# Methanol

Health-based recommended occupational exposure limit

**Gezondheidsraad** **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *methanol*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U 5712/HS/fs/459-S59
Bijlagen : 1
Datum : 21 januari 2010

Geachte minister,

Graag bied ik u hierbij het advies aan over de beroepsmatige blootstelling aan methanol.

Het maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige advieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Dit advies over methanol is opgesteld door de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport en aan de minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Met vriendelijke groet,

prof. dr. J.A. Knottnerus

Bezoekadres
Parnassusplein 5
2511 VX Den Haag
Telefoon (070) 340 70 04
E-mail: h.stouten@gr.nl

Postadres
Postbus 16052
2500 BB Den Haag
Telefax (070) 340 75 23
www.gr.nl

# Methanol

Health-based recommended occupational exposure limit

Dutch Expert Committee on Occupational Safety
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2010/01OSH, The Hague, January 21, 2010

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research...” (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, Agriculture, Nature & Food Quality, and Education, Culture & Science. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

The Health Council of the Netherlands is a member of the European Science Advisory Network for Health (EuSANH), a network of science advisory bodies in Europe.

The Health Council of the Netherlands is a member of the International Network of Agencies for Health Technology Assessment (INAHTA), an international collaboration of organisations engaged with *health technology assessment*.

This report can be downloaded from www.healthcouncil.nl.

Preferred citation:
Health Council of the Netherlands. Methanol. Health-based recommended occupational exposure limit. The Hague: Health Council of the Netherlands, 2010; publication no. 2010/01OSH.



ISBN: 978-90-5549-781-2

# Contents

# Samenvatting en advieswaarde

## Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in lucht waaraan mensen tijdens hun beroepsuitoefening blootgesteld kunnen worden. Deze advieswaarden vormen vervolgens de basis voor grenswaarden – vast te stellen door de minister – waarmee de gezondheid van werknemers beschermd kan worden.

In dit advies bespreekt de commissie de gevolgen van blootstelling aan methanol en stelt zij een gezondheidskundige advieswaarde vast. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór april 2009 zijn verschenen.

## Fysische en chemische eigenschappen

Methanol (CAS nummer 67-56-1) is bij kamertemperatuur een heldere, kleurloze, vluchtige en ontvlambare vloeistof. Zuiver methanol heeft een mild alcoholische geur.

In 2008 werd wereldwijd ongeveer 50 miljoen ton methanol geproduceerd. Daarvan werd ongeveer 75% gebruikt voor de productie van andere chemische

stoffen zoals formaldehyde, methyl *tert*-methylbutylether, *tert*-amylmethylether en azijnzuur, en ongeveer 10% in brandstoffen.

Methanol wordt van nature in het lichaam gevormd; de concentratie methanol in het bloed van mensen kan variëren van ca. 1 tot ca. 2,5 mg/L.

## Monitoring

De Amerikaanse instanties NIOSH en OSHA en de Duitse DFG hebben methoden beschreven voor het bepalen van de concentratie methanol in de lucht op de werkplek. Deze methoden zijn gebaseerd op gaschromatografische analyse (GC-FID).

Voor de bepaling van methanolconcentraties in bloed en urine zijn onder andere door de DFG methoden beschreven die gebruik maken van GC-FID. Ook bepaling in uitademinglucht is op die manier mogelijk.

## Grenswaarden

De huidige wettelijke grenswaarde voor methanol in de lucht op de werkplek bedraagt in Nederland 260 mg/m$^3$ (200 ppm), als tijdgewogen gemiddelde over 8 uur; deze waarde komt overeen met de IOELV van de Europese Unie. Ook in landen als Denemarken, Duitsland, Engeland, Zweden en de Verenigde Staten geldt een norm of aanbeveling van 200 ppm.

Verder geldt er in Nederland een STEL van 520 mg/m$^3$ (400 ppm), als tijdgewogen gemiddelde over 15 minuten, terwijl in bovengenoemde landen die waarde uiteenloopt van 250 ppm (325-350 mg/m$^3$) in Denemarken, Engeland, Zweden en de Verenigde Staten tot 800 ppm (1080 mg/m$^3$) in Duitsland.

In vrijwel alle landen (inclusief Nederland) is een huidnotatie (H) voor methanol van kracht, die aangeeft dat methanol gemakkelijk door de huid in het lichaam wordt opgenomen.

Voor effecten op het nageslacht is methanol in Nederland geclassificeerd in categorie 2 (*dient te worden beschouwd alsof het bij de mens ontwikkelingseffecten veroorzaakt*). De Duitse MAK-commissie heeft methanol geclassificeerd in zwangerschapsrisicogroep C, wat inhoudt dat er geen prenatale toxiciteit te verwachten is bij naleving van de MAK-waarde.

## Kinetiek en toxisch werkingsmechanisme

Na inademing wordt methanol voor 60-85% via de bovenste luchtwegen opgenomen; verschillen tussen mens en dier zijn hierbij te verwaarlozen. Methanol kan

ook via de huid worden opgenomen. In vrijwilligersproeven is bepaald dat de snelheid van opname van methanol door de huid 8,1 mg per $cm^2$ huid per uur bedraagt.

Uit experimenten en modelmatige berekeningen blijkt dat er voor knaagdieren en primaten een lineaire relatie is tussen methanolconcentraties in de lucht en in het bloed bij blootstelling aan methanolconcentraties tot ongeveer 1600 $mg/m^3$ (1200 ppm) en een blootstellingsduur tot 8 uur, waarbij bij ratten en apen de concentraties in het bloed bij blootstelling aan 266 $mg/m^3$ (200 ppm) op het achtergrondniveau blijven. Bij blootstelling aan concentraties hoger dan ongeveer 1600 $mg/m^3$ (1200 ppm) nemen de methanolconcentraties in het bloed lineair toe bij de mens, terwijl er een niet-lineaire toename zal zijn bij proefdieren (het sterkst bij muizen, het minst sterk bij apen).

Na opname verdeelt methanol zich over het lichaam in verhouding tot het watergehalte van weefsels: meer methanol in relatief waterrijke weefsels, minder in relatief waterarme. Methanol wordt in de lever via formaldehyde (in knaagdieren als rat en muis door catalase, in primaten als mens en aap door alcoholdehydrogenase) en formiaat omgezet in kooldioxide. In knaagdieren is de omzetting van methanol in formiaat de snelheidsbepalende stap, in primaten de omzetting van formiaat in kooldioxide. Dit betekent dat bij blootstelling aan hoge concentraties of doses methanol bij knaagdieren ophoping van methanol en bij primaten van formiaat kan optreden. Bij de mens treedt verzadiging van het enzym dat formiaat omzet in kooldioxide, op bij een orale dosis van ongeveer 210 mg/kg lichaamsgewicht.

Na opname in het lichaam worden slechts kleine hoeveelheden methanol onveranderd uitgescheiden via de longen en de nieren. Verreweg het meeste methanol verlaat het lichaam als kooldioxide via de uitgeademde lucht. De metabolieten formaldehyde en formiaat worden in het algemeen gebonden aan lichaamseigen moleculen of worden opgenomen in gebruikelijke stofwisselingsprocessen. De tijd die nodig is om de concentratie van methanol te doen halveren (de halfwaardetijd), bedraagt bij mensen ongeveer 85 minuten tot 3 uur in bloed en ongeveer 85 minuten in urine en uitademingslucht.

De meest geschikte methode om de hoogte van blootstelling aan methanol in de werksituatie te schatten, is overigens het bepalen van methanolconcentraties in urine.

## Effecten

### Bij mensen

Studies over de acute effecten van methanol in de mens zijn beperkt tot casestudies, waarin personen een niet nader gespecificeerde hoeveelheid methanol hebben ingenomen of ingeademd, vaak in combinatie met andere chemicaliën. Zulke blootstellingen kunnen resulteren in een tijdelijke – aan methanol toegeschreven – depressie van het centrale zenuwstelsel. Na een latentietijd van enkele uren tot twee dagen worden dit effect op het zenuwstelsel vaak gevolgd door metabole acidose, effecten op het gezichtsvermogen en sterfte; al deze 'latere' gevolgen worden toegeschreven aan formiaat. Hoeveelheden methanol van minimaal 300 tot 1000 mg methanol/kg lichaamsgewicht kunnen dodelijk zijn. Sterfte is gerelateerd aan methanolconcentraties in het bloed van 1500-2000 mg/L; effecten op het zenuwstelsel en het gezichtvermogen aan concentraties hoger dan respectievelijk 200 en 500 mg/L.

Gecontroleerde inhalatiestudies waarbij relatief kleine groepen gezonde vrijwilligers werden blootgesteld aan methanolconcentraties van 200 ppm (260 $mg/m^3$) gedurende ten hoogste 6 uur, hebben niet geleid tot relevante effecten op het zenuwstelsel of irritatie Er zijn geen gegevens over sensibilisatie door methanol bij mensen.

Er is vrijwel geen onderzoek beschikbaar naar de nadelige effecten als gevolg van langdurige beroepsmatige blootstelling aan methanol, zoals bijvoorbeeld kanker, verminderde vruchtbaarheid en afwijkingen bij het nageslacht, In een Amerikaans onderzoek veroorzaakte blootstelling aan een kopieervloeistof die ongeveer 99% methanol bevatte, gedurende vermoedelijk zo'n 3 jaar: oogirritatie; duizeligheid; hoofdpijn; misselijkheid en onscherp gezichtsvermogen. De methanolconcentraties die in de nabijheid van de kopieermachines werden gemeten over perioden van 15 minuten, waren in 70% van de metingen hoger dan 1064 $mg/m^3$ (800 ppm).

### Bij dieren

Dierexperimenteel onderzoek geeft aan dat contact met methanol als vloeistof kan leiden tot matige huidirritatie maar niet tot overgevoeligheidsreacties. Concentraties hoger dan 29% veroorzaakten oogirritatie en blijvende oogschade.

In onderzoek waarin ratten en muizen gedurende 2¼ tot 8 uur inhalatoir werden blootgesteld, waren de concentraties die sterfte veroorzaakten bij 50% van

de blootgestelde groep (*median Lethal Dose for 50% of subjects*, $LD_{50}$), respectievelijk 79.000 en 130.340 mg/m$^3$ (59.200 en 98.000 ppm). Bij blootstelling via de huid was de $LD_{50}$ voor konijnen 17.000 mg/kg lichaamsgewicht. Bij ratten veroorzaakte occlusieve blootstelling aan 35.000 mg methanol/kg lichaamsgewicht, in tegenstelling tot een hoeveelheid van 45.000 mg/kg lichaamsgewicht, geen sterfte.

In onderzoek waarin proefdieren langdurig inhalatoir werden blootgesteld, werden geen systemische effecten waargenomen. Het betrof onderzoek: bij apen en ratten blootgesteld aan concentraties tot 6650 mg/m$^3$ (6500 ppm), gedurende 6 uur/dag, 5 dagen/week, 4 weken; bij ratten blootgesteld aan 13.300 mg/m$^3$ (10.000 ppm) gedurende 6 weken (alleen longen onderzocht); en vrouwelijke apen blootgesteld aan concentraties tot 2394 mg/m$^3$ (1800 ppm), 2½ uur/dag, gedurende ongeveer 350 dagen (onder andere tijdens paring en zwangerschap).

De (mogelijke) kankerverwekkende eigenschappen van methanol zijn onderzocht bij ratten en muizen. Na inhalatoire blootstelling aan concentraties tot 1330 mg/m$^3$ (1000 ppm), 19-20 uur/dag, 7 dagen/week, gedurende 18-24 maanden, werd geen toename in het voorkomen van tumoren of andere schadelijke effecten waargenomen.

Er is geen overtuigend bewijs dat methanol genotoxisch is, dat wil zeggen schade toebrengt aan het erfelijk materiaal. Hoofdzakelijk negatief waren de resultaten van *in vitro* testen (uitgevoerd met bacteriën, gist, schimmels, zoogdiercellen) gericht op het opsporen van: mutaties; chromosoomafwijkingen en genetische schade; en primaire schade aan het DNA. Bij onderzoek dat *in vivo* bij muizen werd uitgevoerd, leidde inhalatoire blootstelling niet tot een toename van rode bloedcellen of longcellen met micronuclei of tot chromosoomschade in longcellen. Onderzoek waarbij methanol oraal of via injecties in de buikholte werd toegediend leverde strijdige resultaten op; de testen met hogere doses en herhaalde toediening waren negatief.

Tot slot heeft onderzoek uitgevoerd bij apen, ratten en muizen gegevens opgeleverd over eventuele schade aan de voortplantingsorganen en het nageslacht. Bij vrouwelijke apen die ongeveer 350 dagen (voor en tijdens de paring én tijdens de zwangerschap) gedurende 2½ uur/dag werden blootgesteld aan 2394 mg/m$^3$ methanol (1800 ppm ) werden zowel bij de moederdieren als de nakomelingen geen relevante effecten gevonden. Over de eventuele effecten van methanol op het gedrag van de nakomelingen kon geen definitief oordeel geveld worden. De testen werden bij slechts een gering aantal nakomelingen afgenomen en de afwijkingen die gevonden werden, waren gering en vertoonden grote verschillen tussen de nakomelingen. Foetussen van ratten die tijdens de dracht waren blootgesteld aan 13.300 mg/m$^3$ (10.000 ppm) methanol, hadden een ver-

laagd lichaamsgewicht terwijl bij blootstelling aan 26.600 mg/m$^3$ (20.000 ppm) ook afwijkingen aan skelet en ingewanden werden waargenomen. Ook bij muizenfoetussen veroorzaakte methanol afwijkingen: blootstelling aan 2660 mg/m$^3$ (2000 ppm) leidde tot de aanleg van een extra rib en blootstelling aan 6650 mg/m$^3$ (5000 ppm) tot gespleten gehemelte en schedelafwijkingen (exencefalie). Bij concentraties van 1330 mg/m$^3$ (1000 ppm) werden er bij muizen geen ontwikkelingseffecten gezien; bij ratten zelfs bij concentraties tot 6650 mg/m$^3$ (5000 ppm).

## Evaluatie en advies

Op basis van de beschikbare gegevens over de carcinogeniteit heeft de Subcommissie Classificatie Carcinogene Stoffen van de commissie GBBS geconcludeerd dat methanol niet kan worden geclassificeerd (te vergelijken met EU-categorie 'not classifiable'). De subcommissie is verder van mening dat de resultaten uit genotoxiciteitsonderzoek aangeven dat het niet waarschijnlijk is dat methanol genotoxische eigenschappen heeft.

Verder is de commissie – op grond van gebrek aan genotoxische potentie en negatieve inhalatoire carcinogeniteitsstudies – van mening dat het niet waarschijnlijk is dat methanol kankerverwekkende eigenschappen heeft.

Op basis van de beschikbare gegevens over de effecten op de voortplantingsorganen en het nageslacht heeft de Subcommissie Classificatie Reproductietoxische Stoffen al eerder geadviseerd methanol wat 'effecten op de ontwikkeling' betreft, te classificeren in categorie 2 (*stoffen die dienen te worden beschouwd alsof zij bij de mens ontwikkelingsstoornissen veroorzaken*).

De commissie is van mening dat de methanolconcentraties die in het bloed van ratten en muizen gemeten zijn bij de blootstellingsconcentraties die geen (respectievelijk 1000-2170 en circa 100 mg/L) en wel een effect (respectievelijk 1840-2240 en circa 540 mg/L) veroorzaken, dermate hoog zijn dat het niet waarschijnlijk is dat beroepsmatige blootstelling aan methanol leidt tot effecten op het nageslacht.

Om een gezondheidskundige advieswaarde af te kunnen leiden ontbreken geschikte gegevens over de gevolgen van langdurige beroepsmatige blootstelling aan methanol. De commissie neemt daarom de chronische inhalatiestudies met ratten en muizen als uitgangspunt. In deze studies veroorzaakte blootstelling aan methanolconcentraties van 1330 mg/m$^3$, 19-20 uur/dag, 7 dagen/week, gedu-

rende 18-24 maanden, geen schadelijke effecten. De commissie beschouwt de concentratie van 1330 mg/m$^3$ als de *no-observed-adverse-effect level* (NOAEL).

Uitgaande van de NOAEL van 1330 mg/m$^3$ en een onzekerheidsfactor van 10 (voor inter- en intraspeciesverschillen) beveelt de commissie voor methanol een gezondheidskundige limietwaarde aan van 133 mg/m$^3$ (100 ppm). De commissie merkt daarbij op dat in bovengenoemde studies geen concentraties zijn getest die hoger waren dan 1330 mg/m$^3$ en dat de blootstelling vrijwel continu was, zonder blootstellingsvrije herstelperiode; uitgaan van deze studies betekent dus een extra veiligheidsmarge.

De commissie is van mening dat de gezondheidskundige advieswaarde van 133 mg/m$^3$ (100 ppm), gemiddeld over een 8-urige werkdag, werkers ook tegen nadelige effecten op het zenuwstelsel beschermt.

Omdat opname via de huid aanzienlijk kan bijdragen aan de lichaamsbelasting, is de commissie GBBS van mening dat een huidnotatie moet worden toegekend.

## Gezondheidskundige advieswaarde

De Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen van de Gezondheidsraad stelt voor beroepsmatige blootstelling aan methanol een gezondheidskundige advieswaarde voor van 133 mg/m$^3$ (100 ppm), gemiddeld over een achturige werkdag. Ook adviseert zij een huidnotatie.

# Executive summary

## Scope

At request of the Minister of Social Affairs and Employment, the Dutch Expert Committee on Occupational Exposure Safety (DECOS), a committee of the Health Council, proposes health-based recommended occupational exposure limits for chemical substances in the air at the workplace. These recommendations serve as a basis in setting legally binding occupational exposure limits by the minister.

In this report, the committee discusses the consequences of occupational exposure to methanol and recommends a health-based occupational exposure limit. The committee's conclusions are based on scientific papers published prior to April 2009.

## Physical and chemical properties

At room temperature, methanol (CAS number 67-56-1) is a clear, colourless, volatile, flammable liquid. When pure, it has a mild alcoholic odour.

In 2008, global production of methanol amounted to ca. 50 million tonnes/year. About 75% served as a feedstock for the manufacture of chemicals such as formaldehyde, methyl tertiary butyl ether, tertiary amyl methyl ether, and acetic acid (12%), and about 10% is used for fuel applications.

Methanol occurs naturally in the human body; endogenous blood methanol concentrations can range from about 1-2.5 mg/L.

## Monitoring

The US National Institute of Occupational Safety and Health (NIOSH) and Occupational Safety and Health Administration (OSHA) and the Working Group on Analytical Chemistry of the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) of the German Research Foundation (Deutsche Forschungsgemeinschaft - DFG) described methods to determine concentrations of methanol in occupational air. These methods are based on gas chromatographic analysis with flame ionisation detection (GC-FID).

For the determination of methanol in urine and blood, GC-FID methods are described, amongst others, by the Working Group Analytical Chemistry of the MAK Commission of the DFG. This technique enables also the determination of methanol in exhaled breath.

## Limit values

In the Netherlands, the current, legally binding, occupational exposure limit (OEL) for methanol is 260 mg/m$^3$ (200 ppm), as an 8-hour time-weighted average. This value is consistent with the Indicative Occupational Exposure Limit Value (IOELV) of the European Union. Also in countries such as Denmark, Germany, Sweden, the UK, and the USA, a limit value of 200 ppm (260 mg/m$^3$) is set or recommended.

In addition, in the Netherlands, there is a 15-minute Short-Term Exposure Limit (STEL) of 520 mg/m$^3$ (400 ppm), while in the afore-mentioned countries, the STEL ranges from 250 ppm (325-350 mg/m$^3$) in Denmark, Sweden, the UK, and the USA to 800 ppm (1080 mg/m$^3$) in Germany.

In almost every country, including the Netherlands, a skin notation (H) has been designated.

With respect to reproduction toxic effects, methanol is classified in the Netherlands in category 2 (‘substances which should be regarded as if the cause developmental toxicity in humans’). The German MAK-committee has classified methanol in Pregnancy risk group C (‘there is no reason to fear damage to the embryo or foetus when MAK an BAT values are observed’).

## Kinetics and toxic mechanism of action

Inhaled methanol is absorbed for 60-85% through the, especially upper, respiratory tract, with not much difference between humans and animals. After dermal exposure, methanol rapidly diffuses through the skin. In a human volunteer study, a dermal penetration rate of 8.1 mg/cm$^2$/h has been established.

Results from toxicokinetic experiments and modeling indicate that blood methanol levels in rodents and primates increase linearly at 8-hour exposures up to ca. 1600 mg/m$^3$ (1200 ppm), At higher levels, blood methanol levels increase linearly in humans, but non-linearly in laboratory animals (most sharply in mice, less sharply in monkeys). At exposures to ca. 266 mg/m$^3$ (200 ppm), levels in rats and monkeys do not exceed endogenous levels.

Methanol distributes through the body uniformly to body water content. In the liver, methanol is metabolised through formaldehyde (in rodents by catalase; in primates by alcohol dehydrogenase) and formate to carbon dioxide. In rodents, the rate-limiting step in the metabolism of methanol is the oxidation of methanol to formate, while the oxidation of formate to carbon dioxide is rate limiting in primates. As a consequence, exposure to high concentrations or doses of methanol may cause accumulation of methanol in rodents and of formate in primates. In humans, accumulation of formate may occur at methanol doses >210 mg/kg bw.

By far the most of the methanol taken up and distributed is excreted as carbon dioxide and only minor amounts unchanged by the lungs and the kidneys. The methanol metabolites formaldehyde and formate are thought to bind to various endogenous molecules or enter a number of endogenous synthetic pathways. In humans, elimination half-lives of methanol in blood were 1.4-3 h and in urine and breath ca. 1.4 h.

The most suitable biological parameter for biological monitoring of persons exposed to methanol is the methanol concentration in urine.

## Effects

### Human data

Studies addressing acute effects of methanol in humans are limited to case reports in which subjects have ingested or inhaled unspecified high levels of methanol, often in combination with other chemicals. Such exposures can result in a transient depression of the central nervous system (CNS), which is thought

to be a direct effect of methanol. After a latent period of several hours to two days, the CNS depression is often followed by metabolic acidosis, ocular toxicity (blindness), and mortality, which are formate effects. Minimal lethal oral doses are between 300 and 1000 mg/kg bw. Fatalities were reported at blood methanol levels of 1500-2000 mg/L, and CNS and ocular effects at levels above 200 and 500 mg/L, respectively.

In a number of controlled inhalation studies in which relatively small groups of healthy human volunteers were exposed at concentrations not exceeding 200 ppm (266 mg/m$^3$), for 75 to 240 minutes, no irritation or relevant neurophysiological or neurobehavioural effects were observed. No data on sensitising properties of methanol in humans were available.

There were hardly any epidemiological studies addressing occupational chronic exposure to methanol and adverse effects, including carcinogenicity and reproduction toxicity. In one US study, exposure for presumably about 3 years to a duplicator fluid containing 99% methanol at 15-minute average concentrations ranging from 365 to 3080 ppm (485-4096 mg/m$^3$), with 70% exceeding 800 ppm (1064 mg/m$^3$), induced eye irritation, dizziness, headaches, blurred vision, and nausea.

### Animal data

Laboratory animal studies indicated that liquid methanol may cause moderate skin irritation, but no skin sensitisation. Concentrations >29% were irritating and corrosive to the eyes.

For rats and mice, $LC_{50}$ values ranged between 79,000 and 130,340 mg/m$^3$ (59,200 and 98,000 ppm) (exposure duration: 2.25 to 8 hours). The dermal $LD_{50}$ in rabbits was 17,000 mg/kg bw. In rats, no mortality was observed after occlusive application of 35,000 mg/kg bw; amounts of 45,000 mg/kg bw were lethal.

In repeated-dose toxicity studies, no systemic effects were observed in male and female monkeys at exposure to concentrations of 665 to 6650 mg/m$^3$ (500-5000 ppm), 6 hours/day, 5 days/week, for 4 weeks, and in female monkeys to concentrations of 266-2394 mg/m$^3$ (200-1800 ppm), 2.5 hours/day, for ca. 350 days (i.e., during pre-mating, mating, and gestation), or in rats exposed to concentrations of 665 to 6650 mg/m$^3$ (500-5000 ppm), 6 hours/day, 5 days/week, for 4 weeks, or to 13,300 mg/m$^3$ (10,000 ppm) for 6 weeks (only lungs examined).

Well-performed inhalation studies in which rats and mice were exposed to concentrations of 13 to 1330 mg/m$^3$ (10-1000 ppm), 19-20 hours/day, for 18-24 months, did not show evidence of neoplastic or non-neoplastic effects of methanol.

There is no convincing evidence that methanol is a genotoxic compound. The majority of mutagenicity tests in bacteria, yeasts, and mammalian cells, assays on chromosome aberrations and SCEs in mammalian cells, micronucleus tests in mammalian cells, and other tests on DNA or chromosome damage in bacteria and fungi were negative. *In vivo*, exposure by inhalation did not increase the frequency of micronuclei in peripheral blood or lung cells or of SCEs or chromosomal aberrations in lung cells of mice. Both positive and negative results were observed in tests in which methanol was orally or intraperitoneally administered to mice, but tests with higher doses and repeated dosing were negative. A mutation test in fruit flies (*D. melanogaster*) was negative. All four cell transformation assays were negative.

Reproduction toxicity studies were performed with monkeys, rats, and mice. No effects were seen in female monkeys exposed to methanol concentrations of 266-2394 mg/m$^3$ (200-1800 ppm), 2.5 hours/day, during pre-mating, mating, and gestation (ca. 350 days in total), and their offspring. A conclusive judgment of the results of the neurobehavioural tests performed in the monkey offspring was hampered because the changes were small, occurred in the presence of large variations among offspring, and were assessed in a low number of offspring. Exposure of pregnant rats to methanol concentrations of 13,300 and 26,600 mg/m$^3$ (10,000 and 20,000 ppm), 7 hours/day, on gestational days 1-19 and 7-15, respectively, caused decreased fetal weights and at 26,600 mg/m$^3$ also increased incidences of skeletal and visceral malformations. Also in mice, methanol induced developmental effects. Exposure to 2660 mg/m$^3$ (2000 ppm), 7 hours/day, on gestational days 6 through 15, caused increased incidences of cervical ribs at concentrations of 2660 mg/m$^2$ (2000 ppm) and above and of cleft palate and exencephaly at concentrations of 6650 mg/m$^3$ (5000 ppm) and above. The NOAELs for developmental effects were 6650 and 1330 mg/m$^3$ (5000, 1000 ppm) in rats and mice, respectively. No maternal toxicity was observed in the rodent studies.

## Evaluation and recommendation

From the carcinogenicity data, the Subcommittee on the Classification of Carcinogenic Substances of DECOS concludes that methanol cannot be classified with respect to its carcinogenicity (comparable with EU class ‘not classifiable’). The subcommittee is further of the opinion that results from genotoxicity tests indicate that methanol is not likely to have a genotoxic potential.

Based on the lack of a genotoxic potential and negative results in inhalation carcinogenicity studies, DECOS is of the opinion that methanol is not likely to have a carcinogenic potential.

Based on the available data on reproduction toxic effects, the Subcommittee on the Classification of Reproduction Toxic Substances recommended classification of methanol in category 2 ('substances which should be regarded as if they cause developmental toxicity in humans').

Based on the methanol levels measured in the blood of mice and rats at the NOAELs (ca. 100 and 1000-2170 mg/L, respectively) and LOAELs (ca. 540 and 1840-2240 mg/L, respectively) of the reproduction toxicity studies, DECOS is of the opinion that methanol is not likely to induce reproduction toxic effects in occupationally exposed workers.

