

I U C L I D

D a t a S e t

Existing Chemical ID: 67-56-1
CAS No. 67-56-1
EINECS Name methanol
EC No. 200-659-6
Molecular Formula CH4O

Producer Related Part
Company: BASF AG
Creation date: 12-NOV-1992

Substance Related Part
Company: BASF AG
Creation date: 12-NOV-1992

Memo: master

Printing date: 21-JUL-2004
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Chapter (profile): Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, SIDS

1.0.1 Applicant and Company Information

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Flag: Critical study for SIDS endpoint
04-MAY-2004

Type: other: cooperating organisation
Name: Methanol Institute
Country: United States

Flag: Critical study for SIDS endpoint
06-NOV-2002

Type: cooperating company
Name: Methanor v.o.f.
Country: Netherlands

Flag: Critical study for SIDS endpoint
06-NOV-2002

Type: cooperating company
Name: Shell Chemicals Ltd.
Country: United Kingdom

Flag: Critical study for SIDS endpoint
06-NOV-2002

Type: cooperating company
Name: Statoil ASA
Country: Norway

Flag: Critical study for SIDS endpoint
27-MAR-2003

Type: cooperating company
Name: TOTAL Raffinerie Mitteldeutschland GmbH
Country: Germany

Flag: Critical study for SIDS endpoint
02-JUN-2003

Type: cooperating company
Name: Veba Oil Refining & Petrochemicals GmbH
Country: Germany

Flag: Critical study for SIDS endpoint
06-NOV-2002

1.0.2 Location of Production Site, Importer or Formulator

-

1.0.3 Identity of Recipients

-

1.0.4 Details on Category/Template

-

1.1.0 Substance Identification

Mol. Formula: C H4 O
Mol. Weight: 32.042 g/mol

Flag: non confidential, Critical study for SIDS endpoint
02-MAY-2002

1.1.1 General Substance Information

Purity type: typical for marketed substance
Substance type: organic
Physical status: liquid
Purity: >= 99.85 - % w/w
Colour: colourless
Odour: alcoholic

Method: IMPCA 001-98
Remark: Appearance: clear and free of suspended matter
Method: IMPCA 03-98
Flag: non confidential, Critical study for SIDS endpoint
14-NOV-2002

(1) (2)

1.1.2 Spectra

-

1.2 Synonyms and Tradenames

Methanol

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Methanol (8CI, 9CI)

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Methyl alcohol

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Methyl hydroxide

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Methylol

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Monohydroxymethane

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

1.3 Impurities

EINECS-Name: Chloride as Cl-
Mol. Formula: Cl-

Method: IMPCA 002-92
Remark: <= 0.00005 % w/w (<= 0.5 mg/kg)
Flag: non confidential, Critical study for SIDS endpoint
09-DEC-2003 (2)

CAS-No: 7704-34-9
EC-No: 231-722-6
EINECS-Name: sulphur
Mol. Formula: S

Method: ASTM D 3961-89
Remark: <= 0.00005 % w/w (0.5 mg/kg)
Flag: non confidential, Critical study for SIDS endpoint
24-OCT-2002 (2)

CAS-No: 7439-89-6
EC-No: 231-096-4
EINECS-Name: iron
Mol. Formula: Fe

Method: ASTM E 394-94
Remark: total iron
<= 0.00001 % w/w (0.1 mg/kg)
Flag: non confidential, Critical study for SIDS endpoint
24-OCT-2002 (2)

CAS-No: 7732-18-5
EC-No: 231-791-2
EINECS-Name: water
Mol. Formula: H2 O
Contents: <= .1 - % w/w

Method: ASTM E 1064-92
Flag: non confidential, Critical study for SIDS endpoint
15-OCT-2002 (2)

CAS-No: 64-17-5
EC-No: 200-578-6
EINECS-Name: ethanol
Mol. Formula: C2 H6 O
Contents: <= .005 - % w/w

Method: ASTM E 346-94
Remark: (<= 50 mg/kg)
Flag: non confidential, Critical study for SIDS endpoint
09-DEC-2003 (2)

EINECS-Name: Acidity (free acid as acetic acid)
Contents: <= .003 - % w/w

Method: ASTM D 1613-91
Remark: (<= 30 mg/kg)
Flag: non confidential, Critical study for SIDS endpoint
09-DEC-2003 (2)

CAS-No: 67-64-1
EC-No: 200-662-2
EINECS-Name: acetone
Mol. Formula: C3 H6 O
Contents: <= .003 - % w/w

Method: ASTM E 346-94
Remark: (<= 30 mg/kg)
Flag: non confidential, Critical study for SIDS endpoint
24-OCT-2002 (2)

1.4 Additives

1.5 Total Quantity

Remark:

Production volumes in the year 2000:

North America (NAFTA): Production : approx. 7 Mio t
Import : approx. 4 Mio t
-> Total supply: approx. 11 Mio t

use: Formaldehyde : approx. 23%
DMT * : approx. 2%
Acetic Acid : approx. 9%
MTBE/TAME * : approx. 36%
Methyl Methacrylate: approx. 3%
Gasoline/Fuel : approx. 0.2%
Solvents : approx. 6%
Others : approx. 14%
Exports : approx. 7%

South America : approx. 7 Mio t
incl. Trinidad

Western Europe : approx. 3.3 Mio t

Eastern Europe : approx. 0.5 Mio t

Former Soviet Union : approx. 2 Mio t

Africa and Middle East : approx. 5 Mio t

Asia/Pacific : approx. 5 Mio t

World : approx. 35 Mio t/a capacity

* DMT = dimethyl terephthalate
TAME = tert. amylmethylether = 2-methoxy-2-methylbutane
MTBE = tert-butyl methyl ether
Critical study for SIDS endpoint

Flag:

15-OCT-2002

1.6.1 Labelling

Labelling: as in Directive 67/548/EEC
Symbols: (F) highly flammable
(T) toxic
Specific limits: yes
R-Phrases: (11) Highly flammable
(23/24/25) Toxic by inhalation, in contact with skin and if
swallowed
(39/23/24/25) Toxic: danger of very serious irreversible
effects through inhalation, in contact with skin and if
swallowed
S-Phrases: (7) Keep container tightly closed
(16) Keep away from sources of ignition - No smoking
(36/37) Wear suitable protective clothing and gloves
(45) In case of accident or if you feel unwell, seek medical
advice immediately (show the label where possible)
Remark: INDEX-No. 603-001-00-X
Flag: non confidential, Critical study for SIDS endpoint
02-MAY-2002 (3)

1.6.2 Classification

Classified: as in Directive 67/548/EEC
Class of danger: highly flammable
R-Phrases: (11) Highly flammable
Remark: INDEX-No. 603-001-00-X
Flag: non confidential, Critical study for SIDS endpoint
02-MAY-2002 (3)

Classified: as in Directive 67/548/EEC
Class of danger: toxic
R-Phrases: (23/24/25) Toxic by inhalation, in contact with skin and if
swallowed
(39/23/24/25) Toxic: danger of very serious irreversible
effects through inhalation, in contact with skin and if
swallowed
Conc./Class. 1: >= 20% T; R 23/24/25-39/23/24/25
Conc./Class. 2: 10% <= T; R 20/21/22-39/23/24/25
20%
Conc./Class. 3: 3% <= Xn; R 20/21/22-68/20/21/22
10%
Remark: INDEX-No. 603-001-00-X
Flag: non confidential, Critical study for SIDS endpoint
02-MAY-2002 (3)

1.6.3 Packaging

1.7 Use Pattern

Type: type
Category: Wide dispersive use

Flag: non confidential, Critical study for SIDS endpoint
03-MAY-2002

Type: industrial
Category: Basic industry: basic chemicals

Remark: Use as Feedstock for Chemical Syntheses

Approximately 70 % of the methanol produced worldwide is used in chemical syntheses: in order of importance formaldehyde, methyl tert-butyl ether (MTBE), acetic acid, methyl methacrylate, and dimethyl terephthalate. Only a small proportion is utilized for energy production, although this use has great potential.

