200 ppm	2400 ppm	1600 ppm
ERPG-1(AIHA)[a]			200 ppm		
ERPG-2 (AIHA)			1000 ppm		
ERPG-3 (AIHA)			5000 ppm		
EEGL (NRC)[b]	800 ppm	400 ppm	200 ppm		10 ppm [24 hours]
PEL-TWA (OSHA)[c]					200 ppm
PEL-STEL (OSHA)[d]	250 ppm [15 minutes]				
IDLH (NIOSH)[e]		6000 ppm			
REL-TWA (NIOSH)[f]					200 ppm
REL-STEL (NIOSH)[g]	250 ppm [15 minutes]				
TLV-TWA (ACGIH)[h]					200 ppm
TLV-STEL (ACGIH)[i]	250 ppm				
MAK (Germany)[j]					200 ppm
MAK Spitzen-begrenzung (Germany)[k]		1000 ppm			
Einsatztoleranz-wert (Germany)[l]				500 ppm	
MAC (The Netherlands)[m]					200 ppm

[a] **ERPG (Emergency Response Planning Guidelines, American Industrial Hygiene Association)** (AIHA, 1994)
The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor. The ERPG-1 for methanol is based on the threshold for producing headaches and dizziness in workers exposed repeatedly to methanol (Frederick et al., 1984).
The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protective action. The ERPG-2 for methanol is based on observed 1) no toxic effects in workers exposed to 1000-2000 ppm for 0.5 hours or less (Sterner and Fassett, 1958), 2) no serious toxic effects after brief exposures at 3000 ppm (Frederick et al, 1984) or 8000 ppm (Humperdinck, 1941) and 3) no toxic effects in monkeys repeatedly exposed to 5000 ppm (Andrews et al., 1987) or rats repeatedly exposed to 10000 ppm (White et al., 1983).
The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing life-threatening health effects. The ERPG-3 for methanol is based on observed 1) no lethality in workers exposed to 3000 ppm for 15 minutes (Frederick et al., 1984) or 8000 ppm (Humperdinck, 1941) and 2) no toxic effects in monkeys repeatedly exposed to 5000 ppm (Andrews et al., 1987).

[b] **EEGL (Emergency Exposure Guidance Levels, National Research Council)** (NRC, 1985)
is the concentration of contaminants that can cause discomfort or other evidence of irritation or intoxication in or around the workplace, but avoids death, other severe acute effects and long-term or chronic injury. The EEGL for methanol are mainly based on the LC_{Lo} of 1000 ppm in the study on monkeys by McCord (1931), the pharmacokinetic study by Leaf and Zatman (1952) and other observations summarized in the NIOSH Criteria Document (NIOSH, 1976).

[c] **OSHA PEL-TWA (Occupational Health and Safety Administration, Permissible Exposure Limits - Time Weighted Average)** (OSHA, 1994)
is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[d] **OSHA PEL-STEL (Permissible Exposure Limits - Short Term Exposure Limit)** (OSHA, 1994)
is defined analogous to the ACGIH-TLV-STEL.

[e] **IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)** (NIOSH, 1996)
represents the maximum concentration from which one could escape within 30 minutes without any escape-impairing symptoms, or any irreversible health effects. The IDLH for methanol is based on a LC_{Lo} of 37594 ppm for two hours in the mouse (Izmerov et al., 1982).

[f] **NIOSH REL-TWA (National Institute of Occupational Safety and Health, Recommended Exposure Limits - Time Weighted Average)** (NIOSH, 1992)
is defined analogous to the ACGIH-TLV-TWA.

[g] **NIOSH REL-STEL (Recommended Exposure Limits - Short Term Exposure Limit)** (NIOSH, 1992)
is defined analogous to the ACGIH-TLV-STEL.

[h] **ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average)** (ACGIH, 1996)
is the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[i] **ACGIH TLV-STEL (Threshold Limit Value - Short Term Exposure Limit)** (ACGIH, 1996)
is defined as a 15 minute TWA exposure which should not be exceeded at any time during the workday even if the 8-hour TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 minutes and should not occur more than 4 times per day. There should be at least 60 minutes between successive exposures in this range.

[j] **MAK (Maximale Arbeitsplatzkonzentration [Maximum Workplace Concentration], Deutsche Forschungsgemeinschaft [German Research Association], Germany)** (Greim, 1995; DFG, 1999)
is defined analogous to the ACGIH-TLV-TWA.

[k] **MAK Spitzenbegrenzung (Kategorie II,2) [Peak Limit Category II,2]** (DFG, 1999)
constitutes the maximum average concentration to which workers can be exposed for a period up to 30 minutes, with no more than 2 exposure periods per work shift; total exposure may not exceed 8-hour MAK.

[l] **Einsatztoleranzwert [Action Tolerance Levels] (Vereinigung zur Förderung des deutschen Brandschutzes e.V. [Federation for the Advancement of German Fire Prevention])** (Greim, 1996)
constitutes a concentration to which unprotected firemen and the general population can be exposed to for up to 4 hours without any health risks. The value is based on the estimation that the Biologischer-Arbeitsstoff-Toleranzwert [Biological Exposure Index] of 30 mg/l methanol in urine could be reached following a 4-hour exposure to 500 ppm methanol.