Since there were no human studies which allow to assess health effects following chronic exposure to methanol, the committee takes the chronic inhalation studies in rats and mice as starting points for deriving a health-based recommended occupational exposure limit (HBROEL). In these studies, no neoplastic or non-neoplastic effects were seen at exposure to methanol concentrations up to 1330 $mg/m^3$ (1000 ppm), 19-20 hours/day, 7 days/week, for 18-24 months.

Taking the NOAEL of 1330 $mg/m^3$ and applying an assessment factor of 10 for interspecies and intraspecies variation, the committee recommends a health-based occupational exposure limit of 133 $mg/m^3$ (100 ppm) for methanol. Moreover, the committee notes that the NOAEL was the highest concentration level tested and that exposure was almost continuous, and concludes that these aspects provide an additional margin of safety.

The committee is of the opinion that the HBROEL of 133 $mg/m^3$ (100 ppm) will also protect workers from acute neurotoxic/CNS effects.

Since dermal penetration may contribute significantly to the body burden, DECOS considers a skin notation warranted.

### Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Safety of the Health Council recommends a health-based occupational exposure limit for methanol of 133 $mg/m^3$ (100 ppm), as an 8-hour time-weighted average. DECOS also recommends a skin notation.

Chapter 1

# Scope

## 1.1 Background

At request of the Minister of Social Affairs and Employment (Annex A), the Dutch Expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, performs scientific evaluations on the toxicity of existing substances that are used at the workplace. The purpose of the evaluations is to recommend a health-based occupational exposure limit for concentrations in the air, provided the database allows derivation of such a value. In the Netherlands, these recommendations serve as a basis in setting public occupational exposure limits by the minister.

## 1.2 Committee and procedure

This document contains the assessment of DECOS, hereafter called the committee, of the health hazard of methanol. The members of the committee are listed in Annex B.

In 2009, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex C. The committee has taken these comments into account in deciding on the final version of the report.

## 1.3 Data

The committee's recommendations on the health-based occupational exposure limit of methanol have been based on publicly available scientific data. Except for the sections on carcinogenicity, genotoxicity, and reproduction toxicity, the evaluation of the toxicity of methanol builds on the review by the International Programme on Chemical Safety (IPCS), a joint venture of the United Nations Environmental Program (UNEP), the International Labour Organisation (ILO), and the World Health Organization (WHO), published in 1997 in the Environmental Health Criteria (EHC) series.[1] With respect to the data on carcinogenicity and genotoxicity, the original publications were reviewed and evaluated by DECOS's Subcommittee on the Classification of Carcinogenic Substances. The data on reproduction toxicity were taken from the evaluation of the effects of methanol on reproduction and the recommendation for classification by DECOS' Subcommittee on the Classification of Reproduction Toxic Substances, published in 2006.[2]

Additional data were obtained from the on-line databases Medline, Toxline, Toxcenter and Chemical Abstracts (1996 to March 2007), using methanol and CAS number. 67-56-1 and words relating to inhalation exposure as key words, published after 1996. This resulted in a database containing more than 400 references. From this database, relevant papers were selected based on title, keywords, and abstract (when available).

The final search, in Medline (PubMed) was performed on April 7, 2009.

In the sections below, first, data from the WHO/IPCS review are summarised under 'WHO/IPCS data' and the studies to which it is referred are listed in Annex D ('WHO/IPCS references'). Additional information is subsequently presented under 'additional data'.

Chapter 2

# Identity, properties and monitoring

## 2.1 Chemical identity

| | | |
|---|---|---|
| name | : | methanol (ISO)<br>methanol (CAS)<br>methanol (IUPAC) |
| synonyms | : | methyl alcohol, methyl hydroxide, methylol, carbinol, hydroxymethane, monohydroxymethane, wood alcohol, wood spirits, wood naphtha, Columbian spirits, Manhattan spirits, colonial spirit, pyroxylic spirit |
| molecular formula | : | $CH_4O$ |
| structural formula | : |  |
| CAS registry number | : | 67-56-1 |
| EINECS number | : | 200-659-6 |
| EC number | : | 603-001-00-X |
| RTECS number | : | PC 1400000 |

## 2.2 Physical and chemical properties

Methanol is a colourless, volatile, flammable liquid with a mild alcoholic odour when pure. However, the crude product may have a repulsive pungent odour. Methanol is miscible with water, alcohols, esters, ketones, and most other sol-

vents, and forms many azeotropic mixtures. It is only slightly soluble in fats and oils.[1]

Methanol undergoes reactions that are typical of alcohols as a chemical class. The reactions of particular industrial importance include the following: dehydrogenation and oxidative dehydrogenation over silver or molybdenum-iron oxide to form formaldehyde; the acid-catalysed reaction with isobutylene to form tert-butyl methyl ether (MTBE); carbonylation to acetic acid catalysed by cobalt or rhodium; esterification with organic acids and acid derivatives; etherification; addition to unsaturated bonds and replacement of the hydroxyl group.[1]

Physical and chemical properties of methanol are listed in Table 2.1.

*Table 2.1* Physical constants and properties of methanol (data from [1,3-8]).

| | |
|---|---|
| molecular weight | 32.04 |
| melting point | -97.5°C |
| boiling point | 65°C |
| relative density | 0.79 g/cm$^3$ (20°C/4°C) |
| solubility | |
| in water | miscible |
| in ethanol | miscible |
| in ether | miscible |
| in benzene | very soluble |
| in chloroform | soluble |
| Log $P_{octanol/water}$ | -0.82 to -0.68 (experimental); -0.64, -0.63 (estimated) |
| vapour pressure at 20°C | 12.8 kPa |
| relative vapour density | 1.01 |
| flash point | |
| closed cup | 10-12°C |
| open cup | 15.6°C |
| odour threshold | 133 mg/m$^3$ (100 ppm); 13-26,840 mg/m$^3$ (10-20,000 ppm) |
| conversion factors at 20°C, 101.3 kPa | 1 mg/m$^3$ = 0.75 ppm; 1 ppm = 1.33 mg/m$^3$ |
| auto-ignition temperature | 455-470°C |
| explosive limits in air (% by volume) | 6-44% |

## 2.3 EU Classification and labelling

The classification of methanol based on Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP) entered into force on the 20 January 2009, implementing the Globally Harmonised System (GHS), and replacing Directive 67/548/EEC (substances) and Directive 1999/45/EC (preparations)[9] is presented in Table 2.2.

*Table 2.2* EU classification and labelling of methanol.[9]

| classification | | labelling | | specific concentration limits, m-factors |
|---|---|---|---|---|
| hazard class and category code | hazard statement code | pictogram, signal word code | hazard statement code | |
| Flam. Liq. 2 | H225 | GHS02 | H225 | |
| Acute tox. 3 | H331 | GHS06 | H331 | STOT SE 1; |
| Acute tox. 3 | H311 | GHS08 | H311 | H370: C≥10% |
| Acute tox. 3 | H301 | Dgr | H301 | STOT SE 2; |
| STOT SE 1 | H370 | | H370 | H371: 3%≤C≥10% |

H225: highly flammable liquid and vapour
H301: toxic if swallowed
H311: toxic in contact with skin
H331: toxic if inhaled
H370: causes damage to organs
H371: may cause damage to organs

## 2.4 Analytical methods

In this section, well-established, standard methods for detecting and/or measuring and monitoring methanol in occupational air and in biological samples are described.

### *2.4.1 Occupational air samples*

#### WHO/IPCS data

The measurement of methanol in workplace (and ambient) air usually involves a pre-concentration step in which the sample is passed through a solid adsorbent containing silica gel, Tenax GC, Porapak, or activated charcoal, liquid desorption, and gas chromatography with flame ionisation detection (FID) or mass spectrometry (MS). The US National Institute for Occupational Safety and Health (NIOSH) has published methods based on these principles (see below). In addition, methanol can be measured by direct reading instruments, real-time continuous monitoring systems, and passive dosimeters.

#### Additional data

The following methods concerned the determination of methanol in occupational air:

- NIOSH Method 2000. In 1998, NIOSH published an improved version of Method 2000, which had combined and replaced Method S59 and P&CAM.

The method uses a pre-concentration step in which the sample is passed through a solid sorbent tube containing silica gel, liquid (5% solution of 2-propanol in water) desorption, and gas chromatography using flame ionisation detection. The working range is 0.3 to 916 ppm (0.4 to 1200 mg/m$^3$) for a 5-L air sample. The estimated limit of detection is 0.7 µg per sample. At high concentrations of methanol or at high relative humidity, a large silica gel tube is required (700 mg silica gel front section).[10]

- OSHA Method 91. The US Occupational Safety and Health Administration (OSHA) procedure involves sample collection by drawing air through two Anasorb 747 sampling tubes which are connected in series, liquid (carbon disulphide/dimethyl formamide solution) desorption, and gas chromatography with flame ionisation detection. The recommended air volume and sampling rates are 5 L at 0.05 L/min when relative humidity is more than 50% at 25$^o$C and 3 L at 0.05 L/min when relative humidity is less than 50% (at 25$^o$C). The detection limit of the overall procedure is 0.93 µg/sample (186 µg/m$^3$).[11]
- DFG. The Working Group on Analytical Chemistry of the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) of the German Research Foundation (Deutsche Forschungsgemeinschaft - DFG) has developed a method which permits the determination of volatile alcohols (viz., methanol, ethanol, 2-propanol, n-butanol) and 2-butanone in workplace air by adsorption on silica gel, liquid (deionised water), and analysis by head-space gas chromatography with flame ionisation detection. The method was validated with air concentrations equivalent to the German occupational exposure limit of methanol of 270 mg/m$^3$, and could accurately determine 6% of this limit when the given sampling conditions are observed.[12]

### *2.4.2 Biological samples*

#### WHO/IPCS data

A variety of primarily gas chromatographic methods for the determination of methanol in biological samples from normal, poisoned, and occupationally exposed individuals have been described. In these methods, methanol concentrations were measured in exhaled breath, blood, and urine samples.

## Additional data

The Working Group Analytical Chemistry of the MAK Commission of the DFG has published a method for the determination of methanol in urine and blood. Methanol is determined by means of capillary gas chromatography using the headspace technique and a flame ionisation detector (FID).[12]

In its documentation of a Biological Exposure Index (BEI) for methanol, the American Conference of Governmental Industrial Hygienists (ACGIH) refers to a number of gas chromatographic methods for the determination of methanol in urine. These methods, of which some are described in the EHC monograph, include gas chromatography using the headspace technique, flame ionisation detection, and/or direct injection (see e.g.,[12-15]).

Chapter 3

# Sources

## 3.1 Natural occurrence

### WHO/IPCS data

Methanol occurs naturally in humans, animals, and plants. It is a natural constituent in blood, urine, saliva, and expired air, and has also been found in mother's milk. The human diet is a source of the body pool of methanol, as it occurs in fresh fruits and vegetables, fruit juices, and fermented beverages. In addition, it has been suggested that methanol is formed by the activities of the intestinal microflora or by other enzymatic processes.

Natural emission sources of methanol include volcanic gasses, vegetation, microbes, and insects. Methanol is also formed during biological decomposition of biological wastes, sewage, and sludge.

## 3.2 Man-made sources

### *3.2.1 Production*

### WHO/IPCS data

In the 19th century, methanol was mainly produced by dry distillation of wood at about 350 °C. Modern industrial scale methanol production is based exclusively on the catalytic conversion of pressurized synthesis gas (hydrogen, carbon monoxide and carbon dioxide) in the presence of metallic heterogeneous catalysts. Starting materials for synthesis gas production are carbonaceous materials

such as coal, coke, natural gas, petroleum, and fractions obtained from petroleum. About 90% of global methanol production capacity is based on natural gas as the starting material.

Worldwide production capacity of methanol in 1995 was 30.1 million tonnes per year. The largest production takes place in the USA and Canada. The production capacity of methanol in Western Europe between 1978 and 1991 ranged from 2.5 to 3.45 million tonnes per year, the major producers being Germany, the Netherlands and the United Kingdom. It is assumed that about 80% of production capacity is utilized. A shift in methanol production from the developed countries to the developing areas appears to take place.

### Additional data

In the Netherlands, BioMCN has developed an innovative process to produce 'bio-methanol' from crude glycerine. Crude glycerine is a by-product formed during the manufacture of biodiesel. The feasibility of the glycerine-to-methanol process was demonstrated on a pilot plant scale in early 2008 at a former conventional methanol plant site in Delfzijl. In 2009, a newly built larger unit should have a capacity of 200,000 tonnes/year, which can be extended with another three such units, adding up eventually to a capacity of 800,000 tonnes/year.[16]

The Methanol Institute reported that the Caribbean, Persian Gulf, and Asia (China, Taiwan, Japan, South Korea) were the largest methanol-producing regions in 2006 with a production of each 7-8 million tonnes/year. Western-Europe produced about 3.3 million tonnes/year. Global production amounted to ca. 40 million tonnes/year, which is approximately 90% of the total capacity. For 2008, figures for production and capacity were ca. 48 and 61 million tonnes, respectively.[17]

## *3.2.2 Use*

### WHO/IPCS data

Methanol is used in the industrial production of many important organic compounds, such as methyl tertiary butyl ether (MTBE), formaldehyde, acetic acid, glycol methyl ethers, methylamine, methyl halides, and methyl methacrylate. In addition, methanol is a constituent of a large number of commercially available solvents and consumer products including paints, varnishes, paint thinners, cleansing solutions, antifreeze solutions, automotive windshield washer fluids, denaturant for ethanol, and in hobby adhesives. Potentially large use of methanol is directly in fuel, as a replacement for gasoline in gasoline and diesel blends.

## Additional data

Figures presented for 2008 by the Methanol Institute indicate that about 75% of produced methanol serves as a feedstock for chemical synthesis. Manufacture of formaldehyde is the primary use (35%) followed by production of fuel additives (methyl tertiary butyl ether and tertiary amyl methyl ether; 13%) and acetic acid (12%). About 10% is used for fuel applications. More recently developed industrial uses of methanol include its application as a denitrification agent in waste water treatment and as a reagent and solvent in biodiesel production facilities. New applications may be in fuel cells for vehicles and consumer electronic products.[17]

Chapter 4

# Exposure

## 4.1 General population

### WHO/IPCS data

Humans can be exposed to methanol from ambient air, diet, and possibly water. Methanol concentrations in ambient air in different urban air or at dense traffic sites in the US and in Sweden ranged from 0.45 to 100 ppb (0.6 to 133 µg/m$^3$) (Graedel *et al.*, 1986; Jonsson *et al.*, 1985; Snider and Dawson, 1985), whereas ambient air concentrations in remote or rural areas in the USA ranged from 0.5 to 2.6 ppb (0.7 to 3.5 µg/m$^3$) (Cavanaugh *et al.*, 1969; Snider and Dawson, 1985).

Potential methanol exposure levels due to its use in automobile fuel have been modelled for specific conditions of use such as express ways, street canyons, railroad tunnels, and parking garages, assuming 100% of all automobiles fuelled with methanol, under various traffic conditions and meteorological conditions. For these scenarios, exposure levels to methanol ranged from 0.75 to 150 ppm (1 to 200 mg/m$^3$). For personal garages, calculated methanol exposure concentrations ranged from 2.2 to 368 ppm (2.9-490 mg/m$^3$) (Gold and Moulif, 1988; Kavett and Nauss, 1990). Methanol is also present in tobacco smoke. Levels of 180 µg/cigarette have been detected in the vapour phase of mainstream smoke (Guerin *et al.*, 1987; Norman, 1977).

Methanol is available from the intake of dietary fruits and vegetables, fruit juices and fermentation beverages, and from the use of the synthetic sweetener aspartame which on hydrolysis yields 10% of its weight as free methanol, which is available for absorption. Methanol levels in some foods and beverages are summarised in Table 4.1.

*Table 4.1* Methanol levels in some foods and beverages (data from WHO/IPCS[1]).

| sample | methanol level |
|---|---|
| fresh and canned fruit juices | 1-43 mg/L |
| (orange and grapefruit juices) | 10-80 mg/L<br>12-640 mg/L (average: 140 mg/L) |
| neutral spirits | <1500 mg/L |
| beer | 6-27 mg/L |
| wines | 96-321 mg/L |
| distilled spirits | 10-220 mg/L |
| bourbon | 40-55 mg/L |
| 50% grain alcohol | ca. 1 mg/L |
| concentrations permitted in brandies in the USA, Canada, Italy | 6000-7000 mg/L ethanol |
| carbonated beverages | ca. 56 mg/L (originating from aspartame) |
| beans | 1.5-7.9 mg/kg |
| split peas | 3.6 mg/kg |
| lentils | 4.4 mg/kg |

The intake estimates of methanol from these sources vary considerably. Consuming a 12-oz (ca. 350 mL) diet beverage containing aspartame results in a methanol intake of roughly 0.3 mg/kg bw. Excluding exposure from these kind of beverages, daily methanol intake from aspartame can average 0.3-1.1 mg/kg bw (99 percentile: up to 3.4 mg/kg bw). If aspartame would replace all sucrose in the diet, average daily equivalent methanol ingestion could be ca. 0.8 mg/kg bw. The 'background' body burden of methanol was estimated to be 0.5 mg/kg bw (Kavett and Nauss, 1990).

Limited data on methanol in water report levels of 22 µg/L in rainwater (Snider and Dawson, 1985) and levels ranging from 17 to 1050 mg/L in industrial effluents (Jungclaus *et al.*, 1978) in the USA. Levels in finished drinking water were not reported.

### Additional data

Inhalation exposure concentrations as a result from using methanol as an automotive fuel were measured at different locations in a residential home with an attached garage in New Jersey, USA. Measurements (vehicle emission control devices, ventilation, temperatures of ambient air, garage and fuel tank, wind speed) were taken under various conditions, during a 3-hour sampling time. The highest methanol exposure concentration was measured in the garage (1.3 ppm (1.7 mg/m$^3$)), when the charcoal canister hose connection (emission control device) was removed. Under these conditions, maximum methanol concentrations in the room adjacent to the garage and in the remainder of the home were 0.23 ppm (0.31 mg/m$^3$) and 0.11 ppm (0.15 mg/m$^3$), respectively.[18]

An additional source of exposure for the general public forms the use of consumer products containing methanol, such as varnishes, adhesives, and windshield washing fluid. One study investigated the exposure of automobile occupants to methanol through inhalation of vapours of windshield washing fluid. Winter-grade windshield washing fluids containing levels of methanol in concentrations in the range of 44 to 46% were tested in two different cars, under various normal operating conditions. Air samples were collected at 1-minute time intervals and analysed for methanol. Concentrations of methanol ranging from 1.4 ppm (1.9 mg/m$^3$) to 1435 ppm (1909 mg/m$^3$) were recorded, with peak concentrations occurring when the car engine was hot, the heater was on, and ventilation was off.[19]

A dietary source of methanol is aspartame. In the Netherlands, the legislative value for aspartame in soft drinks, fruit juices, concentrates, yoghurt drinks, and drinking chocolates is 600 mg/kg (equivalent to 60 mg methanol/kg). Actual mean levels in products present on the Dutch market ranged from ca. 60 to 145 mg/kg (≈6-15 mg methanol/kg) with maximum levels up to ca. 460 mg/kg (≈46 mg methanol/kg). For yoghurt and throat pastilles, legislative values are 1000 mg/kg (≈100 mg methanol/kg). For these products, actual mean levels were ca. 60 and 100 mg/kg, respectively (≈6, 10 mg methanol/kg) with maximum levels of ca. 200 and 760 mg/kg (≈20-76 mg methanol/kg). For vitamine preparations, the legislative level is 5500 mg/kg (≈550 mg methanol/kg). Actual levels were ca. 6400 mg/kg (≈640 mg methanol/kg) with a maximum of ca. 17,000 mg/kg (≈1700 mg methanol/kg). The overall average daily intake of aspartame of the general population was estimated to be 0.1 mg/kg bw (≈0.01 mg methanol/kg) (95 percentile: 0.5 mg/kg bw; 0.05 mg methanol/kg).[20]

Another dietary source of methanol is dimethyl dicarbonate, which is used as a cold sterilisation agent for tea beverages, sports drinks, fruit or juice sparklers, wines, and wine substitutes. It is unstable in aqueous solutions and primarily breaks down to methanol and carbon dioxide. On a weight basis, 100 mg of dimethyl dicarbonate in a beverage would produce 48 mg methanol. Dietary daily intake of methanol through dimethyl dicarbonate was estimated to be 0.2 mg/kg bw (90% percentile).[21]

## 4.2 Working population

### WHO/IPCS data

At the workplace, duplicator machines and solvent classes such as thinners, degreasers, paints, inks, and adhesives form a source of exposure to methanol. In a chemical plant, 30-minute concentrations ranged from ca. 49 to 303 mg/m$^3$ (37-227 ppm) during the course of a shift (Heinrich and Angerer, 1982). Much higher concentrations were measured in the vicinity of spirit duplicator machines, low-volume copiers using methanol as an 'ink' (475- 4000 mg/m$^3$/356-3000 ppm) (Frederick *et al.* 1984; Kingsley and Hirsch, 1955; NIOSH, 1981), and in a factory producing canned fuel (median: 600 mg/m$^3$ with peaks of 4000-7000 mg/m$^3$/3000-5250 ppm) (Kawai *et al.*, 1991).

### Additional data

The extent of methanol exposure among school workers during spirit duplicator use was evaluated in five schools in North Carolina, USA. Samples were taken in the breathing zone of 48 workers in the duplicating areas, at 11 sampling sites in total. Instantaneous air concentrations were recorded using a gas analyser, every 0.5 to 2 minutes during duplicator use, and were used to calculate 3-minute time-weighted averages. The estimated mean exposure for teachers and teaching assistants were 404±296 ppm (537±394 mg/m$^3$) and 322±248 ppm (428±330 mg/m$^3$), respectively. Most exposure durations lasted between 0.5 and 4.5 minutes, the highest exposure duration was 40.5 to 44.5 minutes. Three measurements of mean inhalation exposure during collating, stapling, and distributing freshly duplicated materials exceeded 200 ppm (266 mg/m$^3$). Dermal exposure to methanol may also take place during handling freshly duplicated materials, but this was not assessed.[22]

The International Agency for Research on Cancer (IARC) set up an international database of exposure measurements of 246 different agents in the pulp, paper, and paper product industries.[23,24] In August 1996, the database included 31,502 measurements from 13 countries, the majority of which were taken from static measuring points, i.e., area samples (61%) and breathing-zone air samples (24%). The measurements varied in duration, purpose, and sample location. Methanol concentrations were measured 301 times, mostly in the pulp production and in the paper product manufacture. In 11% of the measurements, concentrations of methanol exceeded the threshold limit value. Concentrations exceeding 8-hour occupational exposure limits were reported in off-machine

coating areas. Details on the actually measured concentrations were not published, apart from reported measured exposure concentrations in effluent water treatment departments, which were under the detection limit.[23] It is unknown whether this data are representative for the average exposure levels of workers in this industry, due to the lack of background information on the measurements.

The concentration of methanol in wood dust was measured to evaluate whether methanol exposure may play a role in the correlation between the exposure to wood dust and the development of nasal cancer as suggested by epidemiological studies.[25] Concentrations of methanol in wood dust varied from 355 to 452 mg/L, depending on the wood humidity. The authors suggest that *in situ* generation of formaldehyde from methanol plays an important role in the development of nasal cancer. However, the results of this study do not assess the potential methanol exposure concentrations of workers in the wood industry.

Concentrations of chemical compounds including methanol in a petrochemical complex were measured using Open-Path Fourier Transform Infrared Spectroscopy (OP-FTIS) on 11 different manufacturing plants in Taiwan.[26] Continuous and representative monitoring was conducted each year at each plant for three years, with total sample duration of 77 days. Methanol was among the compounds most frequently detected, and was detected at all 11 plants. The mean low estimate for methanol was 23±79 ppb (31±106 µg/m$^3$); the mean high estimate was 24±79 ppb (32±104 µg/m$^3$). The maximum concentration measured was 2449 ppb (3257 µg/m$^3$).

As part of a study investigating the effect of exposure to irritants on the respiratory mucus transportability, methanol concentrations in a foundry from area and personal sampling were measured to range from <10 to 68 mg/m$^3$ (7.5-51 ppm).[27]

Chapter 5

# Kinetics

## 5.1 Absorption

### WHO/IPCS data

The primary routes of methanol exposure are inhalation and ingestion, for both the general and occupational population. No differences exist between the capabilities for absorption of methanol among various animal species.

In an occupational setting, inhalation of methanol is the most common route of entry. Around 60 to 85% of inhaled methanol is absorbed in the lung of humans. A number of studies with human volunteers investigating methanol and/or formic acid (formate) levels in blood and urine after methanol inhalation exposure were reported (see Table 5.1).

One pharmacokinetic model predicted that after exposure to 5000 ppm (6650 mg/m$^3$) methanol vapour, blood methanol concentrations in mice and rats would be 13- to 18-fold and 5-fold higher, respectively, compared to blood concentrations in humans, due to the greater respiration rates of mice and rats (Perkins *et al.*, 1995). Another pharmacokinetic model predicted that at exposure concentrations below 1200 ppm (1596 mg/m$^3$) for 6 hours, the end of exposure blood concentrations of methanol would be similar for Fisher-344 rats, rhesus monkeys, and humans, which would be proportional to atmospheric concentrations. At concentrations above 1200 ppm (1596 mg/m$^3$), the increase of methanol in the blood of rats and monkeys was predicted to become non-linear, whereas for humans blood methanol levels were predicted to increase linearly (Horton *et al.*, 1992).

*Table 5.1* Methanol and formate levels in blood and/or urine of humans exposed by inhalation to methanol (from [1]).

| number of volunteers | exposure conditons | methanol levels | | formate levels | | reference |
|---|---|---|---|---|---|---|
| | | in blood (mg/L ± SD) | in urine (mg/L ± SD) | in blood (mg/L ± SD) | in urine (mg/L ± SD) | |
| not reported | 500-1100 ppm (665-1463 mg/m$^3$), 3-4 h | not reported | ca. 10 to 30 | not reported | not reported | Leaf/Zatman, 1952 |
| 4 | 77, 154, 225 ppm (102, 205, 300 mg/m$^3$), for 8 h | not reported | proportional to concentrations in air | not reported | not reported | Sedivec *et al.* 1981[13] |
| 20 | 37- 231 ppm (49- 307 mg/m$^3$) (arithmetic mean: 111 ppm; 148 mg/m$^3$), for 8 h | increase from <0.6 to 8.9±14.7 | increase from 1.1±0.9 to 21.8±20 | not reported | increase from 12.7± 11.7 to 29.9±28.6 | Heinrich/ Angerer, 1982[14] |
| 6 | 200 ppm (266 mg/m$^3$), for 6 h | increase from 1.8 to 7.0 | not reported | no accumulation above background level (8.1 mg/L) | not reported | Lee *et al.*, 1992 |
| 5 | 200 ppm (266 mg/m$^3$), for 5 days (not further specified) | not reported | not reported | not reported | no accumulation | Franzblau *et al.*, 1993 |
| 26 | 200 ppm (266 mg/m$^3$), for 4 h | not reported | not reported | no increase | no increase | |

Following dermal exposure to methanol in human volunteers, an average skin absorption rate of 0.192 mg/cm$^2$/min has been reported (Dutkiewicz *et al.*, 1980). In another study, the rate of absorption into the skin was found to be higher with M-85 (85% methanol and 15% gasoline) than with pure methanol (Machiele, 1990). In an *in vitro* study, the penetration rate of pure methanol through the epidermis was 10.4 mg/cm$^2$/h (i.e., 0.18 mg/cm$^2$/min) (Scheuplein and Blank, 1971).