Formaldehyde

Formaldehyde is the most important product synthesized from methanol; in 1988, 40 % of the methanol produced worldwide was used to synthesize this product. The processes employed are all based on the oxidation of methanol with atmospheric oxygen.

Methyl tert-butyl ether is produced by reacting methanol with isobutene on acid ion exchangers. Increasing amounts of methanol are used in this form in the fuel sector. The ether is an ideal octane booster and has become extremely important due to the introduction of unleaded grades of gasoline and awareness of the possible harmfulness of aromatic high-octane components. In 1988, 20 % of worldwide methanol production was used for MTBE synthesis; annual increase rates of up to 12 % are expected.

Acetic Acid

Another 9 % of the methanol produced is used to synthesize acetic acid, and annual growth rates of 6 % are estimated. Acetic acid is produced by carbonylation of methanol with carbon monoxide in the liquid phase with cobalt - iodine, rhodium - iodine, or nickel - iodine homogeneous catalysts .

Other Synthesis Products

In the intensive search after the oil crisis for routes to alternative fuels, processes were developed that allowed fuels to be produced from synthesis gas with methanol as an intermediate. Mobil in the United States has contributed decisively to the development of such processes, which involve mainly the reaction of methanol on zeolite catalysts. The most important and, up to now, the only industrially implemented process is methanol to gasoline

(MTG) synthesis.

Methanol is used to synthesize a large number of other organic compounds:

Formic acid	preservatives, pickling agents
Methyl esters of organic acids	solvents, monomers
Methyl esters of inorganic acids	methylation reagents, explosives, insecticides
Methylamines	pharmaceutical precursors, auxiliaries, absorption liquids for gas washing and scrubbing
Trimethylphosphine	pharmaceuticals, vitamins, fragrances, fine chemicals
Sodium methoxide	organic intermediates, catalyst
Methyl halides	organic intermediates, solvents, propellants
Ethylene	organic intermediates, polymers, auxiliaries
Flag: 03-MAY-2002	non confidential, Critical study for SIDS endpoint

(4)

Type: industrial
Category: Basic industry: basic chemicals

Remark: Methanol to Olefins (MTO)

One of the larger potential applications for methanol is the methanol-to-olefins (MTO) process.

In this process, methanol is converted to light olefins, primarily ethylene and propylene.

The quantity of methanol that in theory would be required for a 500,000 ton per year ethylene plant is 7,000 tons per day, or 2,450,000 tons per year.

Obviously, this is larger than any methanol unit currently being built today.

Given the generally lower costs of the traditional cracking technology however, MTO will likely only capture niche markets or serve as "swing" capacity for existing ethylene producers. Nonetheless, the volume potential for methanol is huge.

One of the potential benefits of the MTO process is the ability to separate the olefin production from the natural gas location by building the methanol and the MTO units in

the most financially attractive location.

Flag: non confidential, Critical study for SIDS endpoint (5)
14-NOV-2002

Type: use
Category: other: use as Feedstock for Chemical Syntheses

Remark: World demand for MTBE from Methanol is expected to fall below 8 Mio. t/a equivalent to approx. < 16% of the world demand of methanol in 2004.

Flag: non confidential, Critical study for SIDS endpoint (5)
14-APR-2003

Type: industrial
Category: Fuel industry

Remark: Use as Energy Source

Methanol is a promising substitute for petroleum products if they become too expensive for use as fuels.

Methanol as a Fuel for Otto Engines
The use of methanol as a motor fuel has been discussed repeatedly since the 1920s. Use has so far been restricted to high-performance engines for racing cars and aeroplanes.

Flag: non confidential, Critical study for SIDS endpoint (4)
14-NOV-2002

Type: use
Category: Fuel additives

Flag: non confidential, Critical study for SIDS endpoint
14-NOV-2002

Type: use
Category: other: Other Energy Uses of Methanol

Remark: A use that has been discussed particularly in the United States and implemented in pilot projects is the firing of peak-load gas turbines in power stations (peak shaving). Benefits include simple storage and environmentally friendly combustion in the gas turbine. The use of methanol as a fuel in conventionally fired boilers obviates the need for costly flue gas treatment plants but is not yet economically viable.

Flag: non confidential, Critical study for SIDS endpoint (4)
14-NOV-2002

Type: use
Category: other: Other Energy Uses of Methanol

Remark: Methanol as a Hydrogen Carrier for Fuel Cell Applications

Methanol is a leading candidate to provide the hydrogen necessary to power a range of fuel cell technology applications. Taking an estimate from DaimlerChrysler, by 2020, 4.2 million to 12 million new fuel cell cars will be sold each year. DaimlerChrysler also believes that methanol is the best hydrogen carrier fuel for personal transport. Long before the automotive fuel cell market reaches maturity, consumers will be using cartridges of methanol to fuel direct methanol fuel cell power packs for a range of electronic devices such as cellular phones and laptop computers. Methanol also will be used to power portable fuel cell generators, and even residential fuel cell systems.

Flag: non confidential, Critical study for SIDS endpoint
14-NOV-2002 (6)

Type: use
Category: Intermediates

Flag: non confidential, Critical study for SIDS endpoint
14-NOV-2002

Type: industrial
Category: Paints, lacquers and varnishes industry

Flag: non confidential, Critical study for SIDS endpoint
14-NOV-2002

Type: industrial
Category: Chemical industry: used in synthesis

Flag: non confidential, Critical study for SIDS endpoint
14-NOV-2002

Type: use
Category: Laboratory chemicals

Flag: non confidential, Critical study for SIDS endpoint
14-NOV-2002

Type: use
Category: Solvents

Flag: non confidential, Critical study for SIDS endpoint
14-NOV-2002

Type: use
Category: other

Remark: Methanol's low freezing point and its miscibility with water allow it to be used in refrigeration systems, either in pure form (e.g., in ethylene plants) or mixed with water and glycols. It is also used as an antifreeze in heating and cooling circuits; compared to other commonly used antifreezes (ethylene glycol, propylene glycol, and glycerol), it has the advantage of lower viscosity at low temperature. It is, however, no longer used as an engine antifreeze; glycol-based products are employed instead. Large amounts of methanol are used to protect natural gas pipelines against the formation of gas hydrates at low temperature. Methanol is added to natural gas at the pumping station, conveyed in liquid form in the pipeline, and recovered at the end of the pipeline. Methanol can be recycled after removal of water taken up from natural gas by distillation.