[m] **MAC ([Maximum Workplace Concentration], Dutch Expert Committee for Occupational Standards, The Netherlands)** (MSZW, 1999)
is defined analogous to the ACGIH-TLV-TWA.

8.3. Data Adequacy and Research Needs

Definitive exposure-response data for irreversible or lethal methanol toxicity in humans are not available. However, qualitative information on the human experience affirms that methanol vapor is toxic and can cause irreversible effects (blindness) as well as lethality. Data from occupational exposure studies are often compromised by uncertain quantitation of exposure.

For the derivation of AEGL-3 values studies on lethal effects of inhalation exposure in rodents were not considered appropriate due to the considerable differences in methanol metabolism kinetics and mechanisms of methanol toxicity between primates (humans and monkeys) and rodent species. Since well-described case reports of fatalities after inhalation were not available, the derivation was based on the extensive clinical experience with methanol intoxications. The American Academy of Clinical Toxicology (AACT, 2002) published clinical practice guidelines on the treatment of methanol poisoning. According to these guidelines, peak blood methanol concentrations >500 mg/l indicate serious poisoning for which hemodialysis is recommended. Based on the human experience, a peak blood methanol concentration of 500 mg/l was chosen as the basis for AEGL-3 derivation.

Although methanol intoxication can cause blindness in humans, it is not possible to derive a threshold for this irreversible effect from the available data. However, available reports indicate that blindness results only after live-threatening poisoning. There was thus no basis for the derivation of AEGL-2 on health effects in humans. Therefore, the derivation of AEGL-2 values was based on developmental toxic effects in rodents. A number of teratogenicity studies in mice and rats is available including a two studies reporting developmental toxic effects in mice after single inhalation exposures. There is experimental evidence that developmental toxic effects are caused by methanol itself and not by a

metabolite, such as formate. It therefore was considered adequate to derived AEGL-2 values on the basis of blood methanol concentrations. The total uncertainty factor was applied to the measured end-of-exposure blood methanol concentration. Using a pharmacokinetic model, methanol concentrations in air were calculated which would result in this blood methanol concentration at the end of relevant AEGL time periods. With respect to developmental toxic effects, no information regarding human occupational, accidental or intentional exposure via the inhalation, dermal or oral route is available. More research is needed for an adequate evaluation of the developmental toxic effects of methanol reported in monkeys.

Based on the extremely wide range of reported odor thresholds, the odor threshold data were not considered appropriate for derivation of AEGL-1. A number of high quality, human studies on asymptomatic effects of low methanol concentrations on the central nervous system are available. These studies usually used exposure at 200 ppm which was considered lower than the thresholds for irritation and discomfort. A level of 1000 ppm caused headache and eye irritation is workers and was considered above the discomfort level. Therefore, the pharmacokinetic study by Batterman et al. (1998) employing exposure at 800 ppm was used for the derivation of AEGL-1 values. It has to be noted though, that the study did not formally evaluate and report health effects. In a personal communication by one of the studies‘ coauthors it was stated that none of the subjects reported symptoms. Some uncertainty to this data is conferred by this fact that the evaluation of health effects was not the focus of the study.

With respect to lethal and severe toxic effects, additional inhalation studies on monkeys using single inhalation exposure could support the derived AEGL-2 and AEGL-3 values.

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2552 **APPENDIX A**

2553 **Time Scaling Calculations for AEGLs**

AEGL-1

Key study: Batterman et al. (1998) and Franzblau (1999; 2000; personal communication); Frederick et al. (1984); NIOSH (1980); NIOSH (1981)

Toxicity endpoint: Pharmacologic study exposing 3 female and 12 male subjects to 800 ppm methanol for 8 hours. One of the study's coauthors stated in a personal communication that none of the subjects reported symptoms.

Scaling: $C^3 \times t = k$ for extrapolation to 4, hours, 1 hour and 30 minutes
$k = 800^3\ ppm^3 \times 8\ hours = 4.1 \times 10^9\ ppm^3\ h$
The AEGL-1 for 10 minutes was set at the same concentration as the 30-minute value.

Uncertainty factors: 3 for intraspecies variability

Calculations:

10-minute AEGL-1: 10-min AEGL-1 = 670 ppm (880 mg/m³)

30-minute AEGL-1:
$C^3 \times 0.5\ h = 4.1 \times 10^9\ ppm^3\ h$
C = 2017 ppm
30-min AEGL-1 = 2017 ppm/3 = 670 ppm (880 mg/m³)

1-hour AEGL-1:
$C^3 \times 1\ h = 4.1 \times 10^9\ ppm^3\ h$
C = 1600 ppm
1-hour AEGL-1 = 1600 ppm/3 = 530 ppm (690 mg/m³)

4-hour AEGL-1:
$C^3 \times 4\ h = 4.1 \times 10^9\ ppm^3\ h$
C = 1008 ppm
4-hour AEGL-1 = 1008 ppm/3 = 340 ppm (450 mg/m³)

8-hour AEGL-1: 8-hour AEGL-1 = 800 ppm/3 = 270 ppm (350 mg/m³)

AEGL-2

Key study: Rogers et al. (1993; 1995, abstract; 1997); Rogers (1999, personal communication)