Ingestion of methanol has been the principal route of exposure in the many reported cases of acute poisoning. Oral exposure to methanol leads to rapid absorption from the gastrointestinal tract, with peak absorption occurring in 30 to 60 minutes depending on the presence or absence of food in the stomach (Becker, 1983). Blood and urine concentrations of methanol are highly dependent upon dose, time following exposure, and concomitant ingestion of ethanol. Oral doses of 71 to 84 mg methanol/kg in humans resulted in blood levels of 47 to 76 mg/L blood 2 to 3 hours later (Leaf and Zatman, 1952). No methanol in blood was found 48 hours after intake of small quantities of methanol (10-20 mL; i.e., ca. 110-230 mg/kg bw), but after intake of 50 mL (i.e., ca. 560 mg/kg bw) of methanol, levels of 250 to 1200 mg/L were found in the blood (Lund, 1948). The methanol body burden following ingestion of products sweetened with aspartame (typically 60 to 195 mg/serving) could vary from 6 to 20 mg, due to the hydrolysis of aspartame to methanol (Stegink *et al.*, 1981, 1983).

## Additional data

A study investigating the site and characteristics of methanol absorption in rats demonstrated that absorption of methanol from the lungs occurs almost entirely in the upper respiratory tract. The results suggested further that the absorption of methanol from the lungs in rats depended on the inhalation exposure concentration and duration and ventilation rate. Blood methanol concentration did not in itself affect methanol absorption, but ventilation rate decreased with increasing blood methanol concentration. Rats were exposed for 8 hours to 5000 and 15,000 ppm (6650, 16,625, 19,950 mg/m$^3$) normally, and to 12,500 ppm (16625 mg/m$^3$) by a tracheal cannula while being anaesthetised. After being exposed for 4 hours, some of the rats exposed to 5000 ppm received an intravenous injection of methanol of 4 g/kg (over 2 min) to examine the effect of blood methanol on ventilation and absorption; some of the rats exposed to 15,000 ppm were treated with $CO_2$ or phenobarbital to assess the effect of the ventilation rate on methanol absorption.[28]

Since the publication of the WHO/IPCS document, a number of studies in humans have been published to examine kinetics of methanol after inhalation exposure (Table 5.2).

*Table 5.2* Methanol and formate levels in blood and/or urine of humans exposed by inhalation to methanol; additional data.

| number of volunteers | exposure conditions | methanol levels | | formate levels | | reference |
|---|---|---|---|---|---|---|
| | | in blood (mg/L ± SD) | in urine (mg/L ± SD) | in blood (mg/L ± SD) | in urine (mg/L ± SD) | |
| 26 | 200 ppm (266 mg/m$^3$), for 4 h | increased: from 0.9±0.6 to 6.5±2.7 | significantly increased excretion rates | no significant difference: from 12.7±6.4 to 14.3±12.7 | no significant difference | Chuwers *et al.*, 1995; Osterloh *et al.*, 1996 [29,30] |
| 14<br>8 | 0, 100, 200, 400 ppm (0, 133, 266, 532 mg/m$^3$), for 8 h | not reported | significant increase | not reported | modest increase after exposure to 400 ppm (532 mg/m$^3$) | Franzblau *et al.*, 1997[31] |
| 4 (0.5-2 h); 3, 12 (8 h) | 800 ppm (1064 mg/m$^3$), for 0.5, 1, 2, 8 h | increased:<br>0.5 h: from 2.0±0.9 to 5.3±1.4<br>1 h: from1.3±0.6 to 6.6±1.2<br>2 h: from 1.8±0.7 to 14.0±1.5<br>8 h: from 1.8±0.7 to 30.7±6.9 | increased:<br>0.5 h: from 1.1±0.5 to 3.2±1.2<br>1 h: from 1.5±1.2 to 4.0±1.4<br>2 h: from1.2±0.6 to 11.0±3.2<br>8 h: from 2.0±1.7 to 74.0±33.5 | not reported | not reported | Batterman *et al.*, 1998[32] |
| 8 | 100, 200 ppm (133, 266 mg/m$^3$), for 2 h | increased: from 0.64 to 3.72, and from 0.64 to 7.91 | increased: from 0.32 to 2.56, and from 0.64 to 6.41 | not reported | no significant differences | Ernstgård *et al.*, 2005[33] |

Low level methanol kinetics in humans was studied during and after controlled inhalation exposure to water vapour (controls) or 200 ppm (266 mg/m$^3$) methanol for 4 hours in a randomised double blind study in 26 volunteers. Blood samples were collected at different time points throughout the exposure period and the 4-hour follow-up period. Maximum methanol concentration in blood (6.5±2.7 mg/L) was observed at the end of the exposure period, which was more than a fourfold increase from baseline levels (0.9±0.6 mg/L). The absorption rate constant under these exposure conditions was estimated at 0.87±0.6/h. The area under the curve (AUC) of the serum methanol concentration-time curve from 0 to 8 hours was 35.9±12.6 (mg/L)*h, compared to 9.3±4.7 (mg/L)*h for the control group.[30]

Kinetics of methanol in humans was studied during and after inhalation exposure to 800 ppm (1064 mg/m$^3$) for 30 minutes and 1 and 2 hours in 4 subjects (2 sessions for each concentration for each subject), and for 8 hours in 15 subjects (1 session/subject) Blood samples were collected at several time points during and up to 8 hours following exposure. Baseline blood methanol concentrations ranged from 1.3 to 2.0 mg/L and maximum blood methanol concentrations were 5.3±1.4, 6.6±1.2, 14.0±1.5, and 30.7±6.9 mg/L, respectively, for the four exposure durations. Methanol concentrations in blood lagged some 15-30 minutes behind the termination of exposure. The blood data were used to develop first-order models, which were used to estimate total uptake and uptake rates of methanol for the different exposure durations. Estimated total uptake of methanol was 284, 359, 958, and 5084 mg for the four exposure durations, respectively. The estimated uptake rates of methanol were higher for the 8-hour exposure duration (587 mg/h) than for the short-term exposure durations (346 to 448 mg/h). However, the subjects used in the short-term exposure sessions (up to 2 hours) were different from the subjects used in the 8-hour exposure sessions, which may in part account for the differences found in the uptake rates of methanol.[32]

The uptake of inhaled methanol vapours and possible gender differences in toxicokinetics were studied in four males and four females exposed at three different times to 100 ppm (133 mg/m$^3$) or 200 ppm (266 mg/m$^3$) methanol or to clean air for 2 hours in a stainless steel exposure chamber. During exposure, the volunteers performed light physical exercise at a workload of 50 W on a bicycle ergometer. Samples of blood were taken at different time points during and up to 23 hours after the onset of exposure. Background levels of methanol in blood were 0.3-2.4 mg/L and did not differ between men and women. The concentration of methanol in blood increased from 0.64 to 3.72 mg/L and from 0.64 to 7.91 mg/L after the

2-hour exposure at 100 and 200 ppm (133 and 266 mg/m$^3$) methanol, respectively, and a workload of 50 W. Respiratory uptake fraction throughout exposure was approximately 50% for both exposure levels. The AUC from 0 to 6 hours increased linearly with exposure concentrations, indicating non-saturated first-order kinetics in this exposure range. No gender differences in any of the toxicokinetic parameters were seen.[33]

Model simulations using a physiologically based pharmacokinetic model based on unpublished exhaled breath time course measurements from a study on anaesthetised female cynomolgus monkeys showed that there was no apparent trend between exposure concentration and the relative respiratory uptake, indicating linear absorption kinetics with respect to exposure duration (0.5-2 h) and concentration (10-900 ppm or 13-1197 mg/m$^3$).[34]

A biologically based dynamic model based on data from rats, monkeys, and humans (including two of the studies in humans described above[30,32]) demonstrated that absorption of methanol from the lungs did not appear to be influenced by the exposure level or duration, nor by the pulmonary ventilation rate in exposure concentrations ranging from approximately 77 to 770 ppm (100 to 1000 mg/m$^3$) and exposure durations ranging from 30 minutes to 8 hours. The absorption fraction of methanol used for rats, monkeys, and humans were 0.60, 0.69, and 0.577/h, respectively, although for the simulation of the data of the study in humans by Batterman *et al.* (see [32]), a value ranging from 0.76 to 0.81/h was deemed more appropriate.[35]

Simulations of a kinetic model suggested that an 8-hour inhalation exposure of at least 500 to 2000 ppm (655 to 2660 mg/m$^3$) methanol, without physical activities, would be necessary for blood and urinary formate concentrations to reach reported mean background values in humans.[35] Nevertheless, in one of the toxicokinetic volunteer studies, a modest increase in urinary formate was observed in subjects exposed to 400 ppm (532 mg/m$^3$) methanol for 8 hours.[31] Also, in a group of occupationally exposed subjects (n=20; concentration range: 37-231 ppm (49-307 mg/m$^3$); duration: 8 hours), a small increase in urinary formic acid levels was reported . The median of 20.7 mg/L in the exposed group was 2.5 fold of that in the control group (8.4 mg/L).[14]

*Table 5.3* Half-lives and concentrations of methanol in blood after inhalation in rodents and primates, measured or predicted to occur at the end of a 6-hour exposure period (adapted from OECD[4], and NTP[21]).

| | rat | | mouse | monkey | | human |
|---|---|---|---|---|---|---|
| exposure concentration ($mg/m^3$ (ppm)) | half-life (h) | blood methanol (mg/L) | blood methanol (mg/L) | half-life (h) | blood methanol (mg/L) | blood methanol (mg/L) |
| 0 (background levels) | | 1.8-3 | 1.6 | | 2.4 | 0.6-2.6 |
| 266 (200) | 0.8 | 3.1-7.4 | | 1.1 | 3.9 | 3-8 |
| 665-798 (500-600) | | ca. 10 | | | ca. 10 | ca. 10 |
| 1596 (1200) | 1.0 | 26.6 | ca. 150 | 3.2 | 37.6 | ca. 25 |
| 2660 (2000) | 2.1 | 79.7 | ca. 500 | 2.9 | 64.4 | ca. 50 |
| 6650 (5000) | no data | ca. 400-ca. 880 | ca. 2000 | no data | ca. 240 | ca. 140 |

Based on afore-mentioned data in this section, OECD presented a summarising table (see Table 5.3) which illustrates species differences following inhalation exposure.

These data indicate that, generally, blood levels seem to be similar in rats, monkeys, and humans for 6-hour exposures up to ca. 1596 $mg/m^3$ (1200 ppm). At higher levels, blood methanol levels increase non-linearly in rats and, less steeply, in monkeys and linearly in humans. In mice, levels increase even more sharply (which was thought to be due to their more rapid breathing and higher absorption.[4]

One study was published in which the kinetics of methanol in humans after dermal exposure was investigated. In four volunteers, immersion of one hand in 99.8% methanol for 0, 2, 4, 8, and 16 minutes resulted in high methanol vapour concentrations at exposed skin for several hours, indicating a reservoir effect of the skin. Methanol concentrations in blood slowly raised to a maximum (13.3 mg/L) about 1 hour after exposure and then slowly decayed. From this study, an average dermal permeation rate of 8.1±3.7 $mg/cm^2/h$ (i.e., 0,14±0.6 $mg/cm^2/min$) could be determined.[36,37]

## 5.2 Distribution

### WHO/IPCS data

Methanol distributes readily and uniformly to organs and tissues in direct relation to their water content. The apparent volume of distribution of methanol is 0.6 to 0.7 L/kg, similar to that of ethanol (Haggard and Greenberg, 1939; Yant and Schrenk, 1937).

In animals exposed to methanol, relatively high concentrations (not further specified) were found in blood, vitreous and aqueous humour, bile, urine, kidney, liver, and the gastrointestinal tract.

Relatively low concentrations (not further specified) were found in bone marrow, fatty tissue, brain, and muscle tissue (Bartlett, 1950; Yant and Schrenk, 1937).

Post-mortem analysis of methanol concentrations in body fluids and tissues reported in fatal human cases of methanol poisoning has revealed high concentrations of methanol in cerebrospinal fluid, vitreous humour, and bile. Concentrations in these fluids were higher than blood concentrations. In tissues, the highest concentrations were found in brain, kidney, lung, and spleen and lower concentrations were found in skeletal muscle, pancreas, liver, and heart (Bennet *et al.*, 1950; Whu Chen, 1985).

### Additional data

A biologically based dynamic model based on data from rats, monkeys, and humans determined an apparent volume of distribution for methanol of 0.92, 0.77, and 0.70 L/kg bw, respectively, and larger values for its metabolite formate of 6.4, 4.6, and 4.2 L/kg bw, respectively. The larger value for formate suggests binding to proteins.[35]

## 5.3 Biotransformation

### WHO/IPCS data

After uptake and distribution, most of the methanol is metabolised in the liver to carbon dioxide (96.9%), while a small fraction is excreted directly via the urine (0.6%) and through the lung. In all mammalian species studied, metabolism includes sequential oxidative steps to form formaldehyde, formate, and $CO_2$ (see *figure 5.1*).

The metabolism of methanol to formaldehyde in rats and other non-primate species is mediated by the catalase-peroxidase system. In non-human primates and in humans, the reaction is mediated by alcohol dehydrogenase, and can be significantly inhibited by co-exposure to ethanol, which acts as a competing substrate. Formaldehyde is very rapidly oxidised (with a half-life of ca. 1 min) by many species, including primates, into formate by several enzyme systems including a specific formaldehyde dehydrogenase. Despite differences in enzyme mediation, the conversion of methanol to formate occurs at similar rates in non-human primates and in rats.

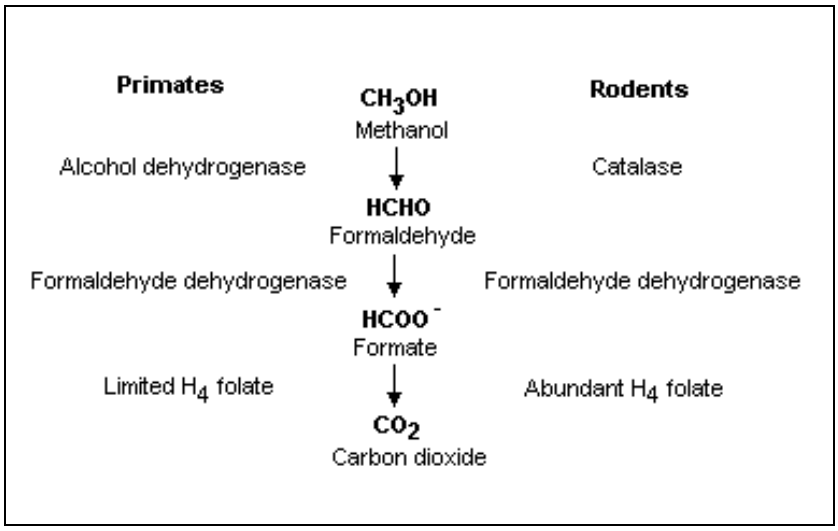


*Figure 5.1* Metabolism scheme of methanol. Major enzymes for primates (left) and rodents (right) are noted. Species differences in methanol toxicity are due primarily due to the metabolic conversion of formate to carbon dioxide, which is rapid in rodents but slow in primates (Medinsky and Dorman, 1994).

The oxidation of formate to $CO_2$ *in vivo* in mammalian species has been shown to be mediated by the tetrahydrofolate-dependent pathway. There are profound differences in the rate of formate oxidation in different species which contribute to the sensitivity to methanol. The folate-mediated oxidation of formate proceeds about twice as slow in non-human primates and humans as in rats. Accumulation of formate in primates is seen to occur at doses of methanol greater than 500 mg/kg bw (Tephly and McMartin, 1984).

The pharmacokinetics of $^{14}$C-methanol and $^{14}$C-formate were studied in normal and folate-deficient female cynomolgus monkeys exposed to 13 to 1200 mg/m$^3$ for two hours. $^{14}$C-methanol-derived formate levels in blood increased by only a small extent in both groups (Medinsky *et al.*, 1997).

### Additional data

The reaction rates of methanol → formate and formate → $CO_2$ were ca. 30 and ca. 73 mg/kg/h, respectively, in rats and ca. 48 and ca. 34 mg/kg/h, respectively, in monkeys, indicating that the conversion of methanol into formate and that of formate in $CO_2$ are rate limiting in rats and monkeys, respectively.[4] These data further indicate that formate accumulation will not occur in rats at any methanol

dose since the maximal formate oxidation rate 73 mg/kg/h equivalent to 1.6 mmol/kg/h) exceeds the maximal rate at which formate is supplied (30 mg or 0.9 mmol/kg/h). In monkeys, on the other hand, sufficiently high methanol doses can result in amounts of formate at a rate that can exceed the metabolic capacity of the folate pathway (34 mg or 0.75 mmol/kg/h).[38]

Assuming that constants for methanol and formate oxidation were similar in non-human primates and humans and that formate is relatively evenly distributed through body water, Kavet and Nauss[38] calculated that the methanol dose saturating the folate pathway would roughly be 210 mg/kg bw.

A biologically based dynamic model based on rat, monkey, and human data indicated that an unspecified substantial fraction of formaldehyde is converted to unobserved forms. This fraction, most plausibly, represents either the formaldehyde that (directly or after oxidation to formate) binds to various endogenous molecules or is incorporated in the tetrahydrofolic-acid-dependent one-carbon pathway to become the building block of a number of synthetic pathways. The model also predicted that the saturation of methanol metabolism appeared to occur at a lower exposure dose in rats than in monkeys and humans. In rats exposed to 2000 ppm (2660 mg/m$^3$) for 6 hours, a Michaelis-Menten affinity constant for methanol metabolism ($K_m$) value of 36.6 mg/L of blood and $V_{max}$ of 19.4 mg/L/h were estimated whereas following a similar exposure in monkeys, no saturation of methanol metabolism was apparent. In addition, it was predicted that no saturation of methanol metabolism occurred in humans exposed to 800 ppm (1064 mg/m$^3$) for 2 hours or to 229 ppm (305 mg/m$^3$) for 8 hours. The estimated metabolic rates differ substantially between rats on the one hand and primates and humans on the other hand for a number of steps in the metabolic clearance of methanol. The metabolic rate constants of methanol to formaldehyde were 0.53, 0.96, and 0.4 h$^{-1}$ for rats, monkeys, and humans, respectively. The metabolism of methanol to formaldehyde is mediated by different enzymes in rats compared to primates and is also saturated at lower doses in rats. The metabolic rate constants for formaldehyde to formate were 14.6 h$^{-1}$ for rats and 7.2 h$^{-1}$ for monkeys and humans. The whole body to exhaled air transfer coefficients combined with metabolic rate constants of formate to $CO_2$ were 0.32 h$^{-1}$ for rats and 0.81 h$^{-1}$ for monkeys and humans. The latter values indicate that formate is cleared twice as slow in monkeys and humans compared to rats.[35]

This biologically based dynamic model appears to be highly useful in extrapolating dosimetrics from animal studies (rats or monkeys) to humans for the purpose of deriving a health-based recommended occupational exposure limit.

Nevertheless, although methanol is metabolised via the same pathway through the same enzymes, in humans and monkeys, there are differences in the quantity of methanol that can be found in the bloodstream. Bouchard *et al.* predicted that an acute exposure to 800/900 ppm (1064/1197 mg/m$^3$) methanol for 2 hours, would result in a blood concentration of methanol that is 4-5 times as high in humans when compared to monkeys (up to 1000 ppm (1330 mg/m$^3$), in steady state concentrations of methanol in the blood).[35]

## 5.4 Elimination

### WHO/IPCS data

The primary route of methanol elimination from the body is via oxidation to formaldehyde and then to formic acid, which may be excreted in the urine or further oxidised to carbon dioxide. In humans, only 2% of a 50 mg/kg bw dose of methanol is excreted unchanged by the lungs and kidneys (Leaf and Zatman, 1952). Methanol has also been found in mother's milk, but no details were reported (Pellizzarri *et al.*, 1982).

The time course of the disappearance of methanol from the circulation is slow relative to ethanol and depends on the combined action of both direct excretion and metabolism. In humans exposed to low levels of methanol orally (<0.1 g/kg) (Leaf and Zatman, 1952) or via inhalation (75-225 ppm (100-300 mg/m$^3$) for 8 hours) (Sedivec *et al.*, 1981), the clearance of methanol obeyed first-order kinetics with a half-time of about 2.5 to 3 hours. The rate at which methanol clears into the urine or into exhaled air is directly proportional to its blood level. At higher doses of methanol, the clearance appears to become saturated, resulting in non-linear elimination kinetics.

Studies investigating formate levels in blood or urine after methanol inhalation exposure in humans are discussed in Section 5.1 and listed in Table 5.1. No accumulation of formate was found in urine or blood of volunteers exposed to 200 ppm (266 mg/m$^3$) methanol for 4 hours, 6 hours, or 5 days (Lee *et al.*, 1992; Franzblau *et al.*, 1993; d'Allessandro *et al.*, 1994). In contrast, a study with 20 subjects occupationally exposed to methanol at concentrations of 37 to 231 ppm (49-307 mg/m$^3$) (average: 111 ppm or 147 mg/ m$^3$) for 8 hours found increased urinary formate levels (mean: 29.9±28.6 mg/L vs. 12.7±11.7 mg/L in unexposed controls) (Heinrich and Angerer, 1982).

After intake of small quantities of methanol (10-20 mL), formate levels in urine were normal within 24 hours. Following intake of large amounts of methanol (50 mL), formate levels in blood of 26 to 78 mg/L were found as well an increased excretion of formate in the urine (540-2050 mg/L) (Lund, 1948).

## Additional data

In a study to evaluate methanol in urine and other potential biological determinants of methanol exposure, volunteer subjects were exposed to 0, 100, 200, and 400 ppm (0, 133, 266, 532 mg/m$^3$) during separate 8-hour sessions in an exposure chamber. Subjects repeated each exposure twice: once while sedentary and once while performing light, intermittent exercise (alternating rest and exercise every 30 minutes) on a bicycle ergometer. During the exercise sessions, it was estimated that overall mean ventilation rate was increased by 50%. Urine samples were collected before, throughout, and immediately after the exposure session. Twenty-two subjects participated in at least one exposure session, and there was a core group of 10 subjects which completed all exposure and exercise combinations. Concentrations of methanol in urine increased linearly with exposure concentrations, but with considerable inter-individual variation. Concentrations of methanol in urine were consistently higher after exposure sessions involving exercise. A modest increase in urinary formate was observed at exposure to 400 ppm (532 mg/m$^3$).[31]

In 26 volunteers exposed to 200 ppm (266 mg/m$^3$) methanol for 4 hours, urinary methanol, but not formate, excretion rate was increased significantly at the end of the exposure period. The overall elimination half-life of methanol in blood plasma was 3.2±2.3 h. However, elimination from plasma fit a mono-exponential model only for half of the subjects during the 4-hour post-exposure follow-up period, with a mean half-life of 2.2 h. Possible contributing factors to the poor fitting of other subjects included dietary intake, endogenous production, or the limited number of sampling times or limited follow-up period.[30]

The committee notes that seven of the subjects included in the study were smokers and were not requested to refrain from smoking before the experiment. Since tobacco smoke has been reported to contain levels of methanol[1], this may be another contributing factor to the poor fitting of the mono-exponential model for plasma elimination.

In a study investigating the kinetics of methanol after short-term inhalation exposure, subjects were exposed to 800 ppm (1064 mg/m$^3$) methanol for 30 minutes, 1, 2, or 8 h. Methanol was measured in blood, urine, and breath samples collected at different time points during and up to 8 hours after exposure. The concentrations of methanol in blood, urine, and breath did not increase linearly with exposure duration due to the fairly rapid clearance of methanol. The half-life

estimates reported were 1.44±0.33 h in blood, 1.55±0.67 h in urine, and 1.40±0.38 h in breath.

The blood data generated in this study closely fit first order models, which could be used to predict that concentrations of methanol asymptotically approach the steady-state level reached after many hours of exposure. For exposure periods of 0.5, 1, 2, and 8 hours, maxima should reach 21%, 38%, 62%, and 98% of the (unspecified) steady-state level, respectively.[32]

Data from the studies above[30,32] and earlier studies have been used to develop and validate a biologically based dynamic model to simulate the uptake and disposition of methanol and its metabolites in rats, monkeys, and humans.[35] The model predicted that the saturation of methanol metabolism appeared to occur at a lower exposure dose in rats than in monkeys and humans. The model further predicted that in monkeys and plausibly humans, a much larger fraction of body formaldehyde is rapidly converted to unobserved forms.

The authors state that the model is able to quantitatively relate the parent compound or the metabolites in biological matrices to the absorbed dose and tissue burdens at any point in time in rats, monkeys, and humans for different exposure situations, thus reducing the uncertainties in the dose-response relationship, animal-to-human, and exposure scenario comparisons.

In volunteers (n=4/sex) exposed at three different times to 0, 100 ppm (133 $mg/m^3$), or 200 ppm (266 $mg/m^3$) methanol during light physical exercise (50 W) in an exposure chamber. the time courses of methanol elimination from the blood, urine, and saliva were parallel after exposure, with half-lives of 1.4, 1.7, and 1.3 h, respectively. Directly after exposure, methanol concentration declined about twice as rapidly in breath as in blood, saliva, and urine with a half-life of 0.8 h, possibly caused by the wash-out effect from the epithelial lining of the respiratory tract. The AUC of methanol in all sample media increased in proportion to exposure up to 200 ppm (266 $mg/m^3$), the highest level tested. These results strongly suggest non-saturated first-order kinetics for this exposure concentration range. The excretion of formic acid was seemingly not affected by exposure up to 200 ppm (266 $mg/m^3$) methanol. No gender differences in any of the toxicokinetic parameters were seen.[33]

It has been suggested that simultaneous exposure to volatile organic compounds may affect the metabolic rate of the chemicals. In a study in rats, simultaneous exposure to methanol and toluene for 6 hours resulted in higher methanol concentrations in blood compared to those in animals exposed to methanol alone.[39] The half-lives of the blood methanol concentrations were comparable

(2.4 and 2.7 h) between the two exposure groups. Urinary formic acid excretion rates were not affected by simultaneous exposure to toluene.[39] Following simultaneous repeated exposure to both compounds (6 hours/day, 5 days/week, 4 weeks), the half-lives of methanol were significantly increased, although more in the group exposed to methanol alone (25.7 h vs. 16.1 h for co-exposure).[39,40]

## 5.5 Biological monitoring

### WHO/IPCS data

Humans have a background body burden of methanol of 0.5 mg/kg bw (Kavett and Nauss, 1990). Ranges of 0.32 to 2.61 mg/L in urine (Sedivec *et al.*, 1981) and of 0.06 to 0.49 mg/m$^3$ in expired air (Eriksen and Kulkarni, 1963) in unexposed, 'normal' subjects have been reported. Another study, however, detected methanol in the expired air at a mean level of 0.5 mg/m$^3$ in normal, healthy, non-smoking subjects (Krotoszynski *et al.*, 1979). An explanation for the large differences in methanol concentration in expired air could not be found.

Urine contains methanol concentrations 20-30% higher than blood, and concentrations depend strictly on the duration and intensity of the methanol exposure. For this reason, urinary methanol concentrations have been suggested to be a reliable parameter for evaluating the degree of methanol exposure, next to direct measurements of methanol in blood.