Methanol is also used as an absorption agent in gas scrubbers. The removal of carbon dioxide and hydrogen sulfide with methanol at low temperature (Rectisol process, Linde and Lurgi) has the advantage that traces of methanol in the purified gas do not generally interfere with further processing.

The use of pure methanol as a solvent is limited, although it is often included in solvent mixtures.

Flag: non confidential, Critical study for SIDS endpoint

14-NOV-2002

(4)

1.7.1 Detailed Use Pattern

1.7.2 Methods of Manufacture

Orig. of Subst.: Synthesis
Type: Production

Remark: The methanol production process involves the catalytic steam reforming of a hydrocarbon feedstock, typically natural gas, to give a gaseous mixture of carbon oxides and hydrogen. This mixture is then compressed and reacted over another catalyst to give methanol and by-products, according to the following reactions.

$$\text{CO} + 2 \text{H}_2 \leftrightarrow \text{CH}_3\text{OH}$$

$$\text{CO}_2 + 3 \text{H}_2 \leftrightarrow \text{CH}_3\text{OH} + \text{H}_2\text{O}$$

The pure product is obtained by fractional distillation. All necessary steps are performed in closed systems.

The gas is reformed over a nickel catalyst. The synthesis gas, containing a mixture of hydrogen, carbon oxides, steam

and residual methane leaves the reformer at about 880oC and 20 bar, and is cooled before being compressed to synthesis pressure.

The synthesis loop comprises a circulator, converter, feed/effluent exchanger, separator, and in some cases a heat recovery exchanger. The optimum operating pressure is typically about 80 bar for large plants, but for smaller plants (less than 600 t/day) a pressure of 50 bar may be economic due to the reduced material requirements.

Synthesis gas is mixed with the recycle gas and preheated in a feed/effluent interchanger. The converter effluent is cooled by passing through the feed/effluent interchanger and dependent on the reactor type, a heat recovery exchanger. Prior to the catchpot, the gas is cooled to about 40oC to condense the product methanol. A purge is taken from the recycle gas to remove inerts (N₂, CH₄, Argon, and surplus hydrogen or carbon oxides) from the loop. This is used as fuel in the reformer. A purge gas expander may be used to recover power from the purge gas as it is let down to the fuel system pressure.

The crude methanol from the separator contains both water and low levels of by-products, which must be removed to achieve required product purity.

Flag: non confidential, Critical study for SIDS endpoint
09-DEC-2003 (7)

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

Type of limit: BAT (DE)
Limit value: 30 other: mg per liter urine

Flag: non confidential, Critical study for SIDS endpoint
03-MAY-2002 (8)

Type of limit: MAK (DE)
Limit value: 200 ml/m³

Flag: non confidential, Critical study for SIDS endpoint
02-MAY-2002 (9)

Type of limit: MAK (DE)
Limit value: 270 mg/m3

Remark: exceeding factor: 4
cutaneous resorption
A risk of fetal damage is not to be feared on compliance of
the MAK and BAT (biological workplace tolerance limit).

Flag: non confidential, Critical study for SIDS endpoint

02-MAY-2002

(9)

1.8.2 Acceptable Residues Levels

-

1.8.3 Water Pollution

Classified by: other: VwVwS (Germany), Annex 2

Labelled by: other: VwVwS (Germany), Annex 2

Class of danger: 1 (weakly water polluting)

Flag: non confidential, Critical study for SIDS endpoint

02-MAY-2002

(10)

1.8.4 Major Accident Hazards

-

1.8.5 Air Pollution

-

1.8.6 Listings e.g. Chemical Inventories

Type: EINECS

Additional Info: EINECS-No. 200-659-6

Flag: non confidential, Critical study for SIDS endpoint

02-MAY-2002

(11)

Type: ENCS

Additional Info: ENCS No. 2-201

Remark: ENCS CLASSIFICATION:

Low Molecular Chain-like Organic Compounds

Flag: non confidential, Critical study for SIDS endpoint

02-MAY-2002

(11)

Type: ECL
Additional Info: ECL Serial No. KE-23193

Remark: KOREAN TCCL DESIGNATION:
ECL Toxic Chemical No. 97-1-80

Flag: non confidential, Critical study for SIDS endpoint
02-MAY-2002 (11)

Type: other: SWISS
Additional Info: SWISS No. G-2063

Remark: SWISS CLASSIFICATION:
Giftliste 1 (List of Toxic Substances 1), 31 May 1999.
Toxic Category 3: Acute oral lethal dose of 50 - 500 mg/kg.

Flag: non confidential, Critical study for SIDS endpoint
02-MAY-2002 (11)

Type: TSCA

Flag: non confidential, Critical study for SIDS endpoint
02-MAY-2002 (11)

Type: DSL

Flag: non confidential, Critical study for SIDS endpoint
02-MAY-2002 (11)

Type: AICS

Flag: non confidential, Critical study for SIDS endpoint
02-MAY-2002 (11)

Type: PICCS

Flag: non confidential, Critical study for SIDS endpoint
02-MAY-2002 (11)

1.9.1 Degradation/Transformation Products

-

1.9.2 Components

-

1.10 Source of Exposure

-

1.11 Additional Remarks

Memo: Conditions to avoid: Avoid temperatures above 40°C

Flag: non confidential, Critical study for SIDS endpoint
03-MAY-2002 (1)

Memo: German "Flammable Liquids" classification (VbF): B

Flag: non confidential, Critical study for SIDS endpoint
14-NOV-2002 (1)

Memo: Hazardous reactions: Formation of explosive gas/air mixtures

Flag: non confidential, Critical study for SIDS endpoint
14-NOV-2002 (1)

1.12 Last Literature Search

-

1.13 Reviews

-

2.1 Melting Point

Value: = -97.8 degree C

Reliability: (4) not assignable
Handbook

Flag: Critical study for SIDS endpoint
26-NOV-2002 (12)

Value: = -98 degree C

Reliability: (4) not assignable
Manufacturer / producer data without proof

Flag: Critical study for SIDS endpoint
13-FEB-2001 (13)

2.2 Boiling Point

Value: = 64.7 degree C

Reliability: (4) not assignable
Handbook

Flag: Critical study for SIDS endpoint
25-OCT-2002 (12)

Value: = 64.7 degree C

Reliability: (4) not assignable
Manufacturer / producer data without proof

Flag: Critical study for SIDS endpoint
13-FEB-2001 (13)

Value: = 64.6 degree C

Reliability: (4) not assignable
Secondary quotation

13-FEB-2001 (14)

2.3 Density

Type: density
Value: = .79 g/cm³ at 20 degree C

Reliability: (4) not assignable
Handbook

Flag: Critical study for SIDS endpoint
13-FEB-2001 (12)

Type: density
Value: = .791 - .792 g/cm³ at 20 degree C

Reliability: (4) not assignable
Manufacturer / producer data without proof

Flag: Critical study for SIDS endpoint
13-FEB-2001 (13)

2.3.1 Granulometry

-

2.4 Vapour Pressure

Value: = 129 hPa at 20 degree C

Reliability: (4) not assignable
Manufacturer / producer data without proof

Flag: Critical study for SIDS endpoint
13-FEB-2001 (13)