Toxicity endpoint: Using repeated 7-hour/day exposures during gestational days 6 to 15, a dose-related, significant increase in cervical ribs was observed at 2000 ppm or higher; other malformations, such as exencephaly and cleft palate occurred dose-dependently at concentrations of 5000 ppm or higher (Rogers et al., 1993). The same type of malformations occurred after a single 7-hour exposure to 10000 ppm (Rogers et al., 1997). In another study of Rogers and coworkers, which has not been formally published up until know, mice were exposed on gestational day 7 to different concentration-time combinations (Rogers et al. 1995, abstract; Rogers, 1999, personal communication). The most sensitive endpoint was cervical rib induction, which occurred at concentration-time products greater than or equal to 15000 ppm · h, but not at concentration-time products below 15000 ppm · h (i.e. no effects were observed after exposure to 2000 ppm x 5 h, 2000 ppm x 7 h and 5000 ppm x 2 h; authors expressed data only as CxT values). In these experiments, the highest no-observed-effect CxT product was 2000 ppm for 7 hours. The corresponding end-of-exposure blood concentration in mice after exposure was measured as 487 mg/l (Rogers et al., 1993). The uncertainty factors were applied to the blood methanol concentration resulting in a concentration of 48.7 mg/l, on which calculations of AEGL-2 exposure concentrations were based.

Scaling: A pharmacokinetic model was used to calculate exposure concentrations that would lead to blood methanol concentrations at the end of periods of 8 hours, 4 hours, 1 hour and 30 and 10 minutes. Calculations are shown in Appendix B, Table 15.

Uncertainty factors: 1 for interspecies variability
10 for intraspecies variability

Calculations: The concentrations calculated using the pharmacokinetic (PK) model were set as AEGL-2 values:

10-minute AEGL-2: 10-min AEGL-2 = 11350 ppm (from PK model) = 11000 ppm (14000 mg/m³)

30-minute AEGL-2: 30-min AEGL-2 = 3980 ppm (from PK model) = 4000 ppm (5200 mg/m³)

1-hour AEGL-2: 1-hour AEGL-2 = 2110 ppm (from PK model) = 2100 ppm (2800 mg/m³)

4-hour AEGL-2: 4-hour AEGL-2 = 730 ppm (from PK model) = 730 ppm (960 mg/m³)

8-hour AEGL-2: 8-hour AEGL-2 = 524 ppm (from PK model) = 520 ppm (680 mg/m³)

AEGL-3

Key study: AACT (2002)

Toxicity endpoint: Case studies reported measured blood methanol concentrations and time periods between intoxication and measurement. Given the time that elapsed until blood sampling, during which part of the methanol was metabolized, it can be concluded that peak blood methanol concentrations have been above 1000 mg/l in all fatal cases. Based on the extensive clinical experience with methanol intoxications, the American Academy of Clinical Toxicology (AACT, 2002) published clinical practice guidelines on the treatment of methanol poisoning. According to these guidelines, peak blood methanol concentrations >500 mg/l indicate serious poisoning for which hemodialysis is recommended. Based on the human experience, a peak blood methanol concentration of 500 mg/l was chosen as the basis for AEGL-3 derivation.

Scaling: A pharmacokinetic model was used to calculate exposure concentrations that would lead to blood methanol concentrations at the end of periods of 8 hours, 4 hours, 1 hour and 30 and 10 minutes. Calculations are shown in Appendix B, Table 16.

Uncertainty factor: 3 for intraspecies variability

Calculations: The concentrations calculated using the pharmacokinetic (PK) model were set as AEGL-3 values:

10-minute AEGL-3: 10-min AEGL-3 = 39500 ppm (from PK model) = 40000 ppm (52000 mg/m³)

30-minute AEGL-3: 30-min AEGL-3 = 13700 ppm (from PK model) = 14000 ppm (18000 mg/m³)

1-hour AEGL-3: 1-hour AEGL-3 = 7220 ppm (from PK model) = 7200 ppm (9400 mg/m³)

4-hour AEGL-3: 4-hour AEGL-3 = 2380 ppm (from PK model) = 2400 ppm (3100 mg/m³)

8-hour AEGL-3: 8-hour AEGL-3 = 1620 ppm (from PK model) = 1600 ppm (2100 mg/m³)

2635 **APPENDIX B**

2636 **Pharmacokinetic Calculations**

Calculation of Exposure Concentrations for Humans

Study: Perkins et al. (1995a)
Pharmacokinetic model for blood methanol concentrations after inhalation exposure.