### Additional data

In its 1983 evaluation of a biological tolerance value (BAT value) for methanol, the German MAK Commission considered the methanol concentration in urine as the most suitable parameter for the biological monitoring of persons exposed to methanol. However, due to a lack of data on a direct relationship between methanol concentration in urine and effect from which a BAT value could be determined, the MAK Commission performed an indirect derivation via the MAK value, taking two studies into consideration. In the first (field) study on employees of a chemical company (see Heinrich and Angerer[14]), an exposure to 100 ppm (133 mg/m$^3$) methanol resulted in urine concentrations of 20 mg/L. Assuming a linear correlation between exposure to concentrations near the MAK value of 200 ppm (266 mg/m$^3$), a urinary methanol excretion of 40 mg/L can be expected. In the second (volunteer) study under laboratory conditions (see Sedivec *et al.*[13]), urinary methanol concentrations of 6.5 and 9.5 mg/L were found in 4 test persons exposed for 8 hours to 154 or 225 ppm (205 or 300 mg/m$^3$) methanol, respectively. These test persons showed ventilation rates of ca. 10 L/min. Taking a ventilation rate of 20 L/min for medium work as a basis, the

results of the volunteer study could be extrapolated to a urinary methanol excretion of ca. 20 mg/L at an exposure to 200 ppm. Based on these two extrapolations, the MAK Commission set the BAT value at 30 mg methanol/L urine (sampling time: second half of a shift at the end of the working week).[41]

In 2005, the American Conference of Governmental Industrial Hygienists (ACGIH) recommended monitoring of methanol in urine as an indicator of recent exposure to methanol. Other indicators such as formic acid in urine and methanol and formic acid in blood were considered less useful. From the human volunteer study of Sedivec *et al.*[13], ACGIH inferred that an 8-hour methanol exposure at 200 ppm when at rest resulted in ca. 8.3 mg/L in end-of-exposure urine and 6.5 mg/L in whole-8-hour-exposure urine, which would correspond to levels of 16.6 and 13 mg/L at a ventilation rate of 21 L/min (10 $m^3$ per 8-hour work shift). Being the mean of these values, 15 mg/L was selected as the Biological Exposure Index (BEI). Toxicokinetic models confirmed that an 8-hour exposure to 200 ppm should yield urinary methanol levels of 9-16 mg/L, depending on the ventilation rates used. According to ACGIH, the volume concentration of methanol correlated to the blood concentration and to recent exposure dose. Measurements, therefore, reflect exposure during the sampling period and should not be corrected for creatinine and specific gravity. Based on the likelihood of uniformity versus non-uniformity of exposures across the shift, end-of-shift versus whole-shift sampling should be conducted.[15]

Methanol concentrations in saliva might also be used as a suitable biomarker of methanol inhalation exposure. In a study exposing 8 volunteers to 100 or 200 ppm (133 or 266 $mg/m^3$) methanol vapours for two hours, consistent and parallel patterns were seen with regard to the methanol time courses in blood, urine, and saliva, whereas the concentration in exhaled air decreased markedly faster.[33]

Skin headspace measurements have also been suggested as a biological exposure indicator that shows the presence and site of a dermal exposure to methanol. In a pilot study in which 4 volunteers immersed one hand in 99.8% methanol for 2 to 16 minutes, measured skin headspace concentrations at unexposed skin were similar to breath concentrations and had a moderate to high correlation with blood levels of methanol.[36]

Background concentrations of urinary methanol concentrations measured in 84 subjects from the city of São Paulo, Brazil that were not occupationally exposed to methanol ranged from <0.84 mg/L (the limit of detection) to 6.0 mg/L (mean:

2.26 ± 1.26 mg/L; geometric mean: 2.10 mg/L). The levels did not differ statistically between male and female subjects.[42]

## 5.6 Summary

During inhalation, methanol is adsorbed by or dissolves into the respiratory airway and lung mucus; during exhalation, methanol desorbs from the respiratory airway and lung mucus and is exhaled. This 'wash-in, wash-out' effect needs to be considered when calculating the bioavailability of methanol after exposure via the lung. The absorption fractions for humans reported range from 50 to 85%, compared to modelled absorption fractions of 60 and 69% for rats and monkeys, respectively. In one study in rats, the absorption fraction appeared to depend on the exposure concentration and duration, but in this study, rats were exposed to much higher concentrations (5115-15,346 ppm or 6650-19,950 mg/$m^3$).

Following dermal exposure to methanol, methanol diffuses rapidly through the skin. Immersion of one hand in 99.8% methanol for up to 16 minutes in 4 volunteers resulted in an absorption rate of 8.1 mg/$cm^2$/h.

Studies in humans indicate that the absorption rate is a linear function of exposure concentration and duration, at least for exposure concentrations ranging from approximately 77 to 770 ppm (100-1000 mg/$m^3$) and exposure durations ranging from 30 minutes to 8 hours. Generally, blood methanol levels seem to be similar in rats, monkeys, and humans at exposures up to ca. 1596 mg/$m^3$ (1200 ppm). At higher levels, blood methanol levels increase non-linearly in rats and, less steeply, in monkeys and linearly in humans. Levels in mice raise even more sharply than in rats.

Methanol distributes readily and uniformly to organs and tissues in direct relation to their water content.

Methanol is metabolised in the liver by sequential oxidative steps to form formaldehyde, formate, and $CO_2$. There are substantial differences between rats, monkeys, and humans with regard to metabolic rates and metabolic saturation in the different steps of the metabolism of methanol. The metabolism of methanol to formaldehyde is mediated by different enzymes in rats compared to primates, but occurs at similar rates. In primates, on the other hand, oxidation of formate is much slower than in rodents. In rats, the conversion of methanol into formate is rate limiting and may result in accumulation of methanol at sufficiently high methanol levels. Since the maximal rate of formate oxidation exceeds the maximal rate of methanol oxidation, formate will not accumulate in rats at any methanol dose. In monkeys, the conversion of formate into $CO_2$ is rate limiting. The formation rate of formate exceeds the rate at which formate can be converted into

$CO_2$, and formate can therefore accumulate at sufficiently high methanol levels. In humans, this may occur at methanol doses >210 mg/kg bw.

By far the most of the methanol taken up and distributed is excreted as $CO_2$ and only minor amounts unchanged by the lungs and the kidneys. Further, the methanol metabolites formaldehyde or formate are thought to bind to various endogenous molecules or enter a number of endogenous synthetic pathways. Studies in which volunteers were exposed to methanol for 30 minutes to 8 hours showed generally similar elimination half-lives of methanol in blood (1.4-3 h) and in urine, breath, and saliva (ca. 1.4 h). In a study in rats, exposure to methanol for 6 hours/day, 5 days/week, for 4 weeks, a half-life of 25.7 h was found for methanol in blood, whereas half-lives after single exposures were similar to those in humans (2.7 h).

In humans, small increases (i.e., compared to background levels) in formate levels in blood or urine have been reported in workers, occupationally exposed to methanol concentrations of 38 to 233 ppm (49-303 mg/m$^3$) (average 112 ppm (145 mg/m$^3$)) for 8 hours, and in volunteers exposed to 532 mg/m$^3$ (400 ppm for 8 hours), while no increased levels were observed in volunteers exposed to 266 mg/m$^3$ (200 ppm).

The most suitable biological parameter for biological monitoring of persons exposed to methanol is the methanol concentration in urine.

Chapter 6

# Mechanism of action

## WHO/IPCS data

The mechanism of action for ocular toxicity of methanol has been attributed to its metabolite formic acid. Formic acid has been hypothesised to produce retinal and optic nerve toxicity by disrupting mitochondrial energy production (Martin-Amat *et al.*, 1977; Sharpe *et al.*, 1982). It has been shown *in vitro* to inhibit the activity of cytochrome oxidase, a vital component of the mitochondrial electron transport chain involved in ATP synthesis (Nicholls, 1975). Inhibition occurs subsequently to the binding of formic acid to the ferric haem iron of cytochrome oxidase. The resulting disrupted retinal energy metabolism probably underlies the visual dysfunction observed after methanol exposure. Several factors may contribute to the unique vulnerability of the retina and optic nerve to the cytotoxic actions of formate. Data from rat studies suggest that toxic actions of methanol on the visual system may be due to the selective accumulation of formate in the vitreous humour and the retina as compared with other regions of the central nervous system (Eells, 1991; Eells *et al.*, 1996). Further, the retina has a very limited metabolic capacity to oxidise and thus detoxify formate (Eells *et al.*,1996). Finally, there may be tissue- and cell-specific differences in mitochondrial populations and in the actions of formate on mitochondrial function (Eells *et al.*, 1995).

In contrast to ocular toxicity, changes in monoamine levels in various brain regions appear to be the direct effect of methanol itself on the monoaminergic neuronal membranes (Jegnathan and Namasivayam, 1989).

## Additional data

The acute toxicity of non-reactive volatile organic compounds (VOCs), including methanol, are generally believed to be due to a general narcotic effect on the body described as non-specific toxicity.

The anaesthetic action of methanol was claimed to be the result of methanol itself and not of its metabolites as administration of 4-methylpyrazole, an alcohol dehydrogenase inhibitor, did not affect the anaesthetic process of methanol in rats. The committee notes, however, that in rats, methanol is metabolised primarily by the catalase-peroxidase system and not, as in humans, by alcohol dehydrogenase (see Section 5.3). The administration of 4-methylpyrazole may, therefore, not have inhibited the metabolism of methanol.

However, the relevance of data generated in rat lethal toxicity studies for the toxicity in humans is questionable. The lethal dose for most non-primate laboratory animals has been reported to be more than six times the average human lethal dose.[43] Initially, a (transient) depression of the central nervous system is observed in humans after toxic exposure to methanol, indicating a possible similar mechanism of action as described for rats. However, after a latent period of several hours to two or more days after exposure, accumulation of formate leads to metabolic acidosis with superimposed toxicity to the visual system. Metabolic acidosis almost never develops in non-primate laboratory animals, due to a more rapid metabolism of formate. Especially individuals with a compromised folate status are thought to be more susceptible to the metabolic acidosis caused by methanol, as folate is involved in the metabolism of formate to $CO_2$ (see Section 5.3).

Some mild irritation effects of methanol have been reported in humans exposed to methanol via inhalation. It has been suggested that pro-inflammatory mediators such as interleukin-8 and interleukin-1β synthesised or released by nasal epithelial cells may play a role in these effects.[44]

Effects of methanol after repeated exposure have not been characterised as well as acute effects. Studies report both general toxicity and developmental and carcinogenic effects in rodents (discussed in Section 7.2), but the mechanism of action underlying these effects is unknown. In addition, there appears to be no consensus on whether the effects are caused by methanol or by its metabolites.

Chapter 7

# Effects

## 7.1 Observations in humans

### *7.1.1 Irritation and sensitisation*

#### WHO/IPCS data

Eye irritation was, amongst others, reported by teacher aides. They worked at or near spirit duplicators using 99% methanol as a duplicator fluid; exposure times varied from 1 hour/day, 1 day/week to 8 hours/day, 5 days/week, presumably for about 3 years. Fifteen-minute breathing zone samples showed methanol concentrations ranging between 365 to 3080 ppm (485-4096 mg/m$^3$), with 15/21 measurements exceeding 800 ppm (1064 mg/m$^3$). While collating and stapling papers impregnated with the fluid up to 3 hours after duplication, 15-minute average concentrations were 180 to 875 ppm (239-1164 mg/m$^3$ (Frederick *et al*., 1984). No data on sensitising properties of methanol in humans were reported.

#### Additional data

In a single-blind controlled study, 12 healthy, non-smoking male students were exposed for 4 hours to either 200 ppm (266 mg/m$^3$) or 20 ppm (27 mg/m$^3$) methanol in an exposure chamber.[44,45] Exposure to 200 ppm (266 mg/m$^3$) of methanol resulted in an increase in interleukin-8 (IL-8) and interleukin-1β (IL-1β) in nasal secretions. No effects were observed on interleukin-6 (IL-6), prostaglandin $E_2$

($PGE_2$), mucociliary clearance, and scores on a subjective symptoms questionnaire relating to irritations before and after exposure. The authors conclude that exposure to 200 ppm (266 mg/m$^3$) caused a sub-clinical inflammatory response.

The committee notes that the relevance of the effects on the two interleukins in nasal secretions to the actual occurrence of a clinically relevant immunological disease is unknown. Actual discomfort as assessed by the questionnaire was not increased after exposure to these concentrations of methanol. The committee therefore regards the exposure concentration of 266 mg/m$^3$ as a NOAEL in this study.

In a study on acute effects on the human EEG, a questionnaire containing amongst others items related to irritation (eyes, nose, throat, and skin) was filled in by 12 subjects before and after 8-hour exposure to methanol concentrations of 27 and 266 mg/m$^3$ (20, 200 ppm). No significant difference in irritation symptom ratings was observed between groups (see Table 7.1).[45]

In only a few toxicokinetic studies, irritation due to inhalation exposure to methanol was addressed.

Ernstgård *et al.* recorded the level of perceived discomfort immediately before, during, and after exposure to 0, 100 ppm (133 mg/m$^3$), or 200 ppm (266 mg/m$^3$) methanol. The subjects (n=4/sex) were exposed two at a time at three different sessions in different exposure orders. Exposure sessions were separated by at least two weeks. Discomfort was scored by means of a questionnaire containing amongst others questions related to irritant symptoms (eyes, nose, throat, or airways) No significant difference in irritation symptom ratings between methanol-exposed and control subjects was noted (see Table 7.1).[33]

Lee *et al.* reported that none of the six subjects (healthy male; age: 29-55 years) had experienced eye irritation during 6-hour exposures to 200 ppm (266 mg/m$^3$).[46] The committee regards the exposure concentration of 200 ppm (266 mg/m$^3$) as a NOAEL for acute irritating effects.

The committee did not find data on sensitising properties of methanol in humans.

### *7.1.2 Toxicity due to acute exposure*

#### WHO/IPCS data

The preponderance of methanol poisonings has resulted from ingestion, for example consumption of adulterated alcoholic beverages. Intoxications from inhalation of methanol occur mostly in the context of intentional inhalation of volatile preparations such as carburettor cleaners.

Acute methanol exposure results in a transient mild depression of the central nervous system (CNS). The initial depressant period is followed by an asymptomatic latent period, which is followed by a syndrome that consists of an uncompensated metabolic acidosis with superimposed toxicity to the visual system. Physical symptoms typically may include headache, dizziness, nausea, and vomiting, followed in more severe cases by abdominal and muscular pain and difficult periodic breathing (Kussmaul breathing), which may progress to coma and death, usually from respiratory distress. Death may occur if patients are not treated for metabolic acidosis, and blindness may result even if treatment for metabolic acidosis is performed (Bennett *et al.*, 1953; Kane *et al.*, 1968; Röe, 1955; Tephly, 1991; Tephly and McMartin, 1984).

The neurotoxic effects of methanol on the visual system can involve both transient and permanent abnormalities (Bennett *et al.*, 1953; Dethlefs and Naraqi, 1978; Kavet and Nauss, 1990). Pallor of the optic disc is an end-stage sign of irreversible effects of the visual system and may appear 1 to 2 months after an acute methanol dosage (or possibly following chronic occupational exposure to methanol vapour) (Bennett *et al.*, 1953; Buller and Wood, 1904; Wood and Buller, 1904).

In the brain, methanol toxicity can cause oedema, necrosis of white and grey matter, atrophy, and haemorrhage (del Carpio-O'Donovan and Glay, 1992; Glazer and Dross, 1993; Gonda *et al*., 1978, Hsieh *et al.*, 1992). In addition, tremor and rigidity, hypokinesia, altered speech, and loss of superficial and proprioceptive sensation of the lower extremities with hyperpathia were reported (Pelletier *et al.*, 1992).

Toxicity has been associated with inhalation of methanol vapours in excess of 800 ppm (1064 mg/m$^3$) (Frederick *et al.*, 1984) (see Section 7.1.1).

Ingestion of 0.3 to 1 g/kg bw is considered the range of a minimum lethal dose for untreated cases of methanol poisoning (Erlanson *et al.*, 1965; Gonda *et al.*, 1978; Röe, 1955), and fatalities have occurred in untreated patients with initial methanol blood levels of 1500-2000 mg/L. CNS and ocular effects seem to appear above methanol blood levels of 200 and 500 mg/L, respectively.

### Additional data

Studies in which volunteers were exposed to methanol concentrations of 27-266 mg/m$^3$ (20-200 ppm) for up to 4 hours are summarised in Table 7.1.

*Table 7.1* Acute neurobehavioural inhalation studies with methanol in human volunteers.

| study design/ population | exposure conditions | end points | remarks/results | (N)OAEL | reference |
|---|---|---|---|---|---|
| counterbalanced, double-blind controlled<br>12 males (age: 22-32 years; healthy);<br>for 12 h prior to experiment, diet restrictions (no alcohol, diet foods and drinks, fruit and fruit juices, and coffee). | 0, 249 mg/m$^3$ (0, 187 ppm), for 75 min | measured before, during, after methanol and control exposure: blood, urinary methanol; plasma formate; oral temperature; blood pressure; subjective mood, alertness, fatigue, workload, and symptom scales; spectral analysis of EEG; visual- and auditory-event-related potentials; contingent negative variation; respiration; cardiac interbeat interval; Symbol Digit substitution task; three-choice reaction time; Stroop colour-word test; simple reaction time; visual function; critical flicker fusion frequency; hand steadiness; visual search task; Gamberale reaction time task; visual tracking task; Sternberg memory task; interval production task, speeded addition task, and 2 dual tasks | controls exposed to filtered air; small number of subjects; only one concentration; short duration; inability to mask methanol odour.<br><br>*results*: slight, significant changes in P200 latency and in reaction time in the Stenberg memory test; higher fatigue levels when exposed; trend toward poorer concentration and less vigour | LOAEL: 249 mg/m$^3$ | Cook *et al.*[47] |
| controlled randomised double-blind; corrected for age, gender, serum folate smoking, alcohol;<br>15 males, 11 female (age: 26-51 years; mean: 35.7±6.8; healthy)<br>for 24 h prior to experiment, diet restrictions | 0, 266 mg/m$^3$ (0, 200 ppm), for 4 h | serum methanol and formate (before, during, after exposure); urinary methanol and formate (before, after exposure); Symbol Digit substitution test; Stroop colour-word test; visual scanning performance 2 and 7 test (all before and during the last 30 min of exposure); Sternberg memory task; Vistech contrast sensitivity vision test; Lanthony D-15 hue colour arrangement test; P300 event-related auditory evoked potentials (all before and after exposure) | controls exposed to water vapour; small number of subjects; only one concentration; inability to mask methanol odour.<br><br>*results*: overall, no significant effect; when considering certain between-subject variables: post-hoc regression analysis of within-condition changes revealed a slight decrease in P300 amplitude and an increase in Symbol Digit score during the control exposure | NOAEL: 266 mg/m$^3$ | Chuwers *et al.*[29,30] |

| | | | | | |
|---|---|---|---|---|---|
| controlled, single-blind cross-over; 12 males (mean age: 26.8±2.1 years; healthy; non-smoking); | 27 ('control'), 266 mg/m$^3$ (20, 200 ppm), for 4 h | questionnaire concerning 17 items on irritation (eyes, nose, throat, skin), breathing difficulties, pre-narcotic symptoms (amongst others headache, dizziness, nausea, fatigue), severity degree scored on an ordinary scale from 0-5; EEG recorded with eyes closed, with eyes open, and during a colour-word test [because of analytical difficulties, no evaluation of measurements of methanol in hourly taken blood samples] | small number of subjects and exposure concentrations<br><br>*results*: no differences in symptom scoring/rating; at 200 ppm: decreased $\theta$-power in EEG suggesting slight excitatory effect (which was weaker than the changes due to circadian rhythm) | NOAEL: 266 mg/m$^3$ | Muttray *et al.*[45] |
| placebo-controlled, cross-over; 4 males, 4 females (age: 20-50 years; healthy, non-smoking) for 48 h prior to experiment diet restrictions (no alcohol, drugs, fruits) | 0, 133, 266 mg/m$^3$ (0, 100, 200 ppm), for 2 h during light exercise (50 W on a bicycle ergometer) | rating level of discomfort immediately before, during 10, 50, 80, and 104 min, after exposure (126 and 210 min) concerning 10 items on irritation (eyes, nose, throat, airways), CNS symptoms (headache, fatigue, nausea, dizziness, feeling of intoxication), difficulty in breathing, smell of solvent; ratings performed on a 100-mm visual analogue scale, graded from 'not at all' (0 mm on the scale) through 'allmost unbearable' (100 mm on the scale) | toxicokinetic study; small number of subjects<br><br>*results*: between exposed and controls, no difference in symptoms ratings | NOAEL: 266 mg/m$^3$ | Ernstgård *et al.*[33] |

In the study of Cook *et al.*[47], exposure of twelve 22-32-year-old male volunteers to methanol concentrations of 249 mg/m$^3$ (187 ppm) for 75 minutes did not result in effects on most of the neurophysiological and neuropsychological end points examined or in an increased reporting of symptoms. However, small but statistically significant changes were found in P200 latency and Sternberg reaction time and in scores for fatigue and concentration. On the other hand, tests for alertness and reaction time were unchanged. The committee notes that Cook *et al.* stated that the aforementioned changes did not exceed normal ranges.

In other studies, exposure to somewhat higher concentrations (266 mg/m$^3$ or 200 ppm) for longer periods (4 or 6 hours) did not affect visual, neurophysiological, and neurobehavioural test outcomes[29,30] or CNS symptom score ratings (e.g., fatigue, dizziness, nausea, etc.).[33,45,46]

Overall, the committee is of the opinion that the negative results in the studies using longer exposure periods[29,30,33,45,46] outweigh the small, statistically significant changes found in the Cook study[47]. Although all these studies were

performed with relatively small groups of healthy volunteers, the committee judges that exposure to 266 mg/m$^3$ (200 ppm) for up to 4 hours will not result in neurotoxic effects in workers.

Based on a limited number of cases (n=22) presented to a regional poison centre in Phoenix, AZ, USA, LoVecchio *et al.* concluded that rarely serious sequelae were observed following abuse of methanol-containing carburettor cleaners via inhalation.[48]

Davis *et al.*[49,50] reviewed a total of 13,524 cases of human poisonings in which methanol was the sole or primary or secondary agent reported to the American Association of Poison Control Centres (AAPCC) Toxic Exposure Surveillance System during 1993-1998. The methanol poisonings occurred at a relative constant rate over this period (average: 2254 cases/year; range: 2035-2486). In approximately 96% of these cases, exposure was by ingestion, ca. 3% via inhalation, and 1.5% ocular. For cases in which a specific product could be identified (76%), 70% were from windshield wiper fluid, 30% from other automotive sources (gasoline enhancer, gas line de-icers, cleaning solutions), and only 3% from pure methanol. Twelve percent had occurred at the workplace. Of these 13,524 cases, 0.5% was lethal and in 1 and 5%, it concerned major and moderate effects, respectively.

Accordingly, numerous case reports of methanol poisoning after ingestion have been reported, which due to lack of added value compared to the studies described in the WHO/IPCS document have not been included in this document.

### *7.1.3 Toxicity due to repeated exposure*

#### WHO/IPCS data

Information based on a limited number of case reports and even fewer epidemiological studies (generally containing unknown levels and/or durations of methanol exposure) suggests that extended exposure to methanol may cause effects qualitatively similar to those observed from relatively high levels of acute exposure, including in some cases CNS and visual disorders (Bennett *et al.*, 1953; Buller and Wood, 1904; Frederick *et al.*, 1984; Greenberg *et al.*, 1938; Kingsley and Hirsch, 1955; Wood and Buller, 1904). Visual disturbances of several types (blurring, constriction of the visible field, changes in colour perception, and temporary or permanent blindness) have been reported in workers who experienced methanol air levels of about 1200 ppm (1600 mg/m$^3$) or more.

CNS effects appear above blood methanol levels of 200 mg/L (6 mmol/L); ocular symptoms appear above 500 mg/L (16 mmol/L).

After occupational exposure to 22 to 25 ppm (29-33 mg/m$^3$) methanol and 40 to 45 ppm (96-108 mg/m$^3$) acetone for 9 months to 2 years, no CNS symptoms or visual anomalies were observed in 19 workers (Greenberg *et al.*, 1938).

Frederick *et al.* (1984) reported, amongst others, headaches, dizziness, blurred vision, and nausea/upset stomach in teacher aides at 15-minute average exposure concentrations ranging from 365 to 3080 ppm (485-4096 mg/m$^3$), with 70% exceeding 800 ppm (1064 mg/m$^3$) (see also Section 7.1.1).

No studies have been reported on the carcinogenicity or reproduction toxicity of methanol in humans.

### Additional data

Infante-Rivard *et al.* carried out a population-based case-control study including 790 incident cases of childhood acute lymphoblastic leukaemia and as many healthy age- and sex-matched controls. Maternal occupational exposure to solvents before and during pregnancy was estimated using the expert method, which involved chemists and industrial hygienists coding each individual's job for specific contaminants. Home exposure to solvents was also evaluated. Infante-Rivard *et al.* did not observe an association between childhood acute lymphoblastic leukaemia and exposure to methanol from two years before pregnancy up to birth (odds ratio - OR: 0.77; 95% confidential interval - CI: 0.41-1.47; adjusted for maternal age and education) or exposure during pregnancy (OR: 0.78; 95% CI: 0.39-1.55; adjusted for maternal age and education).[51]

Rennix *et al.* calculated age-adjusted incidence rates for breast cancer for almost 275,000 enlisted women serving between 1980-1996. For 21 volatile organic chemicals with a potential risk for breast cancer, exposure was assessed based on job title histories and subjective exposure ratings (from 'none' to 'high'). Poisson regression analysis used to evaluate the association between the exposure rating by job title and breast cancer showed that the incidence of breast cancer in the cohort was significantly increased in women younger that 35 years of age, especially among black women, when compared to the age-specific rates in the general population. Women working in occupations with a moderate to high exposure potential to at least one of the substances had a 48% increased risk ($p<0.05$) of breast cancer while on active duty between 1980-1996 when compared to women with low to no exposure. However, no substance-specific, quantitative analyses were made.[52]

Based on the relatively high levels of methanol and methyl acetate found in samples of beech, oak, spruce, and pine, Bleich *et al.* suggested an aetiological role

of methanol in the development of nasal cancer in wood workers due to exposure to wood dust. According to Bleich *et al.*, the permanent release of gaseous materials (e.g., methanol and methyl acetate vapours) from the small wood dust particles adhering to the nasal mucosa and the easy access of these substances to exposed cells would result in a continuous *in situ* generation of formaldehyde.[25] The actual occupational exposure level to methanol was however not measured nor estimated.

Gattas *et al.*[50] performed micronucleus tests in squamous oral cells obtained from pump operators of 28 gas stations in the city of São Paulo, Brasil, at three different periods: before and 1 year after a mixed fuel, containing 33% methanol, 60% ethanol, and 7% gasoline, was introduced (in 1989 and 1992, respectively), and 3 years later when exposure to this mixed fuel had become very low (in 1995). The frequency of micronuclei observed in 79 attendants in 1992 (mean: 3.62±0.39) was significantly increased ($p<0.001$; Mann-Whitney) as compared with those observed in 76 attendants in 1989 (mean: 1.41±0.26) and in 129 attendants in 1995 (mean: 1.20±0.15). These differences were also significant when compared with non-occupationally exposed controls (university employees). For 39 attendants participating in 1989 and 1992, there was a significant increase in the frequency of micronuclei from 1.08 to 3.20 ($p<0.0001$; Mann-Whitney). For the 17 subjects investigated at all three occasions, the average number of micronuclei per individual increased significantly from 1.18 to 3.47 in 1989 and 1992, respectively, and then decreased to 2.06 in 1995. The ANOVA of the three frequencies through the Kruskal-Wallis test revealed that the heterogeneity did not reach statistical significance. Gattas *et al.* performed a stepwise regression analysis of the number of cells with micronuclei and the number of micronuclei per 2000 cells per individual by considering anamnestic data (independent variables), such as previous diseases, number of miscarriages related to operators' partners, use of medicaments and X-rays in the six months before examination, tea and coffee drinking, alcohol intake, smoking and use of psychotropic drugs as semi-quantitative parameters. The number of cells with micronuclei per individual showed a low but significant regression upon the frequency of miscarriages, which was confirmed by square root transformation (a tentative approach for normalising dependent variables), and the number of micronuclei per 2000 cells a positive regression with age and tea drinking, which was also confirmed by square root transformation. Gattas *et al.* further remarked that there was always exposure to benzene and gasoline and that they were not able to disentangle the effects of gasoline, ethanol, and the mixed fuel. In addition, no exposure levels were measured and presented.