Value: = 319 hPa at 38 degree C

Reliability: (4) not assignable
Secondary quotation
13-FEB-2001 (15)

Value: = 552 hPa at 50 degree C

Reliability: (4) not assignable
Manufacturer/producer data
13-FEB-2001 (13)

Value: = 128 hPa at 20 degree C

Reliability: (4) not assignable
Secondary quotation

13-FEB-2001 (16)

2.5 Partition Coefficient

Partition Coeff.: octanol-water

log Pow: = -.82 - -.64

Remark: Compilation of measured log Pow values

Reliability: (2) valid with restrictions
scientifically verified data

Flag: Critical study for SIDS endpoint

26-SEP-2002 (17) (18)

Partition Coeff.: octanol-water

log Pow: = -.77

Reliability: (4) not assignable
Manufacturer / producer data without proof

26-SEP-2002 (13)

Partition Coeff.: octanol-water

log Pow: = -.74

Remark: Recommended value

Reliability: (2) valid with restrictions
Scientifically verified data

26-SEP-2002 (19)

2.6.1 Solubility in different media

Solubility in: Water

Value: at 20 degree C

Descr.: miscible

Remark: pH value: neutral

Reliability: (4) not assignable
Manufacturer / producer data without proof

Flag: Critical study for SIDS endpoint

26-SEP-2002 (13)

Solubility in: Water
Descr.: miscible

Reliability: (4) not assignable
Secondary quotation
26-SEP-2002 (14)

2.6.2 Surface Tension

Value: = 22.55 mN/m at 20 degree C

Reliability: (4) not assignable
Manufacturer / producer data without proof
13-FEB-2001 (20)

2.7 Flash Point

Value: = 9.7 degree C
Type: closed cup

Method: other: Abel-Pensky

Reliability: (2) valid with restrictions
National standard specification without detailed
documentation

Flag: Critical study for SIDS endpoint
13-FEB-2001 (21)

Value: = 11 degree C
Type: closed cup

Method: other: DIN 51755

Reliability: (4) not assignable
Manufacturer / producer data without proof
13-FEB-2001 (13)

2.8 Auto Flammability

Value: = 455 degree C

Method: other: DIN 51 794

Remark: Autoignition temperature
Reliability: (2) valid with restrictions
National standard specification without detailed
documentation

Flag: Critical study for SIDS endpoint
26-SEP-2002 (22)

Value: = 385 degree C at 1013 hPa

Remark: Autoignition temperature
Reliability: (4) not assignable
Manufacturer / producer data without proof

13-FEB-2001 (23)

2.9 Flammability

Result: highly flammable

Reliability: (4) not assignable
Manufacturer / producer data without proof

Flag: Critical study for SIDS endpoint
13-FEB-2001 (13)

2.10 Explosive Properties

Result: not explosive

Remark: because of chemical structure
Reliability: (2) valid with restrictions
expert judgement

Flag: Critical study for SIDS endpoint
13-FEB-2001 (24)

2.11 Oxidizing Properties

Result: no oxidizing properties

Remark: because of chemical structure

Reliability: (2) valid with restrictions
Expert judgement

Flag: Critical study for SIDS endpoint

13-FEB-2001 (24)

2.12 Dissociation Constant

-

2.13 Viscosity

Value: = .59 mPa s (dynamic) at 20 degree C

Reliability: (4) not assignable
Manufacturer / producer data without proof

26-SEP-2002 (13)

2.14 Additional Remarks

Remark: Explosion limits: 5.5 - 36.5 vol.%

Conditions to avoid, substances to avoid:
Hazardous reaction influenced by strong oxidizing agents,
(e.g. nitric acid, chromium trioxide, perchloric acid and salts).
Vapours may form explosive mixture with air.
Explosion hazard when product reacts with metals and forms hydrogen: aluminium, magnesium at elevated temperature.

Hazardous reactions: vapours form explosive mixtures with air

Reliability: Hazardous decomposition products: ignitable gases / vapours
(4) not assignable
Manufacturer / producer data without proof

13-FEB-2001 (13)

Remark: Explosion limits: 5.5 - 36.5 vol.%

Reliability: (4) not assignable
Handbook

13-FEB-2001 (25)

Remark: Explosive limits: 5.5 - 31.0 vol.%
Reliability: (4) not assignable
Secondary quotation
13-FEB-2001 (26)

Remark: Explosive Limits in air: 6.0 - 36.5 vol.%
Reliability: (4) not assignable
Manufacturer / producer data without proof
13-FEB-2001 (23)

3.1.1 Photodegradation

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm³
Rate constant: = .0000000000009 cm³/(molecule * sec)
Degradation: = 50 % after 17.8 day(s)

Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 12-FEB-2001 (27)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH

Method: other (measured)
Test substance: as prescribed by 1.1 - 1.4

Remark: Rate Constant: 0.88 (+/- 0.18)*10⁻¹² cm³/molecule*sec at 298 K
Reliability: (2) valid with restrictions
 13-FEB-2001 (28)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH

Remark: k(298K)= 7.8*10e-13 or 1.4*10e-13 cm³/molecule*sec.
 Temp. dependence of k/cm³/molecule*sec: 3.3*10e-12 exp (-380/T).
 Temp. range/K: 240-300
Reliability: (2) valid with restrictions
 12-FEB-2001 (29)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1000000 molecule/cm³
Rate constant: = .0000000000001 cm³/(molecule * sec)
Degradation: = 50 % after 8 day(s)

Remark: Bimolecular reaction constant
Reliability: (2) valid with restrictions
 12-FEB-2001 (30)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm³
Rate constant: = .0000000000012 cm³/(molecule * sec)
Degradation: = 50 % after 14.6 day(s)

Remark: Reaction at ca. 300 K, smog chamber test
Reliability: (2) valid with restrictions
12-FEB-2001 (31)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 3000000 molecule/cm³

Remark: Atmospheric transformation: 1 to 5 days
Reliability: (2) valid with restrictions
12-FEB-2001 (32)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Rate constant: = .000000000000944 cm³/(molecule * sec)

Remark: at 298 K
Reliability: (1) valid without restriction
12-FEB-2001 (33)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH

Remark: The half-life of methanol in the troposphere for this reaction will obviously depend upon the concentration of hydroxyl radicals. Using an average hydroxyl radical concentration of 1*10⁶ molecules/cm³, half-lives for the reaction ranged from 6.7-13.4 days. However, using the global diurnal mean tropospheric concentration of 5*10⁵ molecules/cm³ quoted in some references, the half-lives ranged from 13.4-17.6 days.
Reliability: (4) not assignable
secondary quotation
13-FEB-2001 (34)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1000000 molecule/cm³
Degradation: = 50 % after 7.3 day(s)

Remark: Reaction at 300 K and atmospheric pressure; $k=1.1 \cdot 10^{-12}$
cm³/molecule*sec
Reliability: (4) not assignable
secondary quotation
13-FEB-2001 (35)

Type: air

Remark: Methanol is listed as hazardous air pollutant under Title
III of CAAA (Clean Air Act Amendments) with an atmospheric
lifetime of 16-18 days.
Reliability: (4) not assignable
secondary literature
15-JAN-1997 (36)

Type: water
INDIRECT PHOTOLYSIS
Sensitizer: OH

Result: Rate constant = $1 \cdot 10^9$ l/mol*sec
13-FEB-2001 (37)

Type: water
INDIRECT PHOTOLYSIS
Sensitizer: OH

Method: other (measured)

Remark: $k(\text{OH}) = 8.46 \cdot 10^9$ liter/mol*sec
OH concentration: $5 \cdot 10^{-14}$ mol/liter
temperature: 298 K
02-DEC-1996 (38)

3.1.2 Stability in Water

Method: other

Remark: Freitag et al. investigated the simulated atmospheric breakdown of methanol adsorbed to silica gel and submitted to 17 hours irradiation with light of wavelength >290 nm. Only 4.1% of the methanol applied to the gel had degraded by the end of exposure period.