Equation:

$$\frac{dC}{dt} = \frac{\Phi \bullet V_h \bullet C_{inh}}{V_d} - \frac{V_{max} \bullet C}{K_m + C}$$

Parameters:

	C	blood methanol concentration [mg/l]
	C_{inh}	methanol concentration in air [mg/l]
	t	time [h]
	Φ	fraction of inhaled methanol absorbed into systemic circulation
	V_h	ventilation rate [l/kg h]
	V_d	volume of distribution [l/kg]
	V_{max}	maximum rate of enzymatic methanol oxidation [mg/l h]
	K_m	Michaelis-Menten constant of enzymatic methanol oxidation [mg/l]

Parameter values: Since the presentation of parameters used for calculations and the reasoning for the parameter values is not clear in the article of Perkins et al. (1995a), for calculations the parameters were not taken over automatically. Instead, the following parameters were used:

TABLE 14: PARAMETERS OF PHARMACOKINETIC MODEL

Parameter	Value used for calculation
Φ	0.7 The mean value of the range (0.53-0.85) reported Leaf and Zatman (1952) and Sedivec et al. (1981) (see Section 4.1.1) was used (value used in Perkins model: 0.75)
V_h (l/kg h)	17.8 (a body weight of 70 kg and a ventilation rate of 10 m^3/8 h for occupational situations were used) (value used in Perkins model: 10.3)
V_d (l/kg)	0.65 The mean value of the range (0.6-0.7) reported by Yant and Schrenk (1937) was used (see Section 4.1.1) (value used in Perkins model: 0.7)
V_{max} (mg/l h)	115 (value used in Perkins model)
K_m (mg/l)	460 (value used in Perkins model)

Procedure: The simulations were performed on a spreadsheet program by converting the differentials to finite differences with a time step of 0.1 hours. For the continuous, instantaneous values for the blood concentration of methanol (C), the value from the previous time step (C_{t-1}) was used. Background blood methanol in humans is approximately 1.0 mg/l (see Table 8 for references) from both endogenous and exogenous sources and this level was used for the initial time step (C_0). Using three significant figures, the lowest exposure concentration was calculated that resulted at or above the desired blood methanol concentration.

Equation:

$$C_t = \frac{\Phi \bullet V_h \bullet C_{inh}}{V_d} \bullet 0.1h - \frac{V_{max} \bullet C_{t-1}}{K_m + C_{t-1}} \bullet 0.1h$$

Calculations: The following exposure concentrations were calculated to result in a blood methanol concentration of 48.7 mg/l in humans:

TABLE 15: CALCULATION OF CONCENTRATIONS FOR INHALATION EXPOSURE I

Exposure time	Calculated exposure concentration (ppm)	Rounded value (ppm)
8 h	524	520
4 h	730	730
1 h	2110	2100
30 min	3980	4000
10 min	11350	11000

Calculations: The following exposure concentrations were calculated to result in a blood methanol concentration of 167 mg/l in humans:

TABLE 16: CALCULATION OF CONCENTRATIONS FOR INHALATION EXPOSURE II

Exposure time	Calculated exposure concentration (ppm)	Rounded value (ppm)
8 h	1620	1600
4 h	2380	2400
1 h	7220	7200
30 min	13700	14000
10 min	39500	40000

Comparison of the Perkins et al. (1995a) and Bouchard et al. (2001) models

In order to demonstrate that the pharmacokinetic model of Perkins et la. (1995a) gives results consistent with newer models, its predictions of methanol concentrations in air, that would lead cause selected blood methanol concentrations were compared with those of the model described by Bouchard et al. (2001). Calculations using the latter model were done by Professor Michele Bouchard, University of Montreal, Canada (Bouchard, personal communication, 2003). Model parameters were chosen as described in the original publication by Professor Bouchard, except that the values for the volume of distribution V_d and for the ventilation rate V_h were adjusted to the used in the Perkins model (see Table 14).

Exposure concentrations in air were calculated for end-of-exposure blood methanol concentrations of 30, 100 and 250 mg/l. As can be seen from the results tables below, both pharmacokinetic models gave consistent results.

TABLE 17: CALCULATION OF METHANOL CONCENTRATIONS RESULTING IN A BLOOD CONCENTRATION OF 30 mg/l

Exposure time	Perkins et al. (1995a) model	Bouchard et al. (2001) model
8 h	330	450
4 h	460	560
1 h	1300	1400
30 min	2500	2600
10 min	7000	7500

TABLE 18: CALCULATION OF METHANOL CONCENTRATIONS RESULTING IN A BLOOD CONCENTRATION OF 100 mg/l

Exposure time	Perkins et al. (1995a) model	Bouchard et al. (2001) model
8 h	1100	1200
4 h	1500	1700
1 h	4400	4600
30 min	8300	8600
10 min	24000	25000

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TABLE 19: CALCULATION OF METHANOL CONCENTRATIONS RESULTING IN A BLOOD CONCENTRATION OF 250 mg/l		
Exposure time	**Perkins et al. (1995a) model**	**Bouchard et al. (2001) model**
8 h	2300	2400
4 h	3500	3600
1 h	11000	11000
30 min	21000	21000
10 min	60000	61000

2723 **APPENDIX C**

2724 **Level of Distinct Odor Awareness**

Derivation of the Level of Distinct Odor Awareness (LOA)

The level of distinct odor awareness (LOA) represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, about 10 % of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odor perception. The LOA derivation follows the guidance given by van Doorn et al. (2002).