The effects of exposure to methanol on reproduction have been reviewed and separately published by DECOS' Subcommittee on Classification of Compounds Toxic to Reproduction. Data and conclusions presented by the subcommittee are summarised below. For detailed information on individual studies, it is referred to the subcommittee's report.[2]

No studies on the effects of methanol on human fertility were presented.

Lorente *et al.*[53] found inconclusive results in a study investigating the role of maternal occupational exposure during pregnancy in the occurrence of oral clefts in 851 women (100 mothers of babies with oral clefts and 751 mothers of healthy referents) who worked during the first trimester of pregnancy. Further two case reports were presented. One of these reported on a woman intoxicated with methanol and given birth to an infant with no signs of distress six days after intoxication.[54] The other case concerned a woman who gave birth to an infant of appropriate weight but representing acute fetal distress with significant metabolic acidosis (with methanol blood levels of ca. 450 mg/L) and neurological after exposure to a mixture of solvents containing methanol.[55]

No studies were found regarding the effects of exposure to methanol on human lactation.

## 7.2 Animal experiments

### *7.2.1 Irritation and sensitisation*

#### WHO/IPCS data

A modified Magnusson-Kligman maximisation test in guinea pigs did not show skin sensitisation or irritation after intracutaneous or percutaneous induction and challenge with 50% methanol solution in distilled water or with Freund's adjuvant (BASF, 1979).

Methanol caused significant conjunctivitis after administration of 100 µL into the lower conjunctival sac of New Zealand White albino rabbits. Initial oedema (chemosis) seen up to 4 hours decreased significantly by 72 hours. Other ocular lesions (iritis and corneal opacity) were much less significant (Jacobs, 1990).

In rats, exposed to methanol concentrations of 500-5000 ppm (665-6650 mg/m$^3$), 6 hours/day, 5 days/week, for 4 weeks, a dose-related increase in mucoid nasal discharge was observed (Andrews *et al.*, 1987).

### Additional data

Exposure to a (lethal) atmosphere saturated by methanol vapours at 20°C (i.e., ≥150,000 mg/m³ or 112,500 ppm); exposure duration not known) caused severe irritation of mucous membranes and milky corneal opacity in rats.[4]

When methanol (dose not specified) was applied under occlusive conditions for up to 20 hours to rabbit skin, no irritating effects were observed. However, in another study, the application of 500 mg methanol to rabbit skin (again under occlusive conditions), moderate skin irritation was reported, either due to prolonged contact or defatting actions of methanol.

In a second Magnusson-Kligman maximisation test performed by BASF (see above 'WHO/IPCS data') using 24 female guinea pigs (in two tests with 12 animals each), a slight skin response (score 1) was observed in 1/12 and 2/12 animals after 24 and 48 hours, respectively.[4]

Instillation of 0.05 mL of undiluted methanol into the eyes of rabbits (n=2) caused slight erythema and corneal opacity and moderate oedema associated with excretion after one hour. These effects were assessed as mild after 24 hours and absent after 8 days.

Using EPA criteria, which were amongst others based on the Draize scoring system, Morgan *et al.*[56] classified methanol as corrosive (i.e., corneal involvement, irritation or eye damage persisting for more than 21 days after treatment) after instillation of 0.1 mL of undiluted methanol into the eyes of rabbits.

Using three to six rabbits, Nagami and Maki[57] determined that the maximum concentration of methanol that did not cause eye irritation was 29%.

With respect to the respiratory tract, the sensory irritation of the upper part was studied by determining the concentration associated with a 50% decrease in the respiratory rate ($RD_{50}$). Using different strains and protocols, Muller and Greff[58] and Kane *et al.*[59] reported $RD_{50}$ values of 33,649 (25,300 ppm) and 55,214 mg/m³ (41,514 ppm), respectively.

## *7.2.2 Toxicity due to single exposure*

### WHO/IPCS data

Dated studies with mice, rats, and dogs investigating the effects of methanol after single exposures by inhalation up to 230 hours at dose levels ranging from 4800 ppm to 152,800 ppm (6384-203,224 mg/m³) found increased rate of respiration, a state of nervous depression followed by excitation, irritation of the mucous membranes, loss of weight, ataxia, partial paralysis, prostration, deep narcosis, convulsions, and death occurring from respiratory failure. Post-mortem examinations showed haem-

orrhage, oedema, congestion and pneumonia in the lungs as well albuminous and fatty degeneration and fatty infiltration of the liver and the kidneys (Eisenberg, 1917; Loewy and von der Heide, 1914; Mashbitz *et al.*, 1936; Scott *et al.*, 1933; Tyson and Schoenberg, 1914; Weese, 1928).

The lethal oral doses ($LD_{50}$) of methanol were 6.2-13.0, 7.3-10, and 8.0 g/kg bw for rats, mice, and dogs, respectively. For monkeys and rabbits, minimum lethal doses of 2-7 and 7.0 g/kg bw, respectively, were reported. In primates, after oral or intraperitoneal administration of 3 to 6 g/kg bw methanol, toxic effects found included metabolic acidosis and ocular toxicity, effects that are not normally found in rodents. Clinically, the signs of toxicity were similar to those noted in humans, including initial CNS depression for 1-2 hours, followed by a latent period of about 12 hours, a progressive weakness, coma, and death (Clay *et al.*, 1975; Cooper and Felig, 1961; Gilger and Potts, 1955; McMartin *et al.*, 1975). An attenuated but prolonged syndrome was produced in monkeys by the administration of an initial methanol dose of 2 g/kg bw and subsequent doses of 0.5-1.0 g/kg bw at 12-24 hour intervals, producing profound ocular toxicity approximately 40-60 hours after the initial dosage (Baumbach *et al.*, 1977; Hayreh *et al.*, 1977; Martin-Amat *et al.*, 1977). Female minipigs treated with a single oral dose of methanol at 1.0 to 5.0 g/kg bw by gavage showed dose-dependent signs of acute methanol intoxication, including mild CNS depression, tremors, ataxia, and recumbency. No optic nerve lesions, toxicologically significant formate accumulation or metabolic acidosis were found (Dorman *et al.*, 1993). Studies in rats have indicated that there are changes in levels of dopamine, norepinephrine, serotonin, and 5-hydroxyindole acetic acid in various brain regions after a single intraperitoneal injection of 3 g methanol/kg bw, which appeared not to be induced by metabolic acidosis, but a direct effect of methanol per se on the monoaminergic neuronal membranes (Jegnathan and Namasivayam, 1989).

### Additional data

For rats, $LC_{50}$ values of 67,000 and 98,000 ppm (89,110 and 130,340 mg/m$^3$) were reported for single 6- and 8-hour exposures, respectively. Clinical signs such as aqueous secretion of eyes and nose, laboured breathing, staggering, apathy, and narcosis, were observed. In mice, the $LC_{50}$ for a single exposure of 2.25 h was ca. 79,000 mg/m$^3$ (59,250 ppm).[4]

The dermal $LD_{50}$ in rabbits was ca. 17,000 mg/kg bw. In rats, no mortality was observed after occlusive application of 35,000 mg/kg bw; amounts of 45,000 mg/kg bw were lethal.

### 7.2.3 *Toxicity due to repeated exposure*

#### WHO/IPCS data

Rhesus monkeys exposed to 3000 ppm (4000 mg/m$^3$) methanol for 21 hours/day survived the 20-day exposure period and rhesus monkeys exposed to 10,000 ppm (13,300 mg/m$^3$) methanol for 21 hours/day survived for more than 4 days. Folate-deficient rats exposed to 3000 ppm (4000 mg/m$^3$) for 20 hours/day, however, did not survive for more than 4 days (Lee *et al.*, 1994). Male and female cynomolgus monkeys *(Macaca fascicularis*; n=3/sex/dose) that were exposed to 500, 2000, and 5000 ppm (665, 2660, and 6650 mg/m$^3$) methanol, 6 hours/day, 5 days/week, for 4 weeks, showed no upper respiratory tract irritation. Gross, microscopic, or ophthalmoscopic examinations did not disclose any ocular effects in the monkeys exposed to 5000 ppm (6650 mg/m$^3$) (Andrews *et al.*, 1987).

In female cynomolgus monkeys (n=8/group) exposed 10, 100, and 1000 ppm (13, 133, 1330 mg/m$^3$), 22 hours/day, for up to 29 months, body weight, haematological and pathological examinations did not reveal any dose-dependent effects except for hyperplasia of reactive astroglias in the nervous system. However, this effect was not correlated to dose or exposure time and was found to be reversible in a recovery test (NEDO, 1982).

In rats, exposure for up to 4 weeks to methanol concentrations of 500-5000 ppm (665-6650 mg/m$^3$; 6 hours/day, 5 days/week) did not exhibit exposure-related effects except for increased discharges around the nose and eyes which were considered reflective of upper respiratory tract irritation. In addition, no effects were found on organ or body weight or after histopathological or ophthalmoscopic examinations (Andrews *et al.*, 1987). In another study, no effects on the lungs was observed in rats following a 6-week exposure to concentrations of up to 10,000 ppm (13,300 mg/m$^3$) (White *et al.*, 1983). In 2 dogs exposed to methanol vapours at 10,000 ppm (13,300 mg/m$^3$), for about 3 minutes in each of 8-hour periods/day for 100 consecutive days, no symptoms, unusual behaviour, or visual toxicity was noticed (Sayers *et al.*, 1944). In contrast, rabbits exposed to 46 ppm (61 mg/m$^3$) methanol for 6 months (duration of exposure/day not reported) exhibited ultrastructural changes in the photoreceptor cells of the retina and Müller fibres (Vendilo *et al.*, 1971).

In two 12-month inhalation studies, Fischer-344 rats (n=20/sex/group) and B6C3F1 mice (n=30/sex/group) were exposed to 10, 100, and 1000 ppm (13, 133, 1330 mg/m$^3$) of methanol to examine non-neoplastic toxic effects. At 1000 ppm (1330 mg/m$^3$), slightly reduced weight gains in male and female rats and a small, not significant increase in relative liver and spleen weights in female rats were observed. In mice, body weights were significantly increased in both males (after 6 months) and females (after 9 months) exposed to 1000 ppm. In addition, the incidence and degree of fatty degeneration of hepatocytes was significantly enhanced in the high-exposure groups of mice. Clinical laboratory results did not show any changes attributable to methanol (Katoh, 1989; NEDO, 1987).

### Additional data

In well-performed carcinogenicity studies by the Japanese New Energy Development Organization (NEDO) (see also Section 7.2.4 for neoplastic effects), specific-antibody-free rats (F344/DuCrj; n=52/sex/group) and mice ($B6C3F_1$; n=52 males/group; 53 females/group) were exposed by whole-body inhalation to concentrations of methanol of 0, 13, 133, and 1330 mg/m$^3$ (0, 10, 100, 1000 ppm), 19.5 hours/day, 7 days/week, for 24 months, and 19.1 hours/day, 7 days/week, for 18 months, respectively. The study protocol was based on OECD guidelines. Body weight and food consumption checks, general observations (appearance, behaviour), ophthalmological, clinical-chemical, haematological, and (histo)pathological examinations, and organ weight checks were performed. Statistical analyses included Student's t-test, Welch's t-test, Armitage's Chi-square test, and Fisher's exact probability test.[60,61]

In rats, treatment did not induce clinical signs of intoxication or changes in mortality rates or haematological or clinical chemistry parameters, or effects on food consumption. In the female rats exposed to 1330 mg/m$^3$, slightly, statistically significantly, decreased body weights (by up to 4%; $p<0.05$) between week 51 and 70 were observed. In the other treatment groups, body weights were not affected. At autopsy, no effect on organ weights were observed. Complete histopathological examination, performed in all rats in the control and high-concentration groups and in those found dead/killed moribund from the other two groups, showed various types of non-neoplastic lesions. They were mostly naturally occurring, age-related changes, and none of them was considered specific to methanol exposure.[61]

In mice, apart from a slight increase in body weights of the males and females exposed to 1330 mg/m$^3$ between month 6 and 12, and a slight decrease in feed consumption in female animals of this group between month 7 and 12, methanol treatment did not induce urinalysis, haematology, clinical chemistry, organ weight, or non-neoplastic changes.[60]

Since in these studies exposure by inhalation to concentrations as high as 1330 mg/m$^3$ (1000 ppm), the highest level tested, ca. 19 hours/day, 7 days/week for 18 or 24 months did not induce significant, biologically relevant non-neoplastic effects in mice or rats, the committee considers 1330 mg/m$^3$ (1000 ppm) to be the NOAEL for chronic effects in rats and mice.

The 12-month inhalation NEDO studies in rats and mice and the 29-month NEDO study in monkeys cited above under 'WHO/IPCS data' were described more comprehensively by other reviewers (e.g., Vyskocil and Viau[43]) and evalu-

ating bodies (OECD[4], German MAK Commission[62]). According to OECD, the body and organ weight effects seen in rats and mice exposed to 1330 mg/m$^3$ for 12 months were within a 5% limit. The fatty degeneration in mice livers was 'moderate', occurred obviously in males only, and was considered incidental because of a high incidence of this lesion in controls as well.[4] However, these studies could not be evaluated critically because of inadequate documentation concerning technical data, histopathological findings, and statistical analyses.[4,38,62] Therefore, the committee is of the opinion that no conclusions can be drawn from these studies. However, the committee notes that no significant effects were found in other NEDO studies in rats and mice exposed to up to 1330 mg/m$^3$ for 24 and 18 months, respectively (see above and Section 7.2.4).

In a reproduction/developmental toxicity study (see Section 7.2.6), female cynomolgus monkeys were exposed to methanol concentrations (up to 1800 ppm (2394 mg/m$^3$), 2.5 hours/day, for a total of about 1 year. No effects were found in the female animals with regard to body weight and clinical observations. Corresponding blood methanol levels were 35 mg/L.[63] The NOAEL in this study is 1800 ppm (2394 mg/m$^3$).

### *7.2.4 Carcinogenicity*

In well-performed inhalation studies (see Section 7.2.3 for experimental details and general toxicity), specific-antibody-free rats (F344/DuCrj; n=52/sex/group) and mice (B6C3F$_1$; n=52/sex/group) were exposed by whole-body inhalation to concentrations of methanol of 0, 13, 133, and 1330 mg/m$^3$ (0, 10, 100, 1000 ppm), 19.5 hours/day, 7 days/week, for 24 months, and 19 hours/day, 7 days/week, for 18 months, respectively.[60,61]

In male rats, there was a suggestion of an increased incidence of proliferative changes involving alveolar epithelium. Incidences of papillary adenocarcinomas, papillary adenomas, and adenomatosis (probably transition to papillary adenoma) combined were 5/52 (0+1+4), 6/50 (0+5+1), 7/52 (0+5+2), and 11/52 (1+6+4) for animals exposed to 0, 13, 133, and 1330 mg/m$^3$, respectively. However, the incidences in the treated groups did not differ statistically significantly from those in controls. In female rats, slight, not statistically significant increases in the incidence of phaeochromocytomas and of hyperplasia of medullary cells were found in the adrenal glands of animals exposed to 1330 and 133 mg/m$^3$, respectively. The incidences of phaeochromocytomas and medullary cell hyperplasia were 2/50, 3/51, 2/49 and 7/52, and 2/50, 3/51, 7/49, and 2/51 for animals exposed to 0, 13, 133, and 1330 mg/m$^3$, respectively.[61]

In mice, there were no significant increases in incidences of any tumour in methanol-treated animals compared to controls.[60]

The committee notes that the Italian European Ramazzini Foundation for Oncology and Environmental Sciences (ERF) has published a carcinogenicity study in which methanol was administered in drinking water for 2 years to male and female Sprague-Dawley rats.[64] However, because of severe flaws (see [65,66]) that bring into question the validity and the relevance of the results of the ERF study, the committee will not present and discuss the data and findings (see Annex E).

No evidence of skin carcinogenicity of methanol was found in a study in female mice where methanol was used as a solvent control. Mice of four strains (n=20/strain; Balb/c, Sencar, CD-1, Swiss) were exposed to 25 µL methanol, twice weekly for 50 weeks, and observed until spontaneous death or killed when moribund.[67]

### *7.2.5 Genotoxicity*

In vitro genotoxicity studies with methanol are summarised in Table 7.2, 7.3, and 7.4. In the majority of the studies, negative results were obtained. Nevertheless, some genotoxic activity of methanol has been found in some of the studies.

In a mutagenicity test with *S. typhimurium* strain TA102, methanol, tested with metabolic activation, induced a reproducible increase in revertants over controls by a maximum factor of 1.5-2 at a dose of 240 mg/plate.[68] In the forward mutation test in *E. coli* strain SA500(*λc*I857Δ431)('RK mutatest'), a 23% methanol solution was not mutagenic (cell survival: 53%), while higher concentrations caused mutagenic and cytotoxic (survival <40%) effects.[69] In a study designed to define the optimal amount of the S9 mix to be used to obtain maximum yield of mutations in the mouse lymphoma L5178Y cell mutation assay, McGregor *et al.* found a positive response for methanol when testing a single concentration of 8 mg/mL at S9-mix concentrations $\geq$2.5 mg/mL. These test conditions were cytotoxic, reducing the relative total growth (RTG) to <30%. Concentrations of methanol of 4-40 mg/mL in the presence of 7.5 mg/mL S9 mix caused mutagenicity and cytotoxicity (RTG <15%), but not in the presence of 2.5 mg/mL S9 mix.[70]

Methanol was reported to be positive in an SCE test in Chinese hamster lung cells when tested without metabolic activation at a concentration of 28.5 mg/mL, which caused 50% growth inhibition, while results were negative at lower levels (i.e., 7.1 and 14.3 mg/mL) or when tested with metabolic activation (NEDO,

1987; cited from OECD[4]). A DNA repair test in *E. coli*., in which the ratio of the minimal inhibitory concentration of the repair-proficient to the minimal inhibitory concentration of the of the repair-deficient strains was taken as indicator for genotoxicity, showed a positive result in the liquid micromethod in the absence of S9 and a negative result the presence of S9. The minimal inhibitory concentrations for methanol were very high: 40 and 20 mg/well, respectively (i.e., about 120 and 60 mg/L). In the 'treat-and-plate' method, results were negative without S9 and ambiguous with S9, while no cytotoxicity was observed in the spot test.[68] In a chromosomal malsegregation assay in *A. nidulans*, cytotoxic concentrations of methanol induced aneuploidy (at 5.0% v/v: 0; at 5.6%: 1.5%; at 6.0%: 3.0%; at 7%: 0; survival: 26, 19, 10, and 5%, respectively).[71]

*Table 7.2 In vitro* genotoxicity tests with methanol: mutation assays (from Greim[62], OECD[4], WHO/IPCS[1], unless otherwise noted).

| test system | concentrations | results[a] | | reference |
|---|---|---|---|---|
| | | -S9 | + S9 | |
| *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | <80 mg/plate | - | n.t. | De Flora, 1981[72] |
| *S. typhimurium* TA97 | ≤7.5 mg/plate | - | - | De Flora *et al.*, 1984[68] |
| TA102 | | - | (+)[b] | |
| *S. typhimurium* TA98, TA1535, TA1537, TA1538 | ≤3.6 mg/plate | - | - | Gocke *et al.*, 1981[73] |
| *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | 0.005-5 mg/plate | - | - | Shimizu *et al.*, 1985 |
| *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | not reported | - | - | Simmon *et al.*, 1977 |
| *S. typhimurium* TA98, TA100, TA1535, TA1537 | 3 ìmol/plate | - | - | Florin *et al.*, 1980 |
| *E. coli* SA500(λcI857Δ431) | 23-31% | + | n.t. | Hayes *et al.*, 1990[69] |
| *E. coli* WP2uvrA | 0.005-5 mg/plate | - | - | Shimizu *et al.*, 1985 |
| *S. pombe* | 5% v/v | - | - | Abbondandolo *et al.*, 1980 |
| *S. cerevisiae* ATCC 26422 | 2-8% | - | n.t. | Hamada *et al.*, 1988 |
| Chinese hamster V79 cells reversion to 8-Aza, 6TG or Quabain resistance | 15.8-63.3 mg/mL | - | - | NEDO, 1987 |
| mouse lymphoma L5178Y cells | ≤48 mg/mL | - | n.t. | Amacher *et al.*, 1980[74] |
| mouse lymphoma L5178Y cells | 10 µL/mlL (8 mg/mL) | n.t. | +[c] | McGregor *et al.*, 1988[70] |
| | 5-50 µL/mL (4-40 mg/mL) | n.t. | -/+[d] | |

[a] n.t.: not tested; - = negative; + = positive; (+) = weakly positive; ± = ambiguous

[b] Methanol, with metabolic activation, produced a reproducible, only 1.5-2-fold increase in revertants over controls at a dose of 240 mg/plate

[c] Tested at S9-mix concentrations of ca. 0.3-7.5 mg/mL; positive response at S9-mix concentrations of ≥2.5 mg/mL. At these concentrations, high cytotoxicity was observed (relative total growth - RTG - <30%).

[d] Negative when tested at standard conditions, i.e., a S9-mix concentration of 2.5 mg/mL; positive when tested at a S9-mix concentration of 7.5 mg/mL. Using the high S9-mix concentration was highly cytotoxic (RTG <15%).

*Table 7.3 In vitro* genotoxicity tests with methanol: cytogenicity assays (from Greim[62], OECD[4], WHO/IPCS[1]).

| test system | concentrations | results[a] | | reference |
|---|---|---|---|---|
| | | -S9 | + S9 | |
| Chinese hamster lung cells; chromosome aberrations | 7.1-28.5 mg/mL | - | - | NEDO, 1987 |
| Chinese hamster lung cells; SCE | 7.1-28.5 mg/mL | +[b] | - | NEDO, 1987 |
| Chinese hamster ovary cells; SCE | 0.1% v/v 1x/d for 8 days | n.t. | - | Obe/Ristow, 1977 |

[a] n.t.: not tested; - = negative; + = positive; (+) = weakly positive; ± = ambiguous
[b] Postive only at 28.5 mg/ml, i.e., highest level tested.

*Table 7.4 In vitro* genotoxicity tests with methanol: other tests (from Greim[62], OECD[4], WHO/IPCS[1], unless otherwise noted).

| test system | concentrations | results[a] | | reference |
|---|---|---|---|---|
| | | -S9 | + S9 | |
| *E. coli* WP2, WP67, CM871; | not reported | | | De Flora *et al.*, 1984[68] |
| DNA repair: liquid micromethod | | (+) | - | |
| 2-h preincubation 'treat-and-plate' test | | - | ± | |
| spot test | | - | n.t. | |
| *E. coli* PQ37; SOS chromotest | 0-100 mM (0-3.2 mg/mL) | - | - | von der Hude *et al.*, 1988[75] |
| *E. coli* $WP2_s$ (λ); prophage induction | 0.15-5% | - | - | DeMarini *et al.*, 1991 |
| *A. nidulans* P1; | 5.2-7% v/v | | | Crebelli *et al.*, 1989[71] |
| chromosomal malsegregation: aneuploidy | | + | n.t. | |
| crossing over | | - | n.t. | |
| *N. crassa*; aneuploidy | not reported | | - | Griffiths, 1981 |
| Chinese hamster V79 cells; micronucleus test | 50 µL/mL ( 40 mg/ml) | - | n.t. | Lasne *et al.*, 1984 |
| Syrian hamster embryo cells; micronucleus test | not reported (but based on range-finding) | -[b] | | Fritzenschaf *et al.*, 1993 |

[a] n.t.: not tested; - = negative; + = positive; (+) = weakly positive; ± = ambiguous
[b] Not clear whether test was performed with or without S9 or both.

*In vivo* studies, almost all performed in mice, are summarised in Table 7.5. Exposure by inhalation to concentrations of 1060-5320 mg/m$^3$ (800-4000 ppm; 6 hours/day, 5 days) did not increase the frequency of micronuclei in peripheral blood cells, of SCEs, chromosomal aberrations, or micronuclei in lung cells, or of synaptonemal complex aberrations in spermatocytes.[76]

Following single oral (1000 mg/kg bw) or repeated intraperitoneal administration (25-100 mg/kg bw/day, 3 days), increases in the incidences of chromosomal aberrations, particularly aneuploidy and centric fusions, respectively, were found[77,78] while a single oral dose (1000 mg/kg bw) caused an increased incidence of SCEs[78]. Oral administration of single doses of 1060-8410 mg/kg bw (NEDO, 1987; cited from OECD[4]) and repeated doses of 2500 mg/kg bw[79], did not cause increases in the frequency of micronuclei. In a separate study, a posi-

tive result was obtained following a single oral dose of 1000 mg/kg bw.[78] Two intraperitoneal micronucleus assays with single doses of 1920-4480[73] and repeated doses of 300-2500 mg/kg bw (API, 1991; cited from OECD[4,80]), respectively, were negative. A sex-linked recessive lethal mutations in male *D. melanogaster* was negative.[73]

*Table 7.5 In vivo* genotoxicity studies with methanol.

| test | species ( strain) | route, concentration/dose | results[a] | reference |
|---|---|---|---|---|
| chromosomal aberrations (in primary lung-cell cultures; in spermatocytes) | mouse (C57Bl/6J) (n=10 males/group) | inhalation; 800-4000 ppm (1060-5320 mg/m$^3$), 6 h/d, 5 d | - | Campbell *et al.*, 1991[76] |
| chromosomal aberrations (in bone marrow polychromatic erythrocytes) | mouse | oral (single dose); 1000 mg/kg bw | +[b] | Pereira *et al.*, 1982[78] |
| chromosomal aberrations (bone marrow) | mouse | intraperitoneal; 25-100 mg/kg bw/d, 3 d | +[c] | Chang *et al.*, 1983[77] |
| SCE (in primary lung-cell cultures) | mouse (C57Bl/6J) (n=10 males/group) | inhalation; 800-4000 ppm (1060-5320 mg/m$^3$), 6 h/d, 5 d | - | Campbell *et al.*, 1991[76] |
| SCE (in bone marrow polychromatic erythrocytes) | mouse | oral (single dose); 1000 mg/kg bw | + | Pereira *et al.*, 1982[78] |
| micronucleus (in primary lung-cell cultures and peripheral blood cells) | mouse (C57Bl/6J) (n=10 males/group) | inhalation; 800-4000 ppm (1060-5320 mg/m$^3$), 6 h/d, 5 d | - | Campbell *et al.*, 1991[76] |
| micronucleus (in bone marrow polychromatic erythrocytes) | mouse | oral (single dose); 1000 mg/kg bw | + | Pereira *et al.*, 1982[78] |
| micronucleus (in bone marrow polychromatic erythrocytes) | mouse (ICR) (n=6/group; sex not reported) | oral (gavage; single dose); 1050-8410 mg/kg bw | - | NEDO, 1987 (cited from OECD[4]) |
| micronucleus (in adult and fetal peripheral blood cells) | mouse (CD-1)[d] | oral (gavage); 2500 mg/kg bw, twice daily during gestational days 6-10 | - | Fu *et al.*, 1996[79] |
| micronucleus (in bone marrow polychromatic erythrocytes) | mouse (NMRI) (n=2/sex/group) | intraperitoneal (single dose); 1920-4480 mg/kg bw | - | Gocke *et al.*, 1981 API, 1991(cited from OECD[4] |
| micronucleus (in bone marrow polychromatic erythrocytes) | mouse (Swiss Webster)[d] (n=10 males/group) | intraperitoneal; 300-2500 mg/kg bw/d, 4 d | - | O'Loughlin *et al.*, 1992[80] |
| sex-linked recessive lethal mutations | *D. melanogaster* (male) | 1000 mM in feed | - | Gocke *et al.*, 1981[73] |

[a] -: negative; +: positive
[b] Particularly aneuploidy
[c] Especially centric fusions
[d] Mice were fed either a normal or folate-deficient diet

*Table 7.6* Cell transformation assays with methanol (from Greim[62]; OECD[4]; WHO/IPCS[1]).

| test system | dose | result | | reference |
|---|---|---|---|---|
| | | - S9 | + S9 | |
| mouse embryo fibroblasts C3H/10T1/2C18, +/- TPA | 5-100 mg/mL | - | - | Ragan/Boreiko, 1981 |
| baby Syrian hamster kidney cells BHK21 C13 | 10-100 µl/ml (8-80 mg/ml) | - | - | Strobel/Greb, 1981 |
| Syrian hamster embryo cells (clonal system) | 0.0625-8% | - | n.t.[a] | Pienta *et al.*, 1977 |
| Syrian hamster embryo cells (infected by Simian adenovirus SA7) | 0.06-1 mg/mL | - | n.t.[a] | Heidelberger *et al.*, 1983 |

[a] n.t. = not tested.