Reliability: (2) valid with restrictions
12-FEB-2001 (39)

Method: other

Remark: Alcohols are generally resistant to hydrolysis.

Reliability: (1) valid without restriction
Handbook

Flag: Critical study for SIDS endpoint
12-FEB-2001 (40)

3.1.3 Stability in Soil

Method: other

Remark: Methanol is completely miscible in water and has a log Kow of -0.77. These properties are indicative of high mobility in soil.

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint
13-FEB-2001 (41) (12)

Type: laboratory

Soil temperature: 6 degree C

Remark: Lokke (1984) studied the adsorption of methanol onto three different soil types at 6 deg C. Only slight methanol adsorption occurred with the two sandy soils tested (percentage organic matter of 0.09% and 0.1% in the samples) and with the clay soil (percentage organic matter was 0.22%). Methanol solutions of concentration 0.1, 1.0, 9 and 90 mg/l were used in 1 hour exposure adsorption studies and adsorption coefficients of between 0.13 and 0.61 were measured for all soil types and at all concentrations. These coefficients indicate that methanol has a low adsorptive capacity on soils.

Reliability: (4) not assignable
12-FEB-2001 (42)

3.2.1 Monitoring Data (Environment)

Medium: air

Remark: concentration: 6-60 ug/m3 (3 locations, 52 samples)
03-FEB-1997 (32)

Type of measurement: other

Medium: other

Remark: Methanol is a naturally occurring compound, being a metabolite in man, animals and plants.
07-JUL-1993 (43)

Type of measurement: other

Remark: Holzer et al. (1977) identified methanol in forest air in Alabama, 20-35 miles away from the closest urban activities/habitation, and ascribed its presence to natural sources.
07-JUL-1993 (44)

Type of measurement: other

Remark: Owens et al. (1969) identified methanol as one of the volatile compounds emitted by alfalfa, and Isidorov et al. (1985) reported that methanol was given off by evergreen cyprus in a survey of the volatile emissions from the foliage of 17 trees characteristic of the forests of Northern Europe and Asia.
07-JUL-1993 (45) (46)

Type of measurement: other

Medium: air

Remark: Jonsson et al. (1985) reported the results of a 1 year study (1982 to 1983) of air quality in Stockholm. Samples were collected at four urban sites in central Stockholm and a more rural site 12 km from the centre of the city. The minimum detectable quantity in the 2-3 litre samples collected was about 0.008 nl/l for a typical oxygenated compound, i.e. about 0.01 ug/m³ methanol. The following methanol levels were reported:

1. Busy street, 8 samples/day collected over 7 days, 56 samples in total, March-May 1983; average: 34.98 ug/m³; range: 5.31-94.84 ug/m³.
2. Quiet street, 8 samples/day collected over 3 days, plus samples taken in early morning and at night, 40 samples in total, August-September 1982; average: 10.96 ug/m³; range: 2.0-28.17 ug/m³.
3. Busy street, 8 samples/day collected over 12 days, 96 samples in total, September-December 1982; average: 10.22 ug/m³; range: 0.76-34.6 ug/m³.
4. Quiet street, 8 samples/day collected over 7 days, plus

samples taken in early morning and at night, 68 samples in total, January-March 1983; average: 5.02 ug/m³; range: 0.59-15.07 ug/m³.

5. Recreation area, 12 km from central Stockholm, 8 samples /day collected over 7 days, 56 samples in total, May-June 1983; average: 11.21 ug/m³; range: 3.21-22.27 ug/m³.

08-JUL-1993 (47)

Type of measurement: other

Medium: other: water and sediment

Remark: Jungclaus et al. (1978) analysed the wastewater and effluent-receiving waters and sediments for a US chemical plant manufacturing a broad range of chemicals, including pharmaceuticals, herbicides and surfactants. The plant was located on a small freshwater river draining into a small brackish cove, which eventually fed into an estuary. Methanol was found at levels of 17-80 mg/l in the waste-water but not in the effluent-receiving waters or associated sediments (no detection limits were reported).

09-JUL-1993 (48)

Type of measurement: other

Medium: other: condensate waters

Remark: Mohr and King (1985) identified methanol in one of four samples of condensate waters taken from a fixed-bed coal gasification process plant. No detection limit was reported but methanol was found in the sample at a level of 1050 mg/l.

08-JUL-1993 (49)

Type of measurement: other

Medium: biota

Remark: Pellizzari et al. (1982) collected 42 samples of mothers' milk from women in four urban areas of the USA. They analysed all the samples by GC/MS and the mass spectra from eight samples were later interpreted manually. Methanol was found in one of these samples, however, no actual level of occurrence was reported.

12-JUL-1993 (50)

Type of measurement: other**Medium:** food

Remark: Methanol has been isolated and identified in the volatile components of the following foodstuffs: baked potatoes (Coleman et al., 1981), roasted filbert nuts (Kinlin et al., 1972), dried legumes (Lovegren et al., 1979). Of these occurrences, only Lovegren et al. quantified the levels of methanol present, as follows: Lima beans (seven samples), common beans (five samples), mung beans (one sample) and soya beans (one sample): mean level =4.2 mg/kg; range: 1.5-7.9 mg/kg.
Split peas (one sample): level =3.6 mg/kg.
Lentils (two samples): mean level =4.4 mg/kg.

08-JUL-1993

(51) (52) (53)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Type: adsorption**Media:** water - air

Remark: The Henry's Law constant of 1.35×10^{-4} atm*m³/mole indicates that volatilization from environmental waters may be significant.

Reliability: (2) valid with restrictions

18-DEC-2003

(54)

Type: volatility**Media:** water - air**Method:** other: calculated according to HENRYWIN, V 3.10**Result:** Henry's Law Constant at 25 °C = 4.27×10^{-6} atm * m³/mol.**Reliability:** (2) valid with restrictions**Flag:** Critical study for SIDS endpoint

01-APR-2004

(55)

Type: volatility**Media:** water - air

Remark: Methanol has a estimated Henry's Law Constant of 4.4×10^{-6} atm*m³/mol at 25 deg C.