For derivation of the odor detection threshold (OT_{50}), a study is available in which the odor threshold for the reference chemical n-butanol (odor detection threshold 0.04 ppm) has also been determined:

Hellman and Small (1974):
odor detection threshold for methanol: 4.26 ppm
odor detection threshold for n-butanol: 0.3 ppm
corrected odor detection threshold (OT_{50}) for methanol: 4.26 ppm * 0.04 ppm / 0.3 ppm = 0.57 ppm

The concentration (C) leading to an odor intensity (I) of distinct odor detection (I=3) is derived using the Fechner function:

$I = k_w * \log (C / OT_{50}) + 0.5$

For the Fechner coefficient, the default of $k_w = 2.33$ will be used due to the lack of chemical-specific data:

$3 = 2.33 * \log (C / 0.57) + 0.5$ which can be rearranged to

$\log (C / 0.57) = (3-0.5) / 2.33 = 1.07$ and results in

$C = (10^{1.07}) * 0.57 = 11.8 * 0.57 = 6.7$ ppm

The resulting concentration is multiplied by an empirical field correction factor. It takes into account that in every day life factors, such as sex, age, sleep, smoking, upper airway infections and allergy as well as distraction, increase the odor detection threshold by a factor of 4. In addition, it takes into account that odor perception is very fast (about 5 seconds) which leads to the perception of concentration peaks. Based on the current knowledge, a factor of 1/3 is applied to adjust for peak exposure. Adjustment for distraction and peak exposure lead to a correction factor of 4 / 3 = 1.33

LOA = C * 1.33 = 6.7 ppm * 1.33 = 8.9 ppm

The LOA for methanol is 8.9 ppm.

2753 **APPENDIX D**

2754 **Derivation Summary for Methanol AEGLs**

ACUTE EXPOSURE GUIDELINES FOR METHANOL
(CAS NO. 67-56-1)

AEGL-1 VALUES				
10 minutes	30 minutes	1 hour	4 hours	8 hours
670 ppm	670 ppm	530 ppm	340 ppm	270 ppm

Reference: Batterman, S.A., A. Franzblau, J.B. D'Arcy, N.E. Sargent, K.B. Gross and R.M. Schreck, 1998. Breath, urine, and blood measurements as biological exposure indices of short-term inhalation exposure to methanol. *International Archives of Occupational and Environmental Health* 71, 325-335; Franzblau, A., University of Michigan School of Public Health, Ann Arbor, Michigan, personal communication, e-mail dated 14 June 1999; Franzblau, A., University of Michigan School of Public Health, Ann Arbor, Michigan, personal communication, e-mail dated 3 October 2000; Frederick, L.J., P.A. Schulte, A. Apol, 1984. Investigation and control of occupational hazards associated with the use of spirit duplicators. *American Industrial Hygiene Association Journal* 45, 51-55; NIOSH, National Institute for Occupational Safety and Health, 1980. Hazard evaluation and technical assistance report TA 80-32. Everett school district, Everett, Washington. National Institute of Occupational Safety and Health, Cincinnatti, OH, USA.; NIOSH, National Institute for Occupational Safety and Health, 1981. Health hazard evaluation report No. HETA-81-177, 178-988, University of Washington, Seattle. National Institute of Occupational Safety and Health, Cincinnatti, OH, USA

Test Species/Strain/Number: Humans / not applicable / in total 7 women and 12 men

Exposure Route/Concentrations/Durations: Inhalation / 0 and 800 ppm / 0.5, 1, 2 and 8 hours

Effects: In this pharmacokinetic study no statement was made on the presence or absence of any signs or symptoms of the methanol exposure. In a personal communication, the second author, Dr. Franzblau, stated that although no formal mechanism of recording symptoms was used, the subjects were generally asked during exposure if they experienced any symptoms. He wrote that individual symptoms were certainly asked of some subjects and that "none of the subjects reported odor, irritation, headache or other non-specific symptoms"; likewise "none of the subjects reported any difficulties or alterations of visual function". Dr. Franzblau wrote that it is possible that some subjects were not queried and that no written notes were made.

Endpoint/Concentration/Rationale: Several experimental human studies are available that used methanol concentrations of about 200 ppm. Chuwers et al. (1995) found no significant effects in a panel of neurophysiological and neuropsychological tests after exposure for 4 hours to 200 ppm. After the same exposure, Muttray et al. (2001) observed electroencephalogram alterations which the authors did not considered adverse; no clinical symptoms were reported by the subjects. Likewise, the NAC/AEGL committee considered these findings as below the threshold for AEGL-1. Batterman et al. (1998) exposed volunteers at a higher level (i.e. 800 ppm for 8 hours). As this was a pharmacokinetic study, health effects were not formally evaluated. In a personal communication the coauthor Dr. Franzblau stated that individual symptoms were asked of some subjects, other subjects were only asked generally if they had symptoms, and that in some exposure sessions subjects might not have been queried. According to Dr. Franzblau, none of the subjects reported symptoms. Since the subjects knew the exposure concentration by means of a meter showing the actual concentration, if might be expected that this would have increased the inclination of subjects to report symptoms.
NIOSH (1980) and Frederick et al. (1984) reported significantly higher frequencies of headaches, dizziness, blurred vision after occupational exposure at 1060 ppm (mean concentration). NIOSH (1981) reported eye irritation in a worker after exposure at 1025 ppm for 25 minutes. Since the 1000-ppm level was considered already a discomfort level, the 800 ppm for 8 hour exposure from the Batterman et al. (1998) study was chosen as a starting point for AEGL-derivation. Since the local irritation effects are determined by the concentration of methanol in air and not to the blood methanol level, calculation of AEGL-1 values was not done using a pharmacokinetic model (as done for AEGL-2 and -3) based on the end-of-exposure blood methanol level of 30.7 mg/l reported by Batterman et al. (1998). Instead, exposure to 800 ppm for 8 hours was used as the basis for AEGL-1 derivation.
Uncertainty Factors/Rationale: Total uncertainty factor: 3 Interspecies: not applicable Intraspecies: 3 - because interindividual variability with regard to slight central nervous system effects (e.g. headache) is likely to exist (although it cannot be quantified exactly from the existing experimental and epidemiological studies) and because subpopulations with a less than optimal folate status may be more susceptible to the health effects of methanol.
Modifying Factor: Not applicable
Animal to Human Dosimetric Adjustment: Not applicable
Time Scaling: $C^n \times t = k$ where the default of n = 3 was used due to the lack of substance-specific data.. For the 10-minute AEGL-1 the 30-minute value was applied because no studies were available that demonstrated the absence of notable discomfort (with respect to irritation) in the general population, including susceptible subpopulations, at 970 ppm (extrapolated value for 10-minute period).