Cell transformation assays with methanol (see Table 7.6 were all negative.)

From these data, DECOS' Subcommittee on the Classification of Carcinogenic Substances concluded that methanol is not likely to have a genotoxic potential (see Annex E).

### 7.2.6 *Reproduction toxicity*

Data on the reproduction toxic effects of methanol have been reviewed and evaluated by DECOS' Subcommittee on the Classification of Reproduction Toxic Substances, and are summarised below. No additional studies were retrieved. For detailed information on individual studies, it is referred to the subcommittee's report.[2]

The committee notes that although *in vitro* embryo culture studies[81,82] showed that formate may have developmental toxic properties in rats or mice, *in vivo* experiments[83] did not demonstrate a significant role of formate in methanol-induced developmental effects in mice. According to the committee, this might indicate that the effects described below might be due to methanol and not to formate. The committee further notes that the developmental effects might also be the consequence of the saturation of catalase (which results in high methanol blood levels associated with developmental effects in rodents). Catalase helps to protect against toxicity induced by reactive oxygen species. In *in vitro* studies, inhibition of teratogenicity caused by reproduction toxic agents such as phenytoin, benzo[a]pyrene, and arsenicals by the addition of catalase to cell cultures has been reported, while in other experiments inhibition of catalase produced a significant increase in malformations in mouse embryo cultures (see Clary[84]).

### Fertility

In female monkeys exposed to methanol concentrations 266-2394 mg/m$^3$ (200-1800 ppm), 2.5 hours/day, during pre-mating, mating, and gestation (ca. 350 days in total), no effects were observed on menstrual cycles, conception rate, and live-birth index. The duration of gestation was decreased (within normal range), and the number of delivery complications requiring Caesarean section was, not dose relatedly, decreased.[63] In a series of inhalation studies with male rats[85-88], inconsistent results concerning the effects of methanol on serum hormone concentrations were observed. In general toxicity studies with monkeys[89] and rats[89,90,91], generally, no effects of inhalation of methanol were seen upon histopathological examination of reproductive organs or on reproductive organ weights. In mice, oral administration of methanol caused a slight, but significant increase in the incidence of banana-like sperm heads[92], but the biological significance of this finding was not clear.

### Developmental toxicity

Prenatal developmental toxic effects of methanol were studied in rats[93], mice[83,94-97], and monkeys[63] after exposure by inhalation, and in rats[96,98-100] and mice[79,101] after oral (gavage or drinking water) administration. Generally, prenatal developmental toxicity evidenced by decreased fetal weight, decreased incidence of live fetuses and increased incidences of resorptions, dead fetuses, exencephaly, neural tube defects, cleft palate and skeletal (cranium, vertebrae, ribs, limb, tail) and visceral (eye, brain, cardiovascular and urinary system) malformations was observed. In a number of these studies[79,93,94,96,99,100,102], methanol induced slight developmental effects without overt signs of maternal toxicity while more severe effects were seen in the presence of maternal toxicity such as decreased body weight (gain), unsteady gait, and neurological symptoms (ataxia, circling, tilted heads, depressed motor activity).[83,93,94,95,97,100,101] In the inhalation study in rats[93], no effects on body weight (gain) or overt methanol-related toxic effects were observed, apart from a slight unsteady gait during the first days of exposure to 26,600 mg/m$^3$ (10,000 ppm). The NOAEL was 5000 ppm (6650 mg/m$^3$), with a LOAEL of 10,000 ppm (13,300 mg/m$^3$), at which decreased fetal weights were induced. The methanol levels in maternal blood corresponding to the NOAEL and LOAEL were 1000-2170 and 1840-2240 mg/L, respectively. The mice studies were generally performed with one methanol concentration, being 13,300 mg/m$^3$ (10,000 ppm) or higher. In the study by Rogers *et al.*[102], mice were exposed to six concentrations ranging from 1330 to 19,950 mg/m$^3$

(1000-15,000 ppm; 7 hours/day, gestational days 6-15): no maternally toxicity was observed at any of the concentrations. For developmental effects, the NOAEL was 1330 mg/m$^3$ (1000 ppm) and the LOAEL 2660 mg/m$^3$ (2000 ppm), at which increased incidences of cervical ribs were seen. The corresponding methanol levels in maternal blood were 97 and 537 mg/L, respectively.

The prenatal developmental toxic effects found *in vivo* were confirmed by a series of mechanistic *in vitro* developmental toxicity studies using rat and mouse whole embryo culture assays.[82,103-109]

Post-natal development studies were performed in rats[110,111] and in monkeys[63] after exposure by inhalation and in rats[112] after oral (drinking water) administration. In the offspring of rats exposed to 5985 mg/m$^3$ (4500 ppm; 6 hours/day, gestational day 6 to post-natal day 21), only subtle neurobehavioural effects were observed in both neonates and adults. There were no effects on dam or pup body weights. Maternal blood methanol levels were 500-800 mg/L; those of pups about twice as high.[111] Except for a small delay in vaginal opening, no effect was seen on any of the developmental parameters measured in the offspring of female rats exposed to 19,950 mg/m$^3$ (15,000 ppm; 7 hours/day, gestational days 7-19). Body weights were decreased in the dams during the first exposure days and in the pups on post-natal days 1, 21, and 35. Maternal serum methanol levels declined from ca. 3800 mg/L on exposure day 1 to ca. 3100 mg/L on exposure day 12.[110] Slight effects on neurobehavioural parameters, in the absence of maternal toxicity, were seen in the offspring of dams treated with ca. 2500 mg/kg bw/day in the drinking water (gestational days 17-17 or 17-19).[112]

The committee has re-evaluated the developmental study with monkeys.[63] In this study, no toxic effects of methanol exposure were found in the mothers at any of the concentrations tested (200-1800 ppm or 266-2394 mg/m$^3$ with corresponding maternal methanol blood levels of 5-35 mg/L). The developmental findings in offspring were small, occurred in the presence of large variations among offspring, and were assessed in a low number of offspring. In view of the above and the large number of neurobehavioural tests that were performed in this study, the committee concludes that this study is inconclusive.

### Lactation

Effects on pup weight and neurodevelopmental toxicity parameters were described in pups exposed to methanol via the lactating mother.[113]

## 7.3 Summary

Acute effects of methanol in humans reported following ingested or inhaled unspecified high levels of methanol, often in combination with other chemicals, included an initial depression of the central nervous system, which after a latent period of several hours to two days is often followed by metabolic acidosis and ocular toxicity. The direct depression effects on the CNS are thought to be caused by methanol, whereas metabolic acidosis and ocular toxicity are attributed to its metabolite, formic acid. Minimal lethal oral doses are between 300 and 1000 mg/kg bw. Fatalities were reported at blood methanol levels of 1500-2000 mg/L, and CNS and ocular effects at levels above 200 and 500 mg/L, respectively.

In a number of controlled inhalation studies in which healthy human volunteers were exposed at concentrations not exceeding 200 ppm (266 mg/m$^3$), for 75 to 240 minutes, no irritation or relevant neurophysiological or neurobehavioural effects were observed.

There were hardly any epidemiological studies addressing occupational chronic exposure to methanol and adverse effects, including carcinogenicity and reproduction toxicity. In one study, exposure for presumably about 3 years to a duplicator fluid containing 99% methanol at 15-minute average concentrations ranging from 365 to 3080 ppm (485-4096 mg/m$^3$), with 70% exceeding 800 ppm (1064 mg/m$^3$) induced eye irritation, dizziness, headaches, blurred vision, and nausea.

Liquid methanol was found not or moderately irritating to the skin of rabbits; it did not have skin sensitising properties in guinea pigs. Depending on the amount of undiluted material instilled, methanol induced mild or severe and persisting damage to the eyes of rabbits; a concentration of 29% was found to be the maximum concentration not causing eye irritation. Sensory irritation of the respiratory tract was examined in mice: depending on strain and protocol, $RD_{50}$ values were 33,649 (25,300 ppm) and 55,214 mg/m$^3$ (41,514 ppm).

For rats and mice, $LC_{50}$ values range between 79,000 and 130,340 mg/m$^3$ (59,200 and 98,000 ppm) (exposure duration: 2.25 to 8 hours). The dermal $LD_{50}$ in rabbits was 17,000 mg/kg bw. In rats, no mortality was observed after occlusive application of 35,000 mg/kg bw; amounts of 45,000 mg/kg bw were lethal. Oral $LD_{50}$ values range between 6200 and 10,000 mg/kg bw for rats, mice, and dogs; for monkeys, minimal doses causing mortality were 2000-7000 mg/kg bw.

In repeated-dose toxicity studies, no effects were observed in male and female monkeys at exposure to concentrations of 665 to 6650 mg/m$^3$ (500-5000

ppm), 6 hours/day, 5 days/week, for 4 weeks, and in female monkeys to concentrations of 266-2394 mg/m$^3$ (200-1800 ppm), 2.5 hours/day, for ca. 350 days (i.e., during pre-mating, mating, and gestation). In rats exposed to concentrations of 665 to 6650 mg/m$^3$ (500-5000 ppm), 6 hours/day, 5 days/week, for 4 weeks, no effects were observed except for increased discharges around nose and eyes while a 6-week exposure to 13,300 mg/m$^3$ (10,000 ppm) did not cause pulmonary toxicity.

Well-performed inhalation studies in which rats and mice were exposed to concentrations of 13 to 1330 mg/m$^3$ (10-1000 ppm), 19-20 hours/day, for 18-24 months, did not show evidence for neoplastic or non-neoplastic effects of methanol.

The majority of mutagenicity tests in bacteria, yeasts, and mammalian cells, assays on chromosome aberrations and SCEs in mammalian cells, micronucleus tests in mammalian cells, and other tests on DNA or chromosome damage in bacteria and fungi were negative. *In vivo*, exposure by inhalation did not increase the frequency of micronuclei in peripheral blood or lung cells or of SCEs or chromosomal aberrations in lung cells of mice. Both positive and negative results were observed in tests in which methanol was orally or intraperitoneally administered to mice, but tests with higher doses and repeated dosing were negative. A mutation assay in fruit flies was negative. All four cell transformation assays were negative.

In reproduction toxicity studies, exposure of pregnant rats to methanol concentrations of 13,300 and 26,600 mg/m$^3$ (10,000 and 20,000 ppm), 7 hours/day, on gestational days 1-19 and 7-15, respectively, caused decreased fetal weights and at 26,600 mg/m$^3$ also increased incidences of skeletal and visceral malformations. These effects were not seen at 6650 mg/m$^3$ (5000 ppm). In mice exposed to 2660 mg/m$^3$ (2000 ppm), 7 hours/day, on gestational days 6 through 15, there were increased incidences of cervical ribs at concentrations of 2660 mg/m$^2$ (2000 ppm) and above and of cleft palate and exencephaly at concentrations of 6650 mg/m$^3$ (5000 ppm) and above which were not seen at 1330 mg/m$^3$ (1000 ppm). No effects were seen in female monkeys exposed to methanol concentrations 266-2394 mg/m$^3$ (200-1800), 2.5 hours/day, during pre-mating, mating, and gestation (ca. 350 days in total); a conclusive judgment of the results of the neurobehavioural tests performed in their offspring was hampered because they were small, occurred in the presence of large variations among offspring, and were assessed in a low number of offspring.

Chapter 8

# Existing guidelines, standards and evaluations

## 8.1 General population

Guidelines for the general population with regard to inhalation exposure to methanol were not found.

## 8.2 Working population

Occupational exposure limits for methanol in some European countries and the USA, listed in the most recent publications available to the committee, are presented in Table 8.1.

*Table 8.1* Occupational exposure limits for methanol in various countries.

| country - organisation | occupational exposure limit | | time-weighted average | type of exposure limit | note[a] | reference[b] |
|---|---|---|---|---|---|---|
| | mg/m$^3$ | ppm | | | | |
| the Netherlands | | | | | | |
| - Ministry of Social Affairs and Employment | 260<br>520 | | 8 h<br>15 min | legally binding | S | 114 |
| Germany | | | | | | |
| - DFG MAK-Kommission | 270<br>1080 | 200<br>800 | 8 h<br>15 min[c] | | S, [d] | 115 |
| - AGS | 270<br>1080 | 200<br>800 | 8 h<br>15 min | | S, [e] | 116 |
| Sweden | 250<br>350 | 200<br>250 | 8 h<br>15 min | | S | 117 |
| Denmark | 260 | 200 | 8 h | | S | 118 |
| United Kingdom | | | | | | |
| - HSE | 266<br>333 | 200<br>250 | 8 h<br>15 min | WEL | S | 119 |
| USA | | | | | | |
| - ACGIH | | 200<br>250 | 8 h<br>15 min | TLV<br>STEL | S | 120 |
| - OSHA | 260 | 200 | 8 h | PEL | | 120 |
| - NIOSH | 260<br>325 | 200<br>250 | 8 h<br>15 min | REL | S | 120 |
| European Union | | | | | | |
| - SCOEL | 260 | 200 | 8 h | IOELV | S | 121 |

a S = skin notation; which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.
b Reference to the most recent official publication of occupational exposure limits.
c Maximum per shift: 4, with a minimum interval between peaks of 1 hour.
d Classified in pregnancy group C: i.e., there is no reason to fear a risk of damage to the embryo or fetus when MAK and Bat values are observed.
e There is no reason to fear a risk of damage to the embryo or fetus when MAK and Bat values are observed.

## 8.3 Evaluations

- European Union
  The committee could not retrieve the documentation for the Indicative Occupational Exposure Limit Value (IOELV) for methanol of 200 ppm (260 mg/m$^3$) as an 8-hour time-weighted average.
- American Conference of Governmental Industrial Hygienists (ACGIH)
  The TLV of 200 ppm (260 mg/m$^3$) and the STEL of 250 ppm (325 mg/m$^3$) are based on the human and animal toxicological responses including sensory irritation, headaches, nausea, and visual disturbances, as well as optic neu-

ropathy, metabolic acidosis, narcosis, and respiratory depression at high exposure levels (1000 to 10,000 ppm from chronic studies).[122]

- Deutsche Forschungsgemeinschaft (DFG)
  In Germany, the MAK value for methanol was re-evaluated in 1999. From essentially the same data as presented in this report, the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area concluded that the prevailing MAK value of 200 ppm (266 mg/m$^3$) did not have an adequate scientific basis. The DFG commission deemed the rodent studies not suitable for the derivation of a MAK value due to differences in metabolism, while the chronic study in monkeys was disregarded because of inadequate methods and documentation. Based on limited occupational data showing effects such as headaches and blurred vision occurring at concentrations of 365-3080 ppm (485 to 4096 mg/m$^3$), but of about 1040 ppm (1436 mg/m$^3$) at average, and on data from volunteer studies showing no adverse effects at 4-hour exposures to 200 ppm (266 mg/m$^3$), the DFG commission inferred that local and systemic effects would not develop at low concentrations and that there would be no need to change the prevailing MAK value. The DFG commission decided to retain this MAK value provisionally.[62]

Chapter 9

# Hazard assessment

## 9.1 Assessment of the health risk

Methanol is readily absorbed following inhalation, ingestion, and dermal application. Methanol is metabolised in the liver by sequential oxidative steps to form formaldehyde, formate, and $CO_2$. The kinetics of methanol metabolism differ both qualitatively and quantitatively between rodents and primates/humans. While the conversion of methanol into formate is rate limiting in rodents, the conversion of formate into $CO_2$ is rate limiting in primates/humans. As a result, sufficiently high doses of methanol cause accumulating blood levels of formate in primates/humans - resulting in formate-characteristic toxicity (metabolic acidosis, ocular toxicity) - and of methanol in rodents. Generally, blood methanol levels are not increased above background levels in rats and monkeys, and slightly (i.e., by a factor 2-3) in humans following single 6-hour exposures up to ca. 266 mg/m$^3$ (200 ppm). At exposures up to ca. 1600 mg/m$^3$ (1200 ppm), blood levels seem to be similar in rats, monkeys, and humans. At higher levels, blood methanol levels increase non-linearly in rats and, less steeply, in monkeys and linearly in humans. Levels in mice raise even more sharply than in rats (see also Table 5.3). Human blood methanol levels of >200, >500, and 1500-2000 mg/L were associated with CNS effects, ocular effects, and mortality, respectively. Formate accumulation due to saturation of the folate pathway may occur in humans at single (bolus) methanol doses of ca. 210 mg/kg bw. The committee notes that such a amount would be taken up at exposure to ca. 17,000 mg/m$^3$

(12,750 ppm) for 1 hour (assuming a 70-kg worker inhales 10 $m^3$ during an 8-hour working day and a pulmonary retention of 70%), and that it is, therefore, unlikely that saturation of the formate metabolism would occur under 'normal' occupational conditions.

In controlled inhalation studies in which human volunteers were exposed at concentrations not exceeding 200 ppm (266 $mg/m^3$), for 75 to 240 minutes, no irritation was observed. In occupational situations, eye irritation was reported at 15-minute average concentrations mostly exceeding 1064 $mg/m^3$ (800 ppm). In animal experiments, liquid methanol was not found to be skin irritating or sensitising; depending on the amount of undiluted material or of the concentration (% dilution) instilled, methanol induced mild or severe and persisting damage to the eyes. Sensory respiratory tract irritation, as measured by the $RD_{50}$, occurred at some tens of thousands of $mg/m^3$.

Acute system effects in humans generally include central nervous system depression, (followed by) metabolic acidosis, ocular toxicity, and death. However, in view of the corresponding blood methanol levels (see afore), the committee is of the opinion that the aforementioned effects will occur only at concentrations in the thousand or ten thousand $mg/m^3$ ranges. In controlled inhalation studies in which human volunteers were exposed at concentrations not exceeding 200 ppm (266 $mg/m^3$), for 75 to 240 minutes, no relevant central nervous system effects were observed. Although all these studies were performed with relatively small groups of healthy volunteers, the committee judges that exposure to 266 $mg/m^3$ (200 ppm) for up to 4 hours will not result in neurotoxic effects in workers.

In acute animal experiments, $LC_{50}$ values range between 79,000 and 130,340 $mg/m^3$ (59,200 and 98,000 ppm) (exposure duration: 2.25 to 8 hours) for rats and mice. The dermal $LD_{50}$ in rabbits was 17,000 mg/kg bw, while in rats, no mortality was observed after occlusive application of 35,000 mg/kg bw; amounts of 45,000 mg/kg bw were lethal. Oral $LD_{50}$ values range between 6200 and 10,000 mg/kg bw for rats, mice, and dogs; for monkeys, minimal doses causing mortality were 2000-7000 mg/kg bw.

There were no human studies which allow the committee to assess health effects following chronic exposure to methanol. In one study, irregular exposure for presumably about 3 years to a duplicator fluid containing 99% methanol at 15-minute average concentrations ranging from 365 to 3080 ppm (485-4096 $mg/m^3$), with 70% exceeding 800 ppm (1064 $mg/m^3$) induced eye irritation, dizziness, headaches, blurred vision, and nausea. However, the committee notes that

symptoms reported are more likely to be related to acute than to repeated exposure. Further, health effects were recorded through a symptoms questionnaire only and no additional physical or clinical examinations were done. The study seems to have been triggered by complaints and may have been subject to responder bias.

In laboratory animal studies, no effects were observed in male and female monkeys at exposure to concentrations of 665 to 6650 mg/m$^3$ (500-5000 ppm), 6 hours/day, 5 days/week, for 4 weeks, and in female monkeys to concentrations of 266-2394 mg/m$^3$ (200-1800 ppm), 2.5 hours/day, for ca. 350 days (i.e., during pre-mating, mating, and gestation). In rats exposed to concentrations of 665 to 6650 mg/m$^3$ (500-5000 ppm), 6 hours/day, 5 days/week, for 4 weeks, no effects were observed except for increased discharges around nose and eyes, which were thought to be indicative of respiratory tract irritation. However, in another study, a 6-week exposure to 13,300 mg/m$^3$ (10,000 ppm) did not cause pulmonary toxicity. These data indicate NOAELs of at least 6650 mg/m$^3$ (5000 ppm) for rats and monkeys exposed 6 hours/day, 5 days/week, for 4 weeks, and of at least 2394 mg/m$^3$ (1800 ppm) in monkeys exposed 2.5 hours/day, for ca. 1 year. The corresponding blood methanol levels might be ca. 400 to ca. 880 mg/L in rats (see Table 5.3) and were 35 mg/L in monkeys.

In well-performed inhalation studies in which rats and mice were exposed to concentrations of 13 to 1330 mg/m$^3$ (10-1000 ppm), 19-20 hours/day, for 18-24 months, no neoplastic or non-neoplastic effects were observed. No specific neurobehavioural tests were performed in these inhalation studies but post-mortem brain histopathology and frequent observations of appearance and behaviour did not reveal CNS effects. The results suggest that methanol is not carcinogenic following inhalation but the committee notes that an MTD (‘maximum tolerable dose’) was not reached in these inhalation studies. From these findings, the Subcommittee on the Classification of Carcinogenic Substances of DECOS concludes that methanol cannot be classified with respect to its carcinogenicity (comparable with EU class ‘not classifiable’).

The majority of *in vitro* genotoxicity tests performed in bacteria, yeast, fungi, mammalian cells, and/or fruit flies on the induction of mutations, chromosome aberrations, SCEs, micronuclei, or other kinds of DNA or chromosome damage were negative. *In vivo* inhalation assays in mice on the induction of micronuclei, chromosome aberrations, or SCEs were negative. Tests following oral or intraperitoneal administration to mice showed both positive and negative results, but tests with higher doses and repeated dosing were negative. Cell transformation

assays were negative. The aforementioned subcommittee is of the opinion that the data indicate that methanol is not likely to be genotoxic.

Based on the lack of a genotoxic potential and negative results in inhalation studies, the committee is of the opinion that methanol is not likely to have a carcinogenic potential.

Reproduction toxicity studies in mice indicate that methanol and not formate is the compound causing developmental effects. No effects were seen in female monkeys exposed to methanol concentrations of 266-2394 mg/m$^3$ (200-1800 ppm), 2.5 hours/day, during pre-mating, mating, and gestation (ca. 350 days in total); a conclusive judgment of the results of the neurobehavioural tests performed in their offspring was hampered because they were small, occurred in the presence of large variations among offspring, and were assessed in a low number of offspring. Exposure of pregnant rats to methanol concentrations of 13,300 mg/m$^3$ (10,000 ppm; 7 hours/day, gestational days 1-19) caused decreased fetal weights in the absence of maternal toxicity. These fetal effects were not seen at 6650 mg/m$^3$ (5000 ppm). In mice exposed to 2660 mg/m$^3$ (2000 ppm; 7 hours/day, gestational days 6-15), there were increased incidences of cervical ribs but no maternal toxicity. The NOAEL for developmental toxicity was 1330 mg/m$^3$ (1000 ppm). Based on these prenatal developmental toxic effects, the Subcommittee on the Classification of Reproduction Toxic Substances recommended classification of methanol in category 2 (‘substances which should be regarded as if the cause developmental toxicity in humans’) and labelling with T;R64 (‘may cause harm to the unborn child’). Based on the methanol levels measured in the blood of mice and rats at the NOAELs (ca. 100 and 1000-2170 mg/L, respectively) and LOAELs (ca. 540 and 1840-2240 mg/L, respectively) of the reproduction toxicity studies presented above, the committee is of the opinion that methanol is not likely to induce reproduction toxic effects in occupationally exposed workers.

## 9.2 Recommendation of the health-based recommended occupational exposure limit

Since there were no human studies which allow to assess health effects following chronic exposure to methanol, the committee takes the laboratory animal studies by Takeda[61] and Matsuura[60] as starting points for deriving a health-based recommended occupational exposure limit (HBROEL). In these studies, general observations (appearance, behaviour), ophthalmological, clinical-chemical, haematological, and complete (histo)pathological examinations, and body and

organ weight checks did not reveal neoplastic or non-neoplastic effects in rats and mice exposed to methanol concentrations up to 1330 mg/m$^3$ (1000 ppm), 19-20 hours/day, 7 days/week, for 18-24 months.

Taking the NOAEL of 1330 mg/m$^3$ and applying an assessment factor of 10 for interspecies and intraspecies variation, the committee recommends a health-based occupational exposure limit of 133 mg/m$^3$ (100 ppm) for methanol. Moreover, the committee notes that the NOAEL was the highest concentration level tested and that exposure was almost continuous, and concludes that these aspects provide an additional margin of safety.

The committee is of the opinion that the HBROEL of 133 mg/m$^3$ (100 ppm) will also protect workers from acute neurotoxic/CNS effects.

## 9.3 Skin notation

According to the committee, a 'skin notation' is warranted if the amount absorbed by both hands and underarms (total surface area: 2000 cm$^2$) during a 1-hour contact with the liquid could amount to more than 10% of the amount absorbed via the lungs following exposure to the occupational exposure limit for eight hours (assuming that this limit is based on systemic effects rather than on local effects).

In a human volunteer study, an average dermal penetration rate of 8.1±3.7 mg/cm$^2$/h was determined.[36,37] Using this rate, it can be calculated that an amount of 16,200 mg methanol (8.1 mg/cm$^2$/h x 2000 cm$^2$ x1 h) can be taken up by dermal contact, which is far more than 10% of the amount absorbed via the lungs at an 8-hour exposure to the proposed health-based occupational exposure limit of 133 mg/m$^3$ (100 ppm).

Therefore, the committee considers a 'skin notation' for methanol warranted.

## 9.4 Groups at extra risk

Folic acid-deficient people might be at extra risk due to the involvement of folate in the metabolism of methanol.

## 9.5 Health-based recommended occupational exposure limit (HBROEL)

The Dutch Expert Committee on Occupational Safety recommends a health-based occupational exposure limit for methanol of 133 mg/m$^3$ (100 ppm), as an 8-hour time-weighted average concentration, and a skin notation.

Chapter 10

# Recommendations for research

The committee recommends more research on the possible neurological effects of methanol following acute and chronic exposure.

# References


1 World Health Organization/International Programme on Chemical Safety (WHO/IPCS). Methanol. Geneva, Switzerland: WHO, 1997; Environmental Health Criteria 196 [cited December 2009]. Available from: http://www.inchem.org/documents/ehc/ehc/ehc196.htm.

2 Health Council of the Netherlands: Committee for Compounds toxic to reproduction. Methanol; evaluation of the effects on reproduction, recommendation for classification. The Hague, the Netherlands: Health Council of the Netherlands, 2006; Publication No. 2006/04OSH [cited December 2009]. Available from: http://www.gezondheidsraad.nl/sites/default/files/06@04OSH.PDF.