Reliability: (4) not assignable

10-OCT-2002

(56)

Type: volatility
Media: water - air
Result: H = 0,461 Pa*m3/mol
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 25-APR-2003 (57)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water
Method: Calculation according Mackay, Level I
Result: water: 84.59 %
 air: 15.38 %
 sediment: 0.0068 %
 soil: 0,0067 %
Reliability: (2) valid with restrictions
 29-APR-2003 (58)

Media: air - biota - sediment(s) - soil - water
Method: Calculation according Mackay, Level III
Remark: Input parameters for fugacity model:
 Molecular weight: 32.04
 Vapor pressure: 129 hPa
 Henry' Law constant: 0.461 Pa*m³/mol
 Log Kow: -0.82
 Water solubility: 1E+006 mg/l
Result:

compartment	mass amount (%)	emission (kg/h)
air	73,3	9093
water	15,6	175
soil	11,1	75
sediment	0,0233	0

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 14-APR-2004 (59)

3.4 Mode of Degradation in Actual Use

-

3.5 Biodegradation

Type: aerobic
Degradation: = 95.6 % after 5 day(s)

Method: other: (BOD of THOD)
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
 12-FEB-2001 (60)

Type: aerobic
Inoculum: domestic sewage
Degradation: = 53.4 % after 5 day(s)

Method: other: BOB-Test (BOD of THOD)
Test substance: as prescribed by 1.1 - 1.4

Remark: BSB 5/10/15/20 of ThSB = 53.4/62.7/69.4/67 %.
 Test substance: 2.5 ppm initial concentration

Reliability: (2) valid with restrictions
 10-OCT-2002 (61)

Type: aerobic
Inoculum: domestic sewage, non-adapted
Degradation: = 76 % after 5 day(s)
Result: readily biodegradable
Kinetic:

5 day(s)	= 76 %
10 day(s)	= 88 %
15 day(s)	= 91 %
20 day(s)	= 95 %

Method: other: closed bottle test (BOD of THOD)
Test substance: as prescribed by 1.1 - 1.4

Remark: concentration: 3-10 mg/l of test substance
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 14-OCT-2002 (62)

Type: aerobic
Inoculum: domestic sewage, non-adapted
Degradation: = 82.5 % after 5 day(s)

Method: other: Respirometric test (BOD of THOD)
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 12-FEB-2001 (63)

Type: aerobic
Inoculum: other bacteria: synthetic sewage
Degradation: = 71.2 % after 5 day(s)

Method: other: Respirometric test (BOD of THOD)
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
12-FEB-2001 (63)

Type: aerobic
Inoculum: activated sludge, industrial
Concentration: 100 mg/l related to Test substance
Degradation: > 90 % after 1 day(s)

Test substance: as prescribed by 1.1 - 1.4

Remark: Matsui et al. (1988) examined the biodegradability of methanol in a batch reactor using activated sludge from the Fukushima industrial wastewater treatment plant, which was acclimated to the wastewater (containing various organic compounds) originating from the Kashima petrochemical complex in Japan. Methanol, at an initial concentration of 100 mg/l, was found to be highly biodegradable, and 91% COD removal and 92% TOC removal was achieved after a 1 day acclimation period and after 1 day's operation of the batch reactor.

Reliability: (2) valid with restrictions
12-FEB-2001 (64)

Type: aerobic
Degradation: = 99 %

Method: OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
secondary quotation
12-FEB-2001 (65)

Type: aerobic
Degradation: = 70.7 - 88.7 % after 5 day(s)

Method: other
Test substance: as prescribed by 1.1 - 1.4

Method: standard dilution method (BOD of THOD) and sea water dilution method
Remark: test concentration: 2.56 ppm
Reliability: (2) valid with restrictions
12-FEB-2001 (66)

Type: aerobic
Inoculum: activated sludge, domestic, non-adapted
Degradation: = 80 %

Method: other: Respirometric test (Sapromat)
Year: 1973
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
Secondary quotation (IUCLID Data Sheet), Data to EU
12-FEB-2001 (67)

Type: aerobic
Inoculum: other bacteria: marine inoculum
Degradation: = 69 % after 5 day(s)

Method: other: closed bottle test (BOD of THOD)
Test substance: as prescribed by 1.1 - 1.4

Remark: BOD 5/10/15/20 of THOD = 69/84/85/97 %.
concentration: 3-10 mg/l of test substance

Reliability: (2) valid with restrictions
12-FEB-2001 (62)

Type: aerobic
Inoculum: other: contaminated soil
Contact time: 30 day(s)
Degradation: ca. 19 % after 30 day(s)

Method: other: see freetext
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Microcosm studies were done in 0.5l-glass bottles with caps containing 150 g soil impacted with relatively high concentrations of solvents inclusive Methanol (MeOH) and approximately 0.4 l groundwater. H2O2 was added as an oxygen source to maintain 1 to 2 mg/l oxygen in solution. In the Microcosms complemented with NH4Cl and Na2HPO4 in ratio COD:N:P= 100:10:2 the result was 19% degradation of MeOH in 30 days.

Reliability: (2) valid with restrictions
01-DEC-1999 (68)

Type: aerobic
Inoculum: other: contaminated soil
Degradation: ca. 100 % after 32 day(s)

Method: other: see freetext
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The continuous-flow reactor consisted of a 30-gal glass reactor and the center portion was filled with site soil and groundwater. Suction and discharge tubing was enveloped in pea stone, perforated baffles were used to separate the zones. Aerobiosis were achieved by addition of H₂O₂.

Result: The half live time for MeOH was : t_{1/2} = 16 days

Reliability: (2) valid with restrictions

01-DEC-1999

(68)

Type: aerobic
Inoculum: activated sludge, adapted
Degradation: = 50 - 80 %

Remark: Swain and Somerville (1978) monitored the biodegradation of methanol in a model activated sludge system. Methanol was not broken down when added transiently (at levels of 0.23% by volume) to the system operating with a retention time of 11 hours. However, adaption of the sludge in the system to 0.1% by volume methanol occurred over a period of several days, and after 2 days acclimation, about 50% of the methanol was utilised. After 6 days acclimation, at least 80% of the methanol had been degraded.

Reliability: (4) not assignable
secondary quotation

12-FEB-2001

(69)

Type: aerobic

Remark: Goldsmith assessed the potential for methanol degradation in subsurface soil and groundwater from sites in Pennsylvania, New York and Virginia under aerobic conditions. Substantial bacterial populations were present in all samples and methanol was readily degraded. A range of methanol concentrations from 1 to 1000 mg/l were used and rates of degradation from 0.8 to 20.4 mg/l/day were recorded in these aerobic studies and in parallel anaerobic studies using material from the same sites. The rates of degradation were generally greater in the anaerobic samples than in the aerobic.

Reliability: (4) not assignable
secondary quotation

12-FEB-2001

(70)

Type: aerobic

Remark: Further details of the study of Goldsmith were reported by Novak et al. They stated that methanol at concentrations up to 1000 mg/l was degraded at all three sites to such an extent that contamination would be reduced to non-measurable amounts within a year. The soils and overlying water used in these tests were not thought to have been previously contaminated. In one aerobic sample of soil from a Pennsylvania site, complete degradation of 100 mg/l methanol occurred in less than 30 days.