Data Adequacy: Some uncertainty to the key study used for AEGL-1 derivation is conferred by the fact that no formal evaluation of health effects was performed and that with regard to effects only a personal communication by one of the key studies‘ coauthors is available, who stated that none of the subjects has reported symptoms. Other controlled studies using comparable exposure concentrations are not available. Other studies describing asymptomatic effects on the central nervous system a lower concentration of about 200 ppm (Chuwers et al., 1995; Muttray et al., 2001) were not used because no dose-response relationships were established and in light of the study of Batterman et al. (1998) and several occupational exposure studies, this exposure concentration is considered lower than the threshold for irritation and discomfort.

ACUTE EXPOSURE GUIDELINES FOR METHANOL
(CAS NO. 67-56-1)

AEGL-2 VALUES				
10 minutes	30 minutes	1 hour	4 hours	8 hours
11000 ppm [a]	4000 ppm	2100 ppm	730 ppm	520 ppm

[a] The 10-minute AEGL-2 value is higher than 1/10 of the lower explosive limit (LEL) of methanol in air (LEL = 55,000; 1/10th LEL = 5500 ppm). Therefore, safety considerations against the hazard of explosion must be taken into consideration.

Reference: Rogers, J.M., M.L. Mole, N. Chernoff, B.D. Barbee, C.I. Turner, T.R. Logsdon and R.J. Kavlock, 1993. The developmental toxicity of inhaled methanol in the CD-1 mouse, with quantitative dose-response modeling for estimation of benchmark doses. *Teratology* 47, 175-188; Rogers, J.M., B.D. Barbee and M.L. Mole, 1995. Exposure concentration and time (C x T) relationships in the developmental toxicity of methanol in mice. *Toxicologist* 15, 164 (abstract); Rogers. J.M. and M.L. Mole, 1997. Critical periods of susceptibility to the developmental toxicity of inhaled methanol in the CD-1 mouse. *Teratology* 55, 364-72; Rogers, J.M., 1999. US-EPA, National Health and Environmental Effects Research Laboratory, Research Trigangle Park, North Carolina, personal communication, letter dated 27 May 1999.

Test Species/Strain/Sex/Number: mouse / CD-1 / pregnant females / variable (see below)

Exposure Route/Concentrations/Durations:
Inhalation exposure to the following concentration-time combinations (number of pregnant females or litters given in brackets) were used:

Rogers et al. (1995); Rogers (1999):
0 ppm (time not given); 2000 ppm x 5 / 7 h; 5000 ppm x 2 / 3 / 5 / 7 h; 10000 ppm x 2 / 3 / 5 / 7 h; 15000 ppm x 1 / 2 / 3 / 5 / 7 h (number of litters 5 to 39 for CxT combinations in methanol-exposed and 106 in control groups)

Rogers et al. (1993):
0 / 1000 / 2000 / 5000 / 7500 / 10000 / 15000 ppm x 7 h/d, bd 6-15 (number of exposed females 20 to 61 per group)

Rogers et al. (1997):
0 / 10000 ppm x 7 h/d for 1 d during period of gd 5-9 / for 2 d during period of gd 6-13 (number of pregnant females 12 to 17 per group)

Effects:
Rogers et al. (1995); Rogers (1999):
- no increased malformations after CxT <15000 ppm · h,
- significantly increased incidences of cervical ribs after CxT ≥15000 ppm · h,
- in addition significantly increased incidences of fetal death, cleft palate and other skeletal defects after CxT ≥70000 ppm · h;

Effects (cont.):
Rogers et al. (1993):
- no increased malformations after 1000 ppm,
- significantly increased incidences of cervical ribs after ≥ 2000 ppm,
- in addition significantly increased incidences of exencephaly and cleft palate after ≥ 5000 ppm,
- in addition significantly increased number of dead fetuses/litter after ≥ 7500 ppm,
- in addition significantly increased number of full-litter resorptions after ≥ 10000 ppm;