3 Lide DR, editor. CRC handbook of chemistry and physics. 86th edition. Boca Raton FL, USA: CRC Press, 2007-2008.

4 Organisation for Economic Co-operation and Development (OECD). Methanol. SIDS initial assessment report for SIAM 19, 2004. [cited August 2008]. Available from: http://cs3-hq.oecd.org/scripts/hpv/.

5 Sangster J. Octanol-water partition coefficients of simple organic compounds. J Phys Chem Ref Data 1989; 18(3): 1111-229.

6 Amoore JE, Hautala E. Odor as an aid to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J Appl Toxicol 1983; 3(6): 272-90.

7 Ruth JH. Odor thresholds and irritation levels of several chemical substances: a review. Am Ind Hyg Assoc J 1986; 47(3): A142-51.

8 SRC. Interactive LogKow (KowWin) demo. [cited April 2009]. Available from: http://www.srcinc.com/what-we-do/databaseforms.aspx?id=385.

9 European Parliament and the Council of the European Union. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Official Journal of the European Union 2008; (L353): 1-1355. [cited August 2009]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:353:0001:1355:EN:PDF.

10 US National Institute for Occupational Safety and Health (NIOSH). NIOSH method 2000: Methanol. [cited March 2008] Available from http://www.cdc.gov/niosh/docs/2003-154/pdfs/2000.pdf.

11 US Department of Labor: Occupational Safety & Health Administration (OSHA). Methyl Alcohol: OSHA Method 91. [cited March 2008]. Available from: http://www.osha.gov/dts/sltc/methods/organic/org091/org091.html.

12 Angerer J, Schaller KH. Alcohols and Ketones. In: Analysis of hazardous substances in biological materials. Weinheim, FRG: Wiley-VCH; 1997: 1-33.

13 Sedivec V, Mraz M, Flek J. Biological monitoring of persons exposed to methanol vapours. Int Arch Occup Environ Health 1981; 48(3): 257-71.

14 Heinrich R, Angerer J. Occupational chronic exposure to organic solvents. X. Biological monitoring parameters for methanol exposure. Int Arch Occup Environ Health 1982; 50(4): 341-9.

15 American Conference of Governmental Industrial Hygienists (ACGIH). Methanol: BEI® 7th edition documentation. Cincinnati OH, USA: ACGIH, 2005.

16 BioMCN. Our product: process. [cited January 2010]. Available from: http://www.biomcn.eu/our-product/process.html.

17 Methanol Institute. Methanol safe handling manual (prepared by Alliance Consulting International, San Diego CA, USA). Arlington VA, USA: Methanol Institute, 2008. [cited February 2009]. Available from: http://www.methanol.org/pdfFrame.cfm?pdf=MethanolSafeHandlingManualOct2008.pdf.

18 Tsai P-Y. Methanol exposure from an M85 fueled vehicle parked in an attached garage [Thesis]. 2000.

19 Becalski A, Bartlett KH. Methanol exposure to car occupants from windshield washing fluid: a pilot study. Indoor Air 2006; 16(2): 153-7.

20 van Rooij L, Konings EJM, Heida P, van Hamersveld I.C.M., van der Wielen J, Kooijman M. Onderzoek naar de kunstmatige zoetstoffen Sacharine, Aspartaam, Acesulfaam-K en Cyclamaat in levensmiddelen. Eindhoven: Voedsel en Waren Autoriteit; 2004.

21 National Toxicology Program (NTP): Center for the Evaluation of Risks to Human Reproduction (CERHR). NTP-CERHR monograph on the potential human reproductive and developmental effects of methanol. Research Triangle Park NC, USA: National institute of Environmental Health Sciences, 2003; NIH Publication No. 03-4479. [cited April 2009]. Available from: http://cerhr.niehs.nih.gov/chemicals/methanol/Methanol_Monograph.pdf.

22 Susi P, Flynn M, Curran P. Methanol exposure among school workers during spirit duplicator use. Appl Occup Environ Hyg 1996; 11(11): 1340-5.

23 Kauppinen T, Teschke K, Savela A, Kogevinas M, Boffetta P. International data base of exposure measurements in the pulp, paper and paper product industries. Int Arch Occup Environ Health 1997; 70(2): 119-27.

24 Teschke K, Ahrens W, Andersen A, Boffetta P, Fincham S, Finkelstein M *et al.* Occupational exposure to chemical and biological agents in the nonproduction departments of pulp, paper, and paper product mills: an international study. Am Ind Hyg Assoc J 1999; 60(1): 73-83.

25 Bleich S, Hapla F, Sprung R. Possible risk to develop nasal cancer by occupational exposure to wood dust containing methanol and methylacetate. Investigations of wood dust using headspace-gas chromatography. Holz Roh- Werkst 1998; 56(6): 367-72.

26 Chan C-C, Shie R-H, Chang T-Y, Tsai D-H. Workers' exposures and potential health risks to air toxics in a petrochemical complex assessed by improved methodology. International Archives of Occupational and Environmental Health 2006; 79(2): 135-42.

27 Di LL, Zocchetti C, Carpinelli G, Capozzi D, Margiotta M, De FG *et al.* Respiratory mucus transportability is impaired in foundry workers: a longitudinal study. Med Lav 1998; 89(4): 323-33.

28 Perkins RA, Ward KW, Pollack GM. Methanol inhalation: site and other factors influencing absorption, and an inhalation toxicokinetic model for the rat. Pharm Res 1996; 13(5): 749-55.

29 Chuwers P, Osterloh J, Kelly T, D'Alessandro A, Quinlan P, Becker C. Neurobehavioral effects of low-level methanol vapor exposure in healthy human volunteers. Environ Res 1995; 71(2): 141-50.

30 Osterloh JD, D'Alessandro A, Chuwers P, Mogadeddi H, Kelly TJ. Serum concentrations of methanol after inhalation at 200 ppm. J Occup Environ Med 1996; 38(6): 571-6.

31 Franzblau A, Batterman SA, Zhou N, Stepien CJ, D'Arcy JB, Sargent NE *et al.* Evaluation of methanol and formate in urine as biological exposure indices of methanol exposure. Appl Occup Environ Hyg 1997; 12(5): 367-74.

32 Batterman SA, Franzblau A, D'Arcy JB, Sargent NE, Gross KB, Schreck RM. Breath, urine, and blood measurements as biological exposure indices of short-term inhalation exposure to methanol. Int Arch Occup Environ Health 1998; 71(5): 325-35.

33 Ernstgård L, Shibata E, Johanson G. Uptake and disposition of inhaled methanol vapor in humans. Toxicol Sci 2005; 88(1): 30-8.

34 Fisher JW, Dorman DC, Medinsky MA, Welsch F, Conolly RB. Analysis of Respiratory Exchange of Methanol in the Lung of the Monkey Using a Physiological Model. Toxicol Sci 2000; 53: 185-93.

35 Bouchard M, Brunet RC, Droz PO, Carrier G. A biologically based dynamic model for predicting the disposition of methanol and its metabolites in animals and humans. Toxicol Sci 2001; 64(2): 169-84.

36 Batterman SA, Franzblau A, Zhou N. Airborne emissions at skin surfaces: a potential biological exposure index. Int Arch Occup Environ Health 1996; 68(4): 268-74.

37 Batterman SA, Franzblau A. Time-resolved cutaneous absorption and penetration rates of methanol in human volunteers. Int Arch Occup Environ Health 1997; 70: 341-51.

38 Kavet R, Nauss KM. The toxicity of inhaled methanol vapors. Crit Rev Toxicol 1990; 21(1): 21-50.

39 Hori Ha, Ishidao T, Oyabu T, Yamato H, Morimoto Y, Tanaka I. Effect of simultaneous exposure to methanol and toluene vapor on their metabolites in rats. J Occup Health 1999; 41(3): 149-53.

40 Ishidao T, Ishimatsu S, Arashidani K, Hori H. Effect of repeated exposure to methanol and toluene vapor on the metabolism of rats. Ind Health 2000; 38(4): 405-7.
41 DFG. Methanol. List of MAK and BAT values 1999; 16.
42 Passarelli MM, Paolielo MM, Matsuo T, Turin CA, Nascimento ES. Methanol reference values in urine from inhabitants of Brazil. Sci Total Environ 1999; 243-244: 349-52.
43 Vyskocil A, Viau C. Proposal for reference concentrations (RfC) for inhalation exposure to methanol. Environ Toxicol Pharmacol 2000; 9(1-2): 9-18.
44 Mann WJ, Muttray A, Schaefer D, Klimek L, Faas M, Konietzko J. Exposure to 200 ppm of methanol increases the concentrations of interleukin-1beta and interleukin-8 in nasal secretions of healthy volunteers. Ann Otol Rhinol Laryngol 2002; 111(7 Pt 1): 633-8.
45 Muttray A, Kurten R, Jung D, Schicketanz KH, Konietzko J. Acute effects on the human EEG after an external exposure to 200 ppm methanol. Int Arch Occup Environ Health 2001; 74(1): 43-8.
46 Lee EW, Terzo TS, D'Arcy JB, Gross KB, Schreck RM. Lack of blood formate accumulation in humans following exposure to methanol vapor at the current permissible exposure limit of 200 ppm. Am Ind Hyg Assoc J 1992; 53(2): 99-104.
47 Cook MR, Bergman FJ, Cohen HD, Gerkovich MM, Graham C, Harris RK *et al.* Effects of methanol vapor on human neurobehavioral measures. Res Rep Health Eff Inst 1991;(42): 1-45.
48 LoVecchio F, Sawyers B, Thole D, Beuler MC, Winchell J, Curry SC. Outcomes following abuse of methanol-containing carburetor cleaners. Hum Exp Toxicol 2004; 23(10): 473-5.
49 Davis LE, Hudson D, Benson BE, Jones Easom LA, Coleman JK. Methanol poisoning exposures in the United States: 1993-1998. J Toxicol Clin Toxicol 2002; 40(4): 499-505.
50 Gattas GJ, Cardoso, Medrado Faria, Saldanha PH. Frequency of oral mucosa micronuclei in gas station operators after introducing methanol. Occup Med (Lond) 2001; 51(2): 107-13.
51 Infante-Rivard C, Siemiatycki J, Lakhani R, Nadon L. Maternal exposure to occupational solvents and childhood leukemia. Environ Health Persp 2005; 113(6): 787-92.
52 Rennix CP, Quinn MM, Amoroso PJ, Eisen EA, Wegman DH. Risk of breast cancer among enlisted army women occupationally exposed to volatile organic compounds. Am J Industr Med 2005; 48(3): 157-67.
53 Lorente C, Cordier S, Bergeret A, De Walle HE, Goujard J, Ayme S *et al.* Maternal occupational risk factors for oral clefts. Occupational Exposure and Congenital Malformation Working Group. Scand J Work Environ Health 2000; 26(2): 137-45.
54 Hantson P, Lambermont JY, Mahieu P. Methanol poisoning during late pregnancy. J Toxicol Clin Toxicol 1997; 35(2): 187-91.
55 Bharti D. Intrauterine cerebral infarcts and bilateral frontal cortical leukomalacia following chronic maternal inhalation of carburetor cleaning fluid during pregnancy. J Perinatol 2003; 23(8): 693-6.
56 Morgan RL, Sorenson SS, Castles TR. Prediction of ocular irritation by corneal pachymetry. Food Chem Toxicol 1987; 25(8): 609-13.
57 Nagami K, Maki E. In vitro cytotoxicity test for estimating non-ocular irritation dose of ophthalmic solutions. Cell Biol Toxicol 1993; 9(2): 107-18.

58 Muller J, Greff G. [Relation between the toxicity of molecules of industrial value and their physico-chemical properties: test of upper airway irritation applied to 4 chemical groups]. Food Chem Toxicol 1984; 22(8): 661-4.

59 Kane LE, Dombroske R, Alarie Y. Evaluation of sensory irritation from some common industrial solvents. Am Ind Hyg Assoc J 1980; 41(6): 451-5.

60 Matsuura I. 18-month Inhalation carcinogenicity study of methanol in B6C3F1 mice: test report. Tokyo: Mitsubishi Chemical Safety Institute; 1985: Test No.: 4A-223.

61 Takeda K. 24-month Inhalation carcinogenicity study on methanol in Fischer rats: test report. Tokyo: Mitsubishi Chemical Safety Institute; 1985: Test No.: 5A-268.

62 Methanol. In: Greim H, editor. Occupational toxicants. Critical data evaluation for MAK values and classification of carcinogens. Weinheim, FRG: Wiley-VCH; 2001: 144-75.

63 Burbacher T, Shen D, Grant K, Sheppard L, Damian D, Ellis S *et al.* Reproductive and offspring developmental effects following maternal inhalation exposure to methanol in nonhuman primates. Res Rep Health Eff Inst 1999; 89: 1-117.

64 Soffritti M, Belpoggi F, Cevolani D, Guarino M, Padovani M, Maltoni C. Results of Long-Term Experimental Studies on the Carcinogenicity of Methyl Alcohol and Ethyl Alcohol in Rats. Ann N Y Acad Sci 2002; 982: 46-69.

65 Rodricks JV. Evaluation of quality of carcinogenicity studies conducted by the Ramazzini Foundation. Methanol Institute. [cited December 2009] Available from: http://www.methanol.org.

66 Cruzan G. Evaluation of the Ramazzini Foundation study of methanol in drinking water in Sprague-Dawley rats. Methanol Institute. [cited December 2009] Available from: http://www.methanol.org.

67 Lijinsky W, Thomas BJ, Kovatch RM. Differences in skin carcinogenesis by methylnitrosourea between mice of several strains. Cancer Lett 1991; 61(1): 1-5.

68 De Flora S., Zanacchi P, Camoirano A, Bennicelli C, Badolati GS. Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutat Res 1984; 133(3): 161-98.

69 Hayes S, Hayes C, Duncan D, Bennett V, Blushke J. Stimulation of mutations suppressing the loss of replication control by small alcohols. Mutat Res 1990; 231(2): 151-63.

70 McGregor DB, Edwards I, Riach CG, Cattanach P, Martin R, Mitchell A *et al.* Studies of an S9-based metabolic activation system used in the mouse lymphoma L5178Y cell mutation assay. Mutagenesis 1988; 3(6): 485-90.

71 Crebelli R, Conti G, Conti L, Carere A. A comparative study on ethanol and acetaldehyde as inducers of chromosome malsegregation in Aspergillus nidulans. Mutat Res 1989; 215(2): 187-95.

72 De Flora S. Study of 106 organic and inorganic compounds in the Salmonella/microsome test. Carcinogenesis 1981; 2(4): 283-98.

73 Gocke E, King MT, Eckhardt K, Wild D. Mutagenicity of cosmetics ingredients licensed by the European Communities. Mutat Res 1981; 90(2): 91-109.

74 Amacher DE, Paillet SC, Turner GN, Ray VA, Salsburg DS. Point mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. II. Test validation and interpretation. Mutat Res 1980; 72(3): 447-74.

75 von der Hude W., Behm C, Gurtler R, Basler A. Evaluation of the SOS chromotest. Mutat Res 1988; 203(2): 81-94.

76 Campbell JA, Howard DR, Backer LC, Allen JW. Evidence that methanol inhalation does not induce chromosome damage in mice. Mutat Res 1991; 260(3): 257-64.

77 Chang LW, McMillan L, Wynne BR, Pereira MA, Colley RA, Ward JB *et al.* The evaluation of six different monitors for the exposure to formaldehyde in laboratory animals. Environ Mutagen 1983; 5: 381.

78 Pereira MA, Chang LW, McMillan L, Ward JB, Legator MS. Battery of short-term tests in laboratory animals to corroborate the detection of human population exposures to genotoxic chemicals. Environ Mutagen 1982; 4: 317.

79 Fu SS, Sakanashi TM, Rogers JM, Hong KH, Keen CL. Influence of dietary folic acid on the developmental toxicity of methanol and the frequency of chromosomal breakage in the CD-1 mouse. Reprod Toxicol 1996; 10(6): 455-63.

80 O'Loughlin K, LeValley S, Mirsalis J, MacGregor J. Erythrocyte micronucleus assay of methanol in normal and folate-deficient swiss mice. Environ Mol Mutagen 1992; 19(Suppl.20): 47.

81 Andrews JE, Ebron-McCoy M, Kavlock RJ, Rogers JM. Developmental toxicity of formate and formic acid in whole embryo culture: a comparative study with mouse and rat embryos. Teratology 1995; 51(4): 243-51.

82 Brown Woodman PD, Huq F, Hayes L, Herlihy C, Picker K, Webster WS. In vitro assessment of the effect of methanol and the metabolite, formic acid, on embryonic development of the rat. Teratology 1995; 52(4): 233-43.

83 Dorman DC, Bolon B, Struve MF, LaPerle KM, Wong BA, Elswick B *et al.* Role of formate in methanol-induced exencephaly in CD-1 mice. Teratology 1995; 52(1): 30-40.

84 Clary JJ. Methanol, is it a developmental risk to humans? Regul Toxicol Pharmacol 2003; 37(1): 83-91.

85 Cameron AM, Nilsen OG, Haug E, Eik Nes KB. Circulating concentrations of testosterone, luteinizing hormone and follicle stimulating hormone in male rats after inhalation of methanol. Arch Toxicol Suppl 1984; 7: 441-3.

86 Cameron AM, Zahlsen K, Haug E, Nilsen OG, Eik Nes KB. Circulating steroids in male rats following inhalation of n-alcohols. Arch Toxicol Suppl 1985; 8: 422-4.

87 Cooper RL, Mole ML, Rehnberg GL, Goldman JM, McElroy WK, Hein J *et al.* Effect of inhaled methanol on pituitary and testicular hormones in chamber acclimated and non-acclimated rats. Toxicology 1992; 71(1-2): 69-81.

88 Lee E, Brady AN, Brabec MJ, Fabel T. Effects of methanol vapors on testosterone production and testis morphology in rats. Toxicol Ind Health 1991; 7(4): 261-75.

89 Andrews LS, Clary JJ, Terrill JB, Bolte HF. Subchronic inhalation toxicity of methanol. J Toxicol Environ Health 1987; 20(1-2): 117-24.

90 Poon R, Chu I, Bjarnason S, Potvin M, Vincent R, Miller RB *et al.* Inhalation toxicity study of methanol, toluene, and methanol/toluene mixtures in rats: effects of 28-day exposure. Toxicol Ind Health 1994; 10(3): 231-45.

91 Poon R, Chu I, Bjarnason S, Vincent R, Potvin M, Miller RB *et al.* Short-term inhalation toxicity of methanol, gasoline, and methanol/gasoline in the rat. Toxicol Ind Health 1995; 11(3): 343-61.

92 Ward JBJ, Hokanson JA, Smith ER, Chang LW, Pereira MA, Whorton EBJ *et al.* Sperm count, morphology and fluorescent body frequency in autopsy service workers exposed to formaldehyde. Mutat Res 1984; 130(6): 417-24.

93 Nelson BK, Brightwell WS, MacKenzie DR, Khan A, Burg JR, Weigel WW *et al.* Teratological assessment of methanol and ethanol at high inhalation levels in rats. Fundam Appl Toxicol 1985; 5(4): 727-36.

94 Bolon B, Dorman DC, Janszen D, Morgan KT, Welsch F. Phase-specific developmental toxicity in mice following maternal methanol inhalation. Fundam Appl Toxicol 1993; 21(4): 508-16.

95 Bolon B, Welsch F, Morgan KT. Methanol-induced neural tube defects in mice: pathogenesis during neurulation. Teratology 1994; 49(6): 497-517.

96 Connelly LE, Rogers JM. Methanol causes posteriorization of cervical vertebrae in mice. Teratology 1997; 55(2): 138-44.

97 Rogers JM, Mole ML. Critical periods of sensitivity to the developmental toxicity of inhaled methanol in the CD-1 mouse. Teratology 1997; 55(6): 364-72.

98 Cummings AM. Evaluation of the effects of methanol during early pregnancy in the rat. Toxicology 1993; 79(3): 205-14.

99 De Carvalho RR, Delgado IF, Souza CA, Chahoud I, Paumgartten FJ. Embryotoxicity of methanol in well-nourished and malnourished rats. Braz J Med Biol Res 1994; 27(12): 2915-23.

100 Youssef AF, Baggs RB, Weiss B, Miller RK. Teratogenicity of methanol following a single oral dose in Long-Evans rats. Reprod Toxicol 1997; 11(4): 503-10.

101 Sakanashi TM, Rogers JM, Fu SS, Connelly LE, Keen CL. Influence of maternal folate status on the developmental toxicity of methanol in the CD-1 mouse. Teratology 1996; 54(4): 198-206.

102 Rogers JM, Mole ML, Chernoff N, Barbee BD, Turner CI, Logsdon TR *et al.* The developmental toxicity of inhaled methanol in the CD-1 mouse, with quantitative dose-response modeling for estimation of benchmark doses. Teratology 1993; 47(3): 175-88.

103 Abbott BD, Logsdon TR, Wilke TS. Effects of methanol on embryonic mouse palate in serum-free organ culture. Teratology 1994; 49(2): 122-34.

104 Abbott BD, Ebron Mccoy M, Andrews JE. Cell death in rat and mouse embryos exposed to methanol in whole embryo culture. Toxicology 1995; 97(1-3): 159-71.

105 Andrews JE, Ebron Mccoy M, Logsdon TR, Mole LM, Kavlock RJ, Rogers JM. Developmental toxicity of methanol in whole embryo culture: a comparative study with mouse and rat embryos. Toxicology 1993; 81(3): 205-15.

106 Andrews JE, Nichols HP, Lamantia A, Rogers JM. Effects of methanol exposure on embryonic retinoic acid concentration. Teratology 1998; 57(4-5): 231-2.

107 Harris C, Wang SW, Lauchu JJ, Hansen JM. Methanol metabolism and embryotoxicity in rat and mouse conceptuses: comparisons of alcohol dehydrogenase (ADH1), formaldehyde dehydrogenase (ADH3), and catalase. Reprod Toxicol 2003; 17(3): 349-57.

108 Harris C, Dixon M, Hansen JM. Glutathione depletion modulates methanol, formaldehyde and formate toxicity in cultured rat conceptuses. Cell Biol Toxicol 2004; 20(3): 133-45.

109 Huang YS, Held GA, Andrews JE, Rogers JM. (14)C methanol incorporation into DNA and proteins of organogenesis stage mouse embryos in vitro. Reprod Toxicol 2001; 15(4): 429-35.

110 Stanton ME, Crofton KM, Gray LE, Gordon CJ, Boyes WK, Mole ML *et al.* Assessment of offspring development and behavior following gestational exposure to inhaled methanol in the rat. Fundam Appl Toxicol 1995; 28(1): 100-10.

111 Stern S, Cox C, Preston R, Sharma A, Inglis GB, Balys M *et al.* Perinatal methanol exposure in the rat. II. Behavioral effects in neonates and adults. Fundam Appl Toxicol 1997; 36(2): 163-76.

112 Infurna R, Weiss B. Neonatal behavioral toxicity in rats following prenatal exposure to methanol. Teratology 1986; 33(3): 259-65.

113 Aziz MH, Agrawal AK, Adhami VM, Ali MM, Baig MA, Seth PK. Methanol-induced neurotoxicity in pups exposed during lactation through mother: role of folic acid. Neurotoxicol Teratol 2002; 24(4): 519-27.

114 Ministry of Social Affairs and Employment (SZW). Wijziging arbeidsomstandighedenregeling. Staatscourant 2006;(252): 23-7.

115 Deutsche Forschungsgemeinschaft (DFG). List of MAK and BAT values 2009. Maximum concentrations and biological tolerance values at the workplace (rep no 45). Weinheim, FRG: Wiley:VCH Verlag; 2009.

116 TRGS 900. Grenzwerte in der Luft am Arbeitsplatz; Technische Regeln für Gefahrstoffe. July 2009. [cited December 2009] Available from: http://www.baua.de/nn_16806/de/Themen-von-A-Z/Gefahrstoffe/TRGS/pdf/TRGS-900.pdf.

117 Swedish National Board of Occupational Safety and Health. Occupational exposure limit values and measures against air contaminants. [cited December 2009] Available from: http://www.av.se/dokument/inenglish/legislations/eng0517.pdf.

118 Arbejdstilsynet. Grænseværdier for stoffer og materialer. [cited December 2009] Available from: http://www.arbejdstilsynet.dk/REGLER/At-vejledninger-mv/Stoffer-og-materialer/At-vejledninger-om-stoffer-og-materialer/C0-Generelt-og-diverse/PDF-C01-Graensevaerdi-for-stoffer-og-mat.aspx?sc_lang=da.

119 Health and Safety Executive (HSE). EH40/2005. Workplace exposure limits: Containing the list of workplace exposure limits for use with the Control of Substances Hazardous to Health Regulations 2002 (as amended). Sudbury (Suffolk), England: HSE Books; 2007.

120 American Conference of Governmental Industrial Hygienists (ACGIH). Guide to occupational exposure values - 2009. Cincinnati OH, USA: ACGIH; 2009.

121 European Commission: Directorate General of Employment and Social Affairs. Consolidated Indicative Occupational Exposure Limits Values (IOELVs). [cited December 2009] Available from: http://ec.europa.eu/social/main.jsp?catId=153&langId=en&intPageId=684.

122 American Conference of Governmental Industrial Hygienists (ACGIH). Methanol: TLV® chemical substances 7th edition documentation. Cincinnati OH, USA: ACGIH, 2009.

## Literature consulted but not cited

Andresen H, Schmoldt H, Matschke J, Flachskampf FA, Turk EE. Fatal methanol intoxication with different survival times – Morphological findings and postmortem methanol distribution. Forensic Sci Int 2008; 179: 206-10.

Aziz MH, Agrawal AK, Adhami VM, Ali MM, Baig MA, Seth PK. Methanol-induced neurotoxicity in pups exposed during lactation through mother: role of folic acid. Neurotoxicol Teratol 2002; 24(4): 519-27.

Caldwell JC, Woodruff TJ, Morello-Frosch R, Axelrad DA. Application of health information to hazardous air pollutants modeled in EPA's cumulative exposure project. Toxicol Ind Health 1998; 14(3): 429-54.

Fang Z, Ionescu P, Chortkoff BS, Kandel L, Sonner J, Laster MJ *et al.* Anesthetic potencies of n-alkanols: results of additivity and solubility studies suggest a mechanism of action similar to that for conventional inhaled anesthetics. Anesth Analg 1997; 84(5): 1042-48.

Frederick LJ, Schulte PA, Apol A. Investigation and control of occupational hazards associated with the use of spirit duplicators. Am Ind Hyg Assoc J 1984; 45: 51-5.

Gaffney S, Moody E, McKinley M, Knutsen J, Madl A, Paustenbach D. Worker exposure to methanol vapors during cleaning of semiconductor waters in manufacturing setting. J Occup Environ Hyg 2008; 5:313-24.

Gulmen MK, Meral D, Hilal ARA, Akcan R, Cekan N. Methanol intoxications in Adana, Turkey. Toxicol Mech Methods 2006; 16(7): 353-7.

Hageman G, van der Hoek J, van Hout M, van der Laan G, Jansen Steur E, de Bruin W *et al.* Parkinsonism, pyramidal signs, polyneuropathy, and cognitive decline after long-term occupational solvent exposure. J Neurol 1999; 246(3): 198-206.

Hovda KE, Hunderi OH, Rudberg N, Froyshov S, Jacobsen D. Anion and osmolal gaps in the diagnosis of methanol poisoning: clinical study in 28 patients. Intensive Care Med 2004; 30(9): 1842-6.

Jones TD. On 'toxicity equivalent factors' and 'relative potency' to account for differential toxicity and carcinogenicity: Concerns about uncommon effects of dose in animal experiments and environmental exposures to humans. Environmetrics 1998; 9(5): 525-39.