Reliability: (4) not assignable
secondary quotation

12-FEB-2001 (71)

Type: aerobic

Remark: Biodegradation of methanol in not heavily polluted aerobic aquifer material: half-live 58-260 days (location: Clay loam Virginia)

Reliability: (4) not assignable
Secondary literature

12-FEB-2001 (72)

Type: anaerobic
Inoculum: other: bacterial population from a sewage sludge
Degradation: = 75 - 80 %

Remark: Bekes et al. (1975) studied the degradation of methanol by the bacterial population from a sewage sludge. Under anaerobic conditions rapid degradation occurred, leading to 75-80% disappearance of the methanol. Rapid liberation of methane and carbon dioxide was observed.

Reliability: (4) not assignable
secondary quotation

12-FEB-2001 (73)

Type: anaerobic
Concentration: 500 mg/l related to Test substance
Degradation: = 66 %

Remark: Chou et al. (1978) studied the acclimation and degradation of several petrochemical wastewater components (including methanol) in an anaerobic fermentation system. Methanol, at a concentration of 500 mg/l, was found to be metabolised by enriched bacterial cultures (developed on acetate substrate) after a period of acclimation. After a 6 day lag period, 66% of the methanol was removed rapidly at a rate of 182 mg/l/day from serum bottle inocula.

Reliability: (2) valid with restrictions
13-FEB-2001 (74)

Type: anaerobic
Degradation: = 83 - 91 % after 3 day(s)

Remark: Oremland et al. (1982) studied the degradation of methanol in anaerobic sediments collected from the upper 15 cm of a salt marsh in San Francisco Bay. The sediments were highly reduced and contained methane and hydrogen sulphide. The sediments were homogenised anaerobically with San Francisco Bay water and 310-340 umol methanol/flask was added to make up the inocula. After 3 days incubation, 83-91% conversion of the methanol had occurred. The products of methanol degradation were methane, CO₂ and water.

Reliability: (2) valid with restrictions
13-FEB-2001 (75)

Inoculum: other: methylotrophic organisms

Remark: In a survey of methylotrophic organisms, i.e. those microorganisms recognised by their ability to grow on compounds that contain no carbon-carbon bonds and to assimilate carbon as formaldehyde or as a mixture of formaldehyde and carbon dioxide, Hanson (1980) identified the following strains/genera of microorganism capable of using methanol as a growth substrate: *Pseudomonas* sp. (including *P. AM1*, *P. C*, *P. 8* and *P. oleovorans*), *Methylobacterium organophilum* XX, *Hyphomicrobium* sp., *Streptomyces* sp., *Rhodopseudomonas acidophila*, *Paracoccus denitrificans*, *Microcyclus aquaticus*, *Thiobacillus novellus*, *Micrococcus denitrificans*, *Achromobacter 1L* and *Mycobacterium* (isolated from activated sewage sludge). Hansen also identified one strain of methanol-oxidising fungus, *Trichoderma lignorum*, and the following genera of methanol-oxidising yeasts: *Pichia*, *Saccharomyces*, *Hansenula*, *Rhodotorula*, *Kloeckera*, *Candida*, *Torulopsis*.

Reliability: (4) not assignable

12-FEB-2001 secondary quotation (76)

Inoculum: other

Remark: Other organisms identified as being capable of utilising methanol as a growth substrate in other reports included: Methanosarcina sp. (anaerobe, transformed methanol to methane), Candida sp. (a yeast isolated from Niger Delta sediment), Mycobacterium sp. (isolated from various samples of Japanese soil) and a yeast isolated from palm wine.

Reliability: (4) not assignable
secondary quotation

12-FEB-2001 (77) (78) (79) (80)

3.6 BOD5, COD or BOD5/COD Ratio

3.7 Bioaccumulation

Species: Cyprinus carpio (Fish, fresh water)

Remark: Gluth et al. studied the accumulation of methanol by carp, Cyprinus carpio, exposed to the chemical for up to 72 hours. The amount of radioactivity derived from the 14-C-labelled chemical was determined in the liver, kidneys, intestine, muscle, blood and gills of carp exposed to methanol at 5 mg/l. The initial uptake of methanol into the different tissue types was the same after 24 hours, and levels remained constant over 72 hours in the liver, kidneys, gills and intestine but dropped slightly in the blood and muscle. Bioaccumulation factors of about 1 were proposed for the fish tissues and organs.

Reliability: (2) valid with restrictions
13-FEB-2001 (81)

Species: Leuciscus idus (Fish, fresh water)
BCF: < 10

Remark: The bioconcentration factor of methanol experimentally measured in fish (golden ide) was less than 10. Based on a log Kow of -0.77 the BCF value for methanol can be estimated to be 0.2 from a recommended regression-derived equation.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
12-FEB-2001 (39) (41) (82)

Species: other: Chlorella fusca (green alga)
Exposure period: 24 hour(s) at 25 degree C
Concentration: 50 µg/l
BCF: = 28400

Method: other
GLP: no data

Method: Algae with a dry weight of 20 mg/200 ml were exposed in a 500 ml Erlenmeyer flask with glass stoppers to 14C-labelled methanol in nutrient solution for at least 24 hours at room temperature (19 - 25°C) under illumination (16 hr/d) and agitation. After 24 hours the algae were removed by centrifugation at 4000 rpm. The 14C activity of the algae was determined by combustion in a Packard Tri Carb Sample Oxidizer and counting in a Packard Tri Carb liquid scintillation counter Model 3385. The bioaccumulation factor on the basis of dry weight is calculated by dividing the chemical concentration of the algae by the final concentration in the water after 24 hours.

Reliability: (3) invalid
The derived high bioconcentration factor is anomalous compared to those for other aquatic organisms. It is likely due to the fact that methanol is taken up very rapidly and metabolized by the algae. The 14C-label, which is measured to calculate the BCF value, is therefore incorporated into the algae in metabolic forms other than methanol. In addition the test was conducted over a wide temperature range.

19-JUL-2004

(83)

3.8 Additional Remarks

Memo: more information see IPCS report no. 196

13-FEB-2001

(84)

Result: Methanol biodegradation has been shown to take place also under anaerobic conditions (all electron acceptors besides oxygen). Microbial growth on methanol with nitrate as the electron acceptor is the second most energetically favored mode of methanol metabolism. There are over 100 wastewater treatment plants in the United States that currently use methanol as a carbon source to remove nitrate (NO3-) from water by anaerobic denitrification.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint

07-MAY-2003

(85)

AQUATIC ORGANISMS**4.1 Acute/Prolonged Toxicity to Fish**

Type: flow through
Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 15400

Method: other
GLP: no data
Test substance: other TS

Remark: Method: Test procedures followed the basic flow-through test method described by the U. S. Environmental Protection Agency (EPA-660/3-75-009, 1975).
The exposure system for fish consisted of several modified proportional diluters with dilution factors ranging from 0.6 to 0.8. Each diluter cycle delivered 1.0 l of water and toxicant, where appropriate, to each of five test concentrations and a control. Each test concentration and control was duplicated by dividing the original volume equally using flow-splitting tanks.
The glass exposure tanks contained 6.3 l water. Diluters were cycled at a rate to yield 5 to 8 tank volumes/day. Exposure samples containing methanol were analyzed by direct aqueous injection gas-liquid chromatography. The percent recovery of methanol-spiked exposure water for bluegill test was 101.5 +/- 2.7% (n = 5).
Bluegills were held in 530-l fiberglass holding tanks at their respective test temperatures and fed a commercial formulation of dry food.
Groups of 10 bluegills were randomly assigned to the exposure tanks at the start of each test.
Fish were not fed 24 h before or during a test.
Average weights of exposed fish were obtained by weighing control fish from each test. Average weight of bluegills was 3.07 g +/- 1.38
Death was the primary endpoint, although effects on equilibrium, behavior and coloration were noted
Median effect concentration, EC50 was 12700 mg/l (11800 - 13700).
Dead fish were counted and removed at 1, 3, 6, 12 and 24 h after initial exposure and at 24-h periods thereafter.
Determination of LC50 and EC50 and their 95% confidence intervals were made using the Trimmed Spearman Karber method (Hamilton et al., 1977). Mortality data from duplicate exposure tanks were combined before LC50 and EC50 determinations were made.

Test condition: Water for all tests was untreated and obtained directly from Lake Superior and heated to mean test temperature. Water temperature of the test: 19.8 +/- 2.3 °C

Mean hardness: 46.6 mg/l CaCO3 (40.4 - 56.3)
 Mean alkalinity: 41.7 mg/l CaCO3 (30.0 - 46.0)
 pH range: 7.04 - 7.97
 Mean dissolved oxygen: 78.8 % (54.3 - 88.9)
Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
 valid with restrictions
Flag: Critical study for SIDS endpoint
 05-DEC-2003 (86)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: 28100

Method: other
GLP: no data
Test substance: other TS

Remark: The test was conducted with Lake Superior water. The flow-through test used a proportional diluter system, in which there were five toxicant concentrations plus a control all in duplicate.
 Individual exposure chambers measured either 26x17x15 cm, or 20x35x15 cm and contained 3.1 l and 6.3 l of water. The diluter system delivered 0.5 l of fresh water alone to the controls or 0.5 l of fresh water plus toxicant in the case of the exposure groups every 15 -19 min.
 Water temperature was maintained at 25 °C and a 16-h light photoperiod was used. Ten to 20 fish of age 30-32 days were placed into each chamber. fish were not fed during the acute test.
 Observations for mortalities and other gross behavioral effects were made at regular intervals through 96 hrs. Several water quality parameters (temperature, dissolved oxygen, hardness, alkalinity, acidity and pH) were monitored throughout the tests.
 Toxicant concentrations were measured daily in acute tests. All twelve chambers were analyzed at 0 and 96 h, with alternating replicates analyzed at 24, 48 and 72 h. Methanol concentrations were determined by using Rhodamine B dye as a tracer. The stock solution contained 25 mg/l which was delivered to the exposure tanks. The concentration of alcohol was calculated from the analysis of Rhodamine B, which was measured a spectrofluorometer.
 The excitation and emission wavelengths were 554 nm and 578 nm respectively. Standard were prepared in Lake Superior water and lake water was used as the reference blank. The exposure tanks contained from 0.1 to 1.3 µg/l Rhodamine B.

Result: LC50 (24h) = 28400 mg/l
 LC50 (48h) = 28400 mg/l

LC50 (72h) = 28400 mg/l
Test substance: methanol, purity > 99 %
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 05-DEC-2003 (87)

Type: flow through
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 20100

Method: other
GLP: no data
Test substance: other TS

Remark: The test was conducted with Lake Superior water. The flow-through test used a proportional diluter system, in which there were five toxicant concentrations plus a control all in duplicate. Individual exposure chambers measured either 26x17x15 cm, or 20x35x15 cm and contained 3.1 l and 6.3 l of water. The diluter cycled every 10-16 min, providing from 6.8 to 10.6 volume additions per day. Water temperature was maintained at approximately 12 °C and a 16-h light photoperiod was used. Ten fish were tested per chamber. Fish were not fed during the acute test. Observations for mortalities and other gross behavioral effects were made at regular intervals through 96 hrs. Several water quality parameters (temperature, dissolved oxygen, hardness, alkalinity, acidity and pH) were monitored throughout the tests. Toxicant concentrations were measured daily in acute tests. All twelve chambers were analyzed at 0 and 96 h, with alternating replicates analyzed at 24, 48 and 72 h. Methanol concentrations in the exposure water were determined by GLC using direct aqueous injection on a Tenax GC packed column and flame-ionization detection

Result: LC50 (24h) = 20300 mg/l
 LC50 (48h) = 20100 mg/l
 LC50 (72h) = 20100 mg/l
Test substance: methanol, purity > 99 %
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 05-DEC-2003 (87)

Type: flow through
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 20100

Method: other: not indicated
GLP: no data
Test substance: other TS

Remark: Method: Flow-through acute toxicity test
 EC50: median effect concentration, EC50=13000 mg/kg
Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
 10-OCT-2002 (86)

Type: other: flow through or static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: g/l **Analytical monitoring:** yes
LC50: 29.4
EC50 : 28.9

Method: other
GLP: no data
Test substance: other TS

Remark: Test organism: fathead minnow, age 28-29 days, overall mean length 21 +/- 2 mm

Test conditions:
 Test temperature: 25 degrees centigrade
 Dissolved oxygen: 7.3 mg/l
 Hardness: 43.5 mg/l CaCO3
 Alkalinity: 46.6 mg/l CaCO3
 Tank volume: 6.3 l
 pH: 7.66

Test concentrations nominal:
 4000; 6700; 11200; 18600; 31000 mg/l

Test substance: EC50: 28900 mg/l/96 h
 methanol, purity > 99 %
Reliability: (2) valid with restrictions
 2.1; accpetable study, meets basic scientific principles

26-SEP-2002 (88)

Type: semistatic
Species: Poecilia reticulata (Fish, fresh water)
Exposure period: 7 day(s)
Unit: mg/l **Analytical monitoring:**
LC50: = 10860

Test substance: Methanol, no further data.
Reliability: (4) not assignable
Secondary quotation
11-OCT-2002 (89) (90)

Type: semistatic
Species: other: Agonus cataphractus
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50: >= 7900 - 26070

Remark: Seawater
Test substance: Methanol, no further data.
Reliability: (4) not assignable
Secondary quotation
11-OCT-2002 (91)

Type: static
Species: Alburnus alburnus (Fish, estuary)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 28000

Method: other: not indicated
GLP: no data
Test substance: other TS

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
11-OCT-2002 (92) (93)

Type: static
Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC : >= 790

Method: other: effect on clinical chemical parameters
GLP: no data
Test substance: other TS

Method: 6 fish were used per group. Exposure time was 6, 24 or 72 hours. The following parameters were examined: serum glucose, serum cortisol, serum protein, serum cholesterol, liver glycogen, muscle glycogen.
Result: Methanol treatment did not affect serum glucose but led to increased serum cortisol, decreased serum protein, decreased serum cholesterol, decreased liver glycogen, increased muscle glycogen.
Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
11-OCT-2002 (94)

Type: static
Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC : = 790

Method: other: effects on clinical chemical parameters were measured
GLP: no data
Test substance: other TS

Method: Exposure time was 72 hours. Exposure temperature