Rogers et al. (1997): several types of malformations were observed. The critical periods differed with maximum effects (% of fetuses per litter affected) on the following exposure days:
- increased resorptions per litter after exposure on gd 7 or on gd 6-7,
- exencephaly after exposure on gd 7 (20 %) or gd 6-7 (30 %),
- cleft palate after exposure on gd 7 (47 %) or gd 6-7 (20 %),
- first cervical vertebra defect after exposure on gd 5 (56 %) or gd 6 (55 %) or gd 6-7 (72 %),
- second cervical vertebra defect after exposure on gd 7 (29 %) or gd 6-7 (22 %),
- cervical ribs on vertebra after exposure on gd 7 (45 %) or gd 6-7 (74 %)

Endpoint/Concentration/Rationale:
Although methanol intoxication can cause blindness in humans, it is not possible to derive a threshold for this effect from the available data. Moreover, available reports indicate that blindness results only after live-threatening poisoning (Naraqi et al., 1979; WHO, 1997; IUCLID, 1996; NIOSH, 1976).
The epidemiological studies evaluating reversible effects on humans, such as slight neurotoxic and irritative effects at the workplace, though evaluating a relevant toxicological endpoint, will not be used for derivation of AEGL-2 values because data on exposure time and exposure concentration were not considered sufficient. However, these reports provide valuable supporting evidence.
The derivation of AEGL-2 values was based on developmental toxic effects in animals. The available data have been reviewed by US-EPA (2001) and NTP-CEHRH (2003) and the developmental toxic effects in rodents were considered relevant for humans.
In mice, repeated 7-hour/day exposures during gestational days 6 to 15 caused a dose-related, significant increase in cervical ribs at 2000 ppm or higher; other malformations, such as exencephaly and cleft palate occurred concentration-dependently at 5000 ppm or higher (Rogers et al., 1993). The same type of malformations was found after a single 7-hour exposure at 10000 ppm (no other concentrations tested) (Rogers et al., 1997). In another study, which has not been formally published up until know, Rogers and coworkers (Rogers et al. 1995, abstract; Rogers, 1999, personal communication) exposed mice on gestational day 7 to different concentration-time combinations. The most sensitive endpoint was cervical rib induction, which occurred at concentration-time products greater than or equal to 15000 ppm · h, but not at concentration-time products below 15000 ppm · h (i.e. no effects were observed at 2000 ppm for 5 h, 2000 ppm for 7 h or 5000 ppm for 2 h; authors expressed data only as CxT values). Thus, while 2000 ppm for 7 hours was a LOEL in the repeated exposure study (Rogers et al., 1993), it was a NOEL after single exposure. Although the single exposure study had shortcomings in the reporting, it was very consistent with the well-documented repeated exposure study. It was therefore considered adequate to use an exposure at 2000 ppm for 7 hours as a starting point for AEGL-2 derivation.
The corresponding end-of-exposure blood concentration was measured as 487 mg/l (Rogers et al., 1993). There is experimental evidence that developmental toxic effects are caused by methanol itself and not by a metabolite, such as formate (Dorman et al., 1995). It therefore was considered adequate to derived AEGL-2 values on the basis of blood methanol concentrations. The total uncertainty factor was applied to the blood methanol concentration resulting in a value of 48.7 mg/l.

Uncertainty Factors/Rationale:
Total uncertainty factor: 10

Interspecies:	1 - because a sensitive species was used for derivation of AEGL-2 values and because toxicokinetic differences between species were accounted for by using a pharmacokinetic model for calculating exposure concentrations.
Intraspecies:	10 - because no information on developmental toxic effects of methanol on humans is available and because also for other chemicals the variability in susceptibility of humans for developmental toxic effects is not well characterized. Moreover, pregnant women are a subpopulation with a less than optimal folate status and, thus, may be more susceptible to the health effects of methanol

Modifying Factor: Not applicable

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling: Using a total uncertainty factor of 10, a blood methanol concentration of 48.7 mg/l was derived as the basis for calculation of exposure concentrations. Application of the uncertainty factor to the blood methanol concentration was preferred because the calculated exposure concentrations in air stayed better in the concentration range for which the pharmacokinetic model was validated and the effect of methanol metabolism for longer exposure periods was more adequately taken into account. In contrast, first calculating exposure concentrations that would lead to a blood methanol level of 487 mg/l, and then applying a factor of 10 to the derived exposure concentration would result in calculation of extremely high concentrations in the fist step at which metabolic pathways would be saturated. After application of the uncertainty factor, concentrations would be below saturation level which would mean that the end-of-exposure methanol levels would vary for the AEGL-2 exposure concentration-time combinations. Using the pharmacokinetic model of Perkins et al. (1995a), inhalation exposure concentrations were calculated for appropriate time periods that would lead to a blood methanol concentration of 48.7 mg/l at the end of the time period. The calculated exposure concentrations were set as AEGL-2 values.
Data Adequacy: The derived AEGL-2 values are supported by the occupational exposure study of Kawai et al. (1991), in which 8-hour mean concentrations of 3000-5500 ppm in 5 samples and 1000-2000 ppm in another 10 samples were measured and resulted in dimmed vision (the authors suggested that visibility was temporarily reduced by fog in the workroom) and nasal irritation, but not in severe or irreversible toxicity.

ACUTE EXPOSURE GUIDELINES FOR METHANOL (CAS NO. 67-56-1)

AEGL-3 VALUES				
10 minutes	30 minutes	1 hour	4 hours	8 hours
#	15000 ppm [a]	7900 ppm [a]	2500 ppm	1600 ppm

[a] The 1-hour AEGL-3 values are higher than 1/10 of the lower explosive limit (LEL) of methanol in air (LEL = 55,000; 1/10th LEL = 5500 ppm). Therefore, safety considerations against the hazard of explosion must be taken into consideration.
[#] The 10-minute AEGL-3 value of 40,000 ppm is higher than 50% of the lower explosive limit of methanol in air (LEL = 55,000 ppm; 50% of the LEL = 27,500 ppm). Therefore, extreme safety considerations against the hazard of explosion must be taken into account.

Reference: AACT, American Academy of Clinical Toxicology Ad Hoc Committee on the Treatment Guidelines for Methanol Poisoning: D.G. Barceloux, G.R. Bond, E.P. Krezelok, H. Cooper, and J.A. Vale, 2002. American Academy of Clinical Toxicology Practice Guidelines on the Treatment of Methanol Poisoning. *Clinical Toxicology* 40, 415-446

Test Species/Strain/Sex/Number: Humans / (not applicable) / (not applicable)

Exposure Route/Concentrations/Durations: Oral / measured blood methanol concentrations are available, but no reliable information on ingested dose / exact information during which time period the methanol dose was consumed is not available, it was assumed that the time period for ingestion was short (up to a few hours)

Effects: In fatal cases, death occurred 1.5-4 days after intoxication; when admitted to hospital (0.5-2 days after intoxication), subjects usually showed severe signs of intoxication (e.g. coma); for all cases measured blood methanol concentrations and time between measurement and intoxication were reported.

Endpoint/Concentration/Rationale: The minimum lethal oral dose of about 1 g/kg reported in review articles by Buller and Wood (1904) and Röe (1982) was not used as the basis for AEGL derivation because the value was not sufficiently supported by data in these articles. However, the reported minimum lethal oral dose which corresponds to a peak blood methanol level of about 1540 mg/l is supported by information from case studies on intoxication with methanol only (i.e. without concomitant ethanol consumption) (Naraqi et al., 1979; Erlanson et al., 1965; Bennett et al., 1955; Gonda et al., 1978; Meyer et al., 2000). These studies reported measured blood methanol concentrations and time periods between intoxication and measurement. Given the time that elapsed until blood sampling, during which part of the methanol was metabolized, it can be concluded that peak blood methanol concentrations have been above 1000 mg/l in all fatal cases (see Figure 2). Based on the extensive clinical experience with methanol intoxications, the American Academy of Clinical Toxicology (AACT, 2002) published clinical practice guidelines on the treatment of methanol poisoning. According to these guidelines, peak blood methanol concentrations >500 mg/l indicate serious poisoning for which hemodialysis is recommended. Based on the human experience, a peak blood methanol concentration of 500 mg/l was chosen as the basis for AEGL-3 derivation

Uncertainty Factors/Rationale: Total uncertainty factor: 3 Interspecies: not applicable Intraspecies: 3 - because because clinical experience with methanol intoxications is mainly based on cases involving adult men while much less data is available for women, children or elderly persons, and because subpopulations with a less than optimal folate status may be more susceptible to the health effects of methanol
Modifying Factor: Not applicable
Animal to Human Dosimetric Adjustment: Not applicable
Time Scaling: Using a total uncertainty factor of 3, a blood methanol concentration of 167 mg/l was derived as the basis for calculation of exposure concentrations. Application of the uncertainty factor to the blood methanol concentration was preferred because the calculated exposure concentrations in air stayed better in the concentration range for which the pharmacokinetic model was validated and the effect of methanol metabolism for longer exposure periods was more adequately taken into account. In contrast, first calculating exposure concentrations that would lead to a blood methanol level of 500 mg/l and then applying a factor of 3 to the derived exposure concentration would result in calculation of extremely high concentrations in the fist step at which metabolic pathways would be saturated. Using the pharmacokinetic model of Perkins et al. (1995a), inhalation exposure concentrations were calculated for appropriate time periods that would lead to a blood methanol concentration of 167 mg/l at the end of the time period. The calculated exposure concentrations were set as AEGL-3 values.
Data Adequacy: AEGL-3 values were based on studies reporting lethality in humans after oral intoxication. Available studies on lethal effects of inhalation exposure in rodents were not considered appropriate due to the considerable differences between primates (humans and monkeys) and rodent species in the kinetics of methanol metabolism and the mechanisms of methanol toxicity. The derived values are supported by the occupational exposure study of Kawai et al. (1991) (no effects more severe than dimmed vision (the authors suggested that visibility was temporarily reduced by fog in the workroom) and nasal irritation occupational exposure against up to 3000-5500 ppm during an 8-hour work shift) and by studies on monkeys (Andrews et al., 1987) (no toxic effects after repeated exposure to 5000 ppm for 6 hours/day). In teratogenicity studies in mice, no fetal death was found after single or repeated exposure to 5000 ppm for 7 hours (measured blood methanol concentration was 2126 mg/l at the end of exposure) (Rogers et al., 1993; 1995; Rogers, 1999). This blood methanol concentration is about 11-fold higher than the blood methanol concentration of 185 mg/l, which was used to derive AEGL-3 values.