Kapur BM, Vandenbroucke AC, Adamchik Y, Lehotay DC, Carlen PL. Formic acid, a novel metabolite of chronic ethanol abuse, causes neurotoxicity, which is prevented by folic acid. Alcohol Clin Exp Res 2007; 31(12): 2114-20.

Lévy A, Bailey B, Letarte A, Dupuis C, Lefebre M. Unproven ingestion: an unrecognized bias in toxicological case series. Clin Toxicol 2007; 45: 946-9.
Morgan MS. The Biological Exposure Indices: A key component in protecting workers from toxic chemicals. Environ Health Perspect 1997; 105, suppl 1: 105-15.
Olszowy Z, Plewka A, Czech E, Nowicka J, Plewka D, Nowaczyk G *et al.* Effect of L-carnitine supplementation on xenobiotic-metabolizing hepatic enzymes exposed to methanol. Exp Toxicol Pathol 2005; 57(5-6): 427-35.
Paasma R, Hovda KE, Tikkerberi A, Jacobsen D. Methanol mass poisoning in Estonia: outbreak in 154 patients. Clin Toxicol (Phila) 2007; 45: 152-7
Paasma R, Hovda KE, Jacobsen D. Methanol poisoning and long term sequelae – a six years follow-up after a large methanol outbreak. BMC Clin Pharmacol 2009; 9:5 Parthasarathy NJ, Kumar RS, Manikandan S, Devi RS. Methanol-induced oxidative stress in rat lymphoid organs. J Occup Health 2006; 48(1): 20-7.
Parthasarathy NJ, Kumar RS, Karthikeyan P, Devi RS. In vitro and in vivo study of neutrophil functions after acute methanol intoxication in albino rats. Toxicol Environ Chem 2005; 87(4): 559-66.
Parthasarathy NJ, Kumar RS, Sheela Devi R. Effect of methanol intoxication of rat neutrophil functions. J Immunotoxicol 2005; 2(2): 115-21.
Shakhov V, McCallum G, Siu M, Wells PG. Methanol metabolism and oxidative DNA damage in rabbits. Birth Defects Res A Clin Mol Teratol 2007; 79: 419.
Siu MT, McCallum G, Shakhov G, Wells PG. Methanol metabolism and oxidative DNA damage in mice. Birth Defects Res A Clin Mol Teratol 2007; 79: 418.
Soysal D, Yersal Kabayegit O, Yilmaz S, Tatar E, Ozatli T, Yildiz B *et al.* Transdermal methanol intoxication: a case report. Acta Anaesthesiol Scand 2007; 51: 779-80.
Sweeney LM. Comparing occupational and environmental risk assessment methodologies using pharmacokinetic modeling. Hum Ecol Risk Assess 2000; 6(6): 1101-24.
Starr TB, Festa JL. A proposed inhalation reference concentration for methanol. Regul Toxicol Pharmacol 2003; 38(2): 224-31.
Verslegers WR, De Deyn PP. Ernstige intoxicatie met methanol door inhalatie. Commentaar. Ned Tijdschr Geneeskd 2006; 150(37): 2057.
Wallace EA, Green AS. Methanol toxicity secondary to inhalant abuse in adult men. Clin Toxicol (Phila) 2009; 47: 239-42
Yasugi T, Kawai T, Mizunuma K, Horiguchi S, Iwami O, Iguchi H *et al.* Formic acid excretion in comparison with methanol excretion in urine of workers occupationally exposed to methanol. Int Arch Occup Environ Health 1992; 64: 329-37.

# Annexes

Annex **A**

# Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

- The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:
- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit,

or, in the case of genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10-4 and 10-6 per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the committee is given in Annex B.

Annex **B**

# The committee

- G.J. Mulder, *chairman*
  emeritus professor of toxicology; Leiden University, Leiden
- R.B. Beems
  toxicologic pathologist; formerly employed at the National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard, advisor
  toxicologist; Shell International BV, The Hague
- J.J.A.M. Brokamp, *advisor*
  Social and Economic Council, The Hague
- D.J.J. Heederik
  professor of risk assessment in occupational epidemiology; Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R. Houba
  occupational hygienist; Netherlands Expertise Centre for Occupational Respiratory Disorders, Utrecht
- H. van Loveren
  professor of immunotoxicology; Maastricht University, Maastricht, and National Institute for Public Health and the Environment, Bilthoven
- T.M. Pal
  occupational physician; Netherlands Centre for Occupational Diseases, University of Amsterdam, Amsterdam

- A.H. Piersma
  professor of reproductive toxicology; Utrecht University, Utrecht, and National Institute for Public Health and the Environment, Bilthoven
- H.P.J. te Riele
  professor of molecular biology; VU University Amsterdam, Amsterdam
- I.M.C.M. Rietjens
  professor of toxicology; Wageningen University and Research Centre, Wageningen
- H. Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague
- G.M.H. Swaen
  epidemiologist; Dow Benelux N.V., Terneuzen
- R.C.H. Vermeulen
  epidemiologist/environmental hygienist; Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R.A. Woutersen
  toxicologic pathologist, TNO Quality of Life, Zeist, and professor of translational toxicology, Wageningen University and Research Centre, Wageningen
- P.B. Wulp
  occupational physician; Labour Inspectorate, Groningen
- J.T.J. Stouten, *scientific secretary*
  Health Council of the Netherlands, The Hague

The first draft of this report was prepared in 2007 by the National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, by contract with the Health Council of the Netherlands.

### The Health Council and interests

Members of Health Council Committees – which also include the members of the Advisory Council on Health Research (RGO) since 1 February 2008 – are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be rele-

vant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

# Comments on the public draft

A draft of the present report was released in 2009 for public review. The following persons and organisations have commented on the draft review:

- V. Gálvez Pérez, Centro Nacional de Nuevas Tecnologías, Madrid, Spain
- R.D. Zumwalde, National Institute for Occupational Safety and Health, Cincinnati OH, USA.

Annex **D**

# WHO/IPCS references*


Abbondandolo A, Bonatti S, Corsi C, Corti G, Fioro R, Leporini C, Mazzacccaro A, & Nieri R (1980) The use of organic solvents in mutagenicity testing. Mutat Res, 79: 141-150.

Andrews LS, Clary JJ, Terrill JB, & Bolte HF (1987) Subchronic inhalation toxicity of methanol. J Toxicol Environ Health, 20: 117-124.

Bartlett GR (1950) Inhibition of methanol oxidation by ethanol in the rat. Am J Physiol, 163: 619-621.

BASF (1979) [Report on the comparative testing on sensitizing effects in guinea pigs, modified maximization test.] Ludwigshafen, Germany, BASF AG, 11 pp (Unpublished report) (in German).

Baumbach Gl, Cancilla PA, Martin-Amat G, Tephly TR, McMartin KE, Makar Ab, Hayreh M, & Haryeh SS (1977) Methyl alcohol poisoning: IV. Alterations of the morphological findings of the retina and optic nerve. Arch Ophthalmol, 95: 1859-1865.

Becker CE (1983) Methanol poisoning. J Emerg Med, 1: 51-58.

Bennett IL, Cary FH, Mitchell GL, & Cooper MN (1953) Acute methyl alcohol poisoning: a review based on experiences in an outbreak of 323 cases. Medicine, 32: 431-463.

Buller F & Wood CA (1904) Poisoning by wood alcohol. J Am Med Assoc, 43: 1058-1062.

Cavanaugh LA, Schadt CF, & Robinson E (1969) Atmospheric hydrocarbon and carbon monoxide measurements at Point Barrow, Alaska. Environ Sci Technol, 3: 251-257.


* World Health Organization/International Programme on Chemical Safety (WHO/IPCS). Methanol. Geneva, Switzerland: WHO, 1997; Environmental Health Criteria 196 [cited December 2009]. Available from: http://www.inchem.org/documents/ehc/ehc/ehc196.html.

Clay KL, Murphy RC, & Watkins WD (1975) Experimental methanol toxicity in the primate: analysis of metabolic acidosis. Toxicol Appl Pharmacol, 34: 49-61.
Cooper JR & Felig P (1961) The biochemistry of methanol poisoning: II. Metabolic acidosis in the monkey. Toxicol Appl Pharmacol, 3: 202-209.
D'Alessandro A, Osterloh JD, Chuwers P, Quinlan PJ, Kelly TJ, & Becker CE (1994) Formate in serum and urine after controlled methanol exposure at the threshold limit value. Environ Health Perspect, 102: 178-181.
Del Carpio-O'Donovan L & Glay J (1992) Subarachnoid hemorrhage resulting from methanol intoxication: Demonstrated by computed tomography. Can Assoc Radiol J, 43: 263-299.
Dethlefs R & Naraqi S (1978) Ocular manifestations and complications of acute methyl alcohol intoxication. Med J Aust, 2: 483-485.
Dorman DC, Dye JA, Nassise MP, Ekuta J, Bolon B, & Medinsky MA (1993) Acute methanol toxicity in minipigs. Fundam Appl Toxicol, 20: 341-347.
Dutkiewicz B, Konczalik J, & Karwacki W (1980) Skin absorption and per os administration of methanol in men. Int Arch Occup Environ Health, 47: 81-88.
Eells JT (1991) Methanol- induced visual toxicity in the rat. J Pharmacol Exp Ther, 257: 56-63.
Eells JT, Salzman MM, & Trusk TC (1995) Inhibition of retinal mitochondrial function in methanol intoxication. Toxicologist, 15: 21-23.
Eells JT, Salzman MM, Lewandowski MF, & Murray TG (1996) Formate induced alterations in retinal function in methanol-intoxicated rats. Toxicol Appl Pharmacol, 140: 58-69.
Eisenberg AA (1917) Visceral changes in wood alcohol poisoning by inhalation. Am J Public Health, 7: 765-771.
Eriksen SP & Kulkarni AB (1963) Methanol in normal human breath. Science, 141: 639-640.
Erlanson P, Fritz H, Hagstam KE, Liljenberg B, Tryding N, & Voigt G (1965) Severe methanol intoxication. Acta Med Scand, 177: 393-408.
Franzblau A, Lee EW, Schreck RM, D'Arcy JB, Santrock J, & Levine SP (1993) Absence of formic acid accumulation in urine following five days of methanol exposure. Appl Occup Environ Hyg, 8: 883-888.
Frederick LJ, Schulte PA, & Apol A (1984) Investigation and control of occupational hazards associated with the use of spirit duplicators. Am Ind Hyg Assoc J, 45: 51-55.
Gilger AP & Potts AM (1955) Studies on the visual toxicity of methanol: V. The role of acidosis in experimental methanol poisoning. Am J Ophthamol, 39: 63-86.
Glazer M & Dross P (1993) Necrosis of the putamen caused by methanol intoxication: MR findings. Am J Roentgenol, 160: 1105-1106.
Gold MD & Moulif CE (1988) Effects of emission standards on methanol vehicle-related ozone, formaldehyde and methanol exposure. Presented at 81st Meeting of Air Pollution Control Association, Dallas, TX, June 19-24. Pittsburgh, Pennsylvania, Air Pollution Control Association.
Gonda A, Gault H, Churchill D, & Hollomby D (1978) Hemodialysis for methanol intoxication. Am J Med, 64: 749-757.

Graedel TE, Hawkins DT, & Claxton LD ed. (1986) Atmospheric chemical compounds: Sources, occurrence and bioassay. New York, London, Academic Press, pp 512-514, 557.
Greenberg L, Mayers MR, Goldwater LJ, & Burke WJ (1938) Health hazards in the manufacture of “fused collars”: II. Exposure to acetone-methanol. J Ind Hyg Toxicol, 20: 148-154.
Griffiths AJF (1981) Neurospora and environmentally induced aneuploidy. In: Stich HF & San RHC ed. Short-term tests for chemical carcinogens. Berlin, Heidelberg, New York, Springer-Verlag, pp 187-199.
Guerin MR, Higgins CE, & Greist WH (1987) The analysis of the particulate and vapour phases of tobacco smoke. In: O'Neill IK, Brunnemann KD, Dodet B, & Hoffmann D ed. Environmental carcinogens: Methods of analysis and exposure measurement, Volume 9. Lyon, International Agency for Research on Cancer, pp 115-139 (IARC Scientific Publications No. 81).
Haggard HW & Greenberg LA (1939) Studies on the absorption, distribution and elimination of alcohol: IV. The elimination of methylalcohol. J Pharmacol Exp Ther, 66: 479-496.
Hayreh MS, Hayreh SS, Baumbach GL, Cancilla P, Martin-Amat G, Tephly TR, McMartin KE, & Makar AB (1977) Methyl alcohol poisoning: III. Ocular toxicity. Arch Ophthamol, 95: 1851-1858.
Heidelberger C, Freeman AE, Pienta RJ, Sivak A, Bertram DS, Casto BC, & Dunkel VC (1983) Cell transformation by chemical agents: A review and analysis of the literature. Mutat Res, 114: 283-385.
Heinrich R & Angerer J (1982) Occupational chronic exposure to organic solvents. Int Arch Occup Environ Health, 50: 341-349.
Horton VI, Higuchi MA, & Rickert DE ( 1992) Physiologically based pharmacokinetic model for methanol in rats, monkeys and humans. Toxicol Appl Pharmacol, 117: 26-36.
Hsieh FY, Leu TM, & Chia LG (1992) Bilateral putaminal necrosis caused by methanol poisoning: A case report. Chin Med J (Taipei), 49: 283-288.
Jacobs GA (1990) OECD eye irritation tests on three alcohols: Acute toxicity data. J Am Coll Toxicol, 1: 56-57.
Jeganathan PS & Namasivayam A (1989) Methanol induced monoamine changes in hypothalamus and striatum of albino rats. Alcohol, 6: 451-454.
Jonsson A, Persson KA, & Grigoriadis V (1985) Measurements of some low molecular-weight oxygenated, aromatic and chlorinated hydrocarbons in ambient air and in vehicle emissions. Environ Int, 11: 383-392.
Jungclaus GA, Lopez-Avila V, & Hites RA (1978) Organic compounds in an industrial wastewater: A case study of their environmental impact. Environ Sci Technol, 12: 88-96.
Kane Rl, Talbert W, Harlan J, Sizemore G, & Cataland S (1968) A methanol poisoning outbreak in Kentucky. Arch Environ Health, 17: 119-129.
Katoh M (1989) New Energy Development Organization data. Presented at the Methanol Vapors and Health Effects Workshop: What we know and what we need to know - Summary Report. Washington, DC, ILSI Risk Science Institute/US Environmental Protection Agency/Health Effects Institute/ American Petroleum Institute, p A-7.

Kavet R & Nauss KM (1990) The toxicity of inhaled methanol vapors. CRC Crit Rev Toxicol, 21: 21-50.
Kawai T, Yasugi T, Mizunuma K, Horiguchi S, Hirase Y, Uchida Y, & Ikeda M (1991) Methanol in urine as a biological indicator of occupational exposure to methanol vapor. Int Arch Occup Environ Health, 63: 311-318.
Kingsley WH & Hirsch FG (1955) Toxicological considerations in direct process spirit duplicating machines. Compens Med, 6: 7-8.
Krotoszynski BK, Bruneau GM, & O'Neill HJ (1979) Measurement of chemical inhalation exposure in urban populations in the presence of endogenous effluents. J Anal Toxicol, 3: 225-234.
Leaf G & Zatman LJ (1952) A study of the conditions under which methanol may exert a toxic hazard in industry. Br J Ind Med, 9: 19-31.
Lee EW, Terzo TS, D'Arcy JB, Gross KB, & Schreck RM (1992) Lack of blood formate accumulation in humans following exposure to methanol vapors at the current permissible exposure limit of 200 ppm. Am Ind Hyg Assoc J, 53: 99-104.
Loewy A & von der Heide R (1914) [The uptake of methyl alcohol by inhalation.] Biochem Ztg, 65: 230-252 (in German).
Lund A (1948) Excretion of methanol and formic acid in man after methanol consumption. Acta Pharmacol, 4: 205-212.
Machiele PA (1990) A health and safety assessment of methanol as an alternative fuel. In: Kohl WL ed. Methanol as an alternative choice. Washington, DC, The Johns Hopkins Foreign Policy Institute, pp 217-239.
McMartin KE, Makar AB, Martin-Amat G, Palese M, & Tephly TR (1975) Methanol poisoning I. The role of formic acid in the development of metabolic acidosis in the monkey and the reversal by 4-methylpyrazole. Biochem Med, 13: 319-333.
McMartin KE, Martin-Amat G, Makar AB, & Tephly TR (1977) Methanol poisoning: V. The role of formate metabolism in the monkey. J Pharmacol Exp Ther, 201: 564-572.
Martin-Amat G, Tephly TR, McMartin KE, Makar AB, Hayreh MS, Hayreh SS, Baumbach G, & Cancilla P (1977) Methyl alcohol poisoning: II. Development of a model for ocular toxicity in methyl alcohol poisoning using the rhesus monkey. Arch Ophthamol, 95: 1847-1850.
Mashbitz LM, Sklianskaya RM, & Urieva FI (1936) The relative toxicity of acetone, methyl alcohol and their mixtures: II. Their action on white mice. J Ind Hyg Toxicol, 18: 117-122.
Medinsky MA & Dorman DC (1994) Assessing risks of low-level methanol exposure. CIIT Act, 14(7): 1-7.
Medinsky MA, Dorman DC, Bond JA, Moss OR, Janszen DB, & Everitt JI (1997) Pharmacokinetics of methanol and formate in female cynomolgus monkeys exposed to methanol vapours. Cambridge, Massachusetts, Health Effects Institute (Research Report No. 77).
NEDO (1982) Toxicological research of methanol as a fuel for power station: Summary report on tests with monkeys, rats and mice. Tokyo, Japan, New Energy Development Organization.

NEDO (1987) Toxicological research of methanol as a fuel for power station: Summary report on tests with monkeys, rats and mice. Tokyo, Japan, New Energy Development Organization, pp 1-296.
Nicholls P (1975) Formate as an inhibitor of cytochrome c oxidase. Biochem Biophys Res Commun, 67: 610-616.
Norman V (1977) An overview of the vapor phase, semivolatile, and non-volatile components of cigarette smoke. Recent Adv Tob Sci, 3: 25-58.
Obe G & Ristow H (1977) Acetaldehyde, but not ethanol induces sister chromatid exchanges in Chinese hamster cells *in vitro.* Mutat Res, 56: 211-213.
Pelletier J, Habib MH, Khalil R, Salamon G, Bartoli D, & Jean P (1992) Putaminal necrosis after methanol intoxication. J Neurol Neurosurg Psych, 55: 234-235.
Pellizzari ED, Hartwell TD, Harris BSH III, Waddell RD, Whitaker DA, & Erikson MD (1982) Purgable organic compounds in mother's milk. Bull Environ Contam Toxicol, 28: 322-328.
Perkins RA, Ward KW, & Pollack GM (1995) A pharmacokinetic model of inhaled methanol in humans and comparison to methanol disposition in mice and rats. Environ Health Perspect, 103: 716-733.
Pienta RJ, Poiley JA, & Lebherz WB III (1977) Morphological transformation of early passage Golden Syrian hamster embryo cells derived from cryopreserved cultures as a reliable *in vitro* bioassay for identifying carcinogens. Int J Cancer, 19: 642-655.
Röe O (1955) The metabolism and toxicity of methanol. Pharmacol Rev, 7: 399-412.
Scheuplein RJ & Blank IH (1971) Permeability of the skin. Physiol Rev, 51: 702-747.
Scott E, Helz MK, & McCord CP (1933) The histopathology of methyl alcohol poisoning. Am J Clin Pathol, 3: 311-319.
Sedivec V, Mraz M, & Flek J (1981) Biological monitoring of persons exposed to methanol vapors. Int Arch Occup Environ Health, 48: 257-271.
Sharpe JA, Hostovsky M, Bilbao JM, & Rewcastle NB (1982) Methanol optic neuropathy: A histopathological study. Neurology, 32: 1093-1100.
Simmon VF, Kauhanen K, & Tardiff RG (1977) Mutagenic activity of chemicals identified in drinking water. In: Scott D, Bridges BA, & Sobels FH ed. Progress in genetic toxicology. Amsterdam, Elsevier/North Holland Press, vol 2, pp 249-268.
Snider JR & Dawson GA (1985) Tropospheric light alcohols, carbonyls and acetonitrile: Concentrations in the Southwestern United States and Henry's law data. J Geophys Res, 90: 3797-3805.
Stegink LD, Brummel MC, McMartin KE, Martin-Amat G, Filer LJ Jr, Baker GL, & Tephly TR (1981) Blood methanol concentrations in normal adult subjects administered abuse doses of aspartame. J Toxicol Environ Health, 7: 281-290.
Stegink LD, Brummel MC, Filer LJ Jr, & Baker GL (1983) Blood methanol concentrations in one-year-old infants administered graded doses of aspartame. J Nutr, 113: 1600-1606.
Tephly TR (1991) Mini review-the toxicity of methanol. Life Sci, 48: 1031-1041.

Tephly TR & McMartin KE (1984) Methanol metabolism and toxicity. In: Stegink LD & Filer LJ Jr ed. Aspartame: Physiology and biochemistry. New York, Basel, Marcel Dekker, pp 111-140.
Tyson HH & Schoenberg MJ (1914) Experimental researches in methyl alcohol inhalation. J Am Med Assoc, 63: 915-921.
Vendilo MV, Egorov YL, & Feldman NG (1971) [The effects of methanol and of some higher alcohols on the retina of the eyes (an electron-microscope investigation).] Gig Tr Prof Zabol, 15: 17-21 (in Russian).
Weese H (1928) Vergleichende Untersuchungen uber die Wirksamkeit und Giftigkeit der Dampfe niederer aliphatischer Alkohole. Arch Exptl Pathol Pharmacol, 135: 118-130 [in German].
White LR, Martinsen ABL, & Nilsen OG (1983) Biochemical and cytological studies of rat lung after inhalation of methanol vapour. Toxicol Lett, 17: 1-5.
Wood CA & Buller F (1904) Poisoning by wood alcohol. J Am Med Assoc, 43: 973-977.
Wu Chen NB, Donoghue ER, & Schaffer MI (1985) Methanol intoxication: Distribution in postmortem tissues and fluids including vitreous humor. J Forensic Sci, 30: 213-216.
Yant WP & Schrenk HH (1937) Distribution of methanol in dogs after inhalation and administration by stomach tube and subcutaneously. J Ind Hyg Toxicol, 19: 337-345.

Annex **E**

# Subcommittee on the Classification of Carcinogenic Substances

## E.1 Evaluation of data on carcinogenicity and genotoxicity

In studies by the Japanese New Energy Development Organization (NEDO), rats and mice of both sexes were exposed to methanol concentrations of 13, 133, and 1330 mg/m$^3$ (10, 100, 1000 ppm), approximately 19 hours/day, 7 days/week, for 24 (rats) or 18 (mice) months.[1,2] The subcommittee concludes that methanol was not carcinogenic in these experiments since no methanol-related, statistically significant increases in tumour incidences were observed. The subcommittee is of the opinion that the studies were well performed but notes that no exposure concentrations were included that induced some kind of toxicity (i.e. a maximum tolerated dose or MTD).

The subcommittee discussed a carcinogenicity study performed by the Italian European Ramazzini Foundation for Oncology and Environmental Sciences (ERF). In this study, methanol was administered in the drinking water for 2 years to male and female Sprague-Dawley rats.[3] The subcommittee notes that the ERF's claims of GLP compliance could not be confirmed by GLP compliance monitoring authorities. In addition, ERF performed its studies according to an unusual, non-guideline design. Animals were not sacrificed immediately after terminating exposure, but kept until natural death. This complicates histopathological evaluations because of increase in background pathology and higher probability of autolytic changes. Further, ERF only poorly reported results (such as dosages and non-neoplastic and neoplastic lesions) and was not very willing to

allow a peer review of the pathology slides. ERF used Sprague-Dawley rats that have been isolated and inbred at the ERF for decades. These rats were not specific pathogen free, which could make them very susceptible to chronic respiratory system infections. Respiratory system infections can cause lung lymphomas such as seen in the ERF methanol study. Finally, the subcommittee notes the uncertainty about the correctness of the diagnosis of some tumour types, such as for instance the ear duct tumours seen in the methanol study (see evaluations by EFSA[4] and for the National Petrochemical and Refiners Association and Methanol Institute[5,6]). The subcommittee is of the opinion that this study had severe flaws which brought into question the validity and the relevance of the results. Therefore, the subcommittee decides not to consider the ERF study in the evaluation of the carcinogenicity properties of methanol.

With respect to genotoxicity, the subcommittee concludes that the majority of the *in vitro* tests were negative. Some positive results were obtained at high, cytotoxic doses. *In vivo* inhalation tests were negative. Both positive and negative results were observed when methanol was orally or intraperitoneally administered but tests with higher doses and repeated dosing were negative. Overall, the subcommittee is of the opinion that methanol is not likely to have a genotoxic potential.

## E.2 Recommendation for classification

The subcommittee concludes that there is only one valid carcinogenicity study. The results of this inhalation study suggest that methanol is not carcinogenic in rats and mice following inhalation. However, a final judgement is hampered because the study did not include exposure concentrations that induced some kind of toxicity (i.e., a maximum tolerated dose or MTD).

Overall, the subcommittee concludes that methanol cannot be classified with respect to its carcinogenicity (comparable with EU class ‘not classifiable’).

## E.3 References


1 Matsuura I. 18-month Inhalation carcinogenicity study of methanol in B6C3F1 mice: test report. Tokyo: Mitsubishi Chemical Safety Institute; 1985: Test No.: 4A-223. [cited February 2009]. Available from: http://www.methanol.org.

2 Takeda K. 24-month Inhalation carcinogenicity study on methanol in Fischer rats: test report. Tokyo: Mitsubishi Chemical Safety Institute; 1985: Test No.: 5A-268. [cited February 2009]. Available from: http://www.methanol.org.

3 Soffritti M, Belpoggi F, Cevolani D, Guarino M, Padovani M, Maltoni C. Results of Long-Term Experimental Studies on the Carcinogenicity of Methyl Alcohol and Ethyl Alcohol in Rats. Ann N Y Acad Sci 2002; 982: 46-69.

4 European Food Safety Authority (EFSA): Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing aids and Materials in contact with Food (AFC) on a request from the Commission related to a new long-term carcinogenicity study on aspartame. The EFSA Journal 2006; 356: 1-44. [cited February 2009]. Available from: http://www.efsa.europa.eu/en/scdocs/doc/afc_op_ej356_aspartame_en1,3.pdf.

5 Rodricks JV. Evaluation of quality of carcinogenicity studies conducted by the Ramazzini Foundation. Methanol Institute. [cited February 2009]. Available from: http://www.methanol.org.

6 Cruzan G. Evaluation of the Ramazzini Foundation study of methanol in drinking water in Sprague-Dawley rats. Methanol Institute. [cited February 2009]. Available from: http://www.methanol.org.

## E.4 The Subcommittee

- G.J. Mulder, *chairman*
  emeritus professor of toxicology, Leiden University
- P.J. Boogaard
  toxicologist, Shell International BV, The Hague
- M.J.M. Nivard
  molecular biologist and genetic toxicologist, Leiden University Medical Centre, Leiden
- G.M.H. Swaen
  epidemiologist, Dow Benelux NV, Terneuzen
- R.A. Woutersen
  toxicologic pathologist, TNO Quality of Life, Zeist, and professor of translational toxicology, Wageningen University and Research Centre, Wageningen
- A.A. van Zeeland
  professor of molecular radiation dosimetry and radiation mutagenesis, Leiden University Medical Centre, Leiden
- E.J.J. van Zoelen
  professor of cell biology, Radboud University Nijmegen, Nijmegen
- A.S.A.M. van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague
- J.M. Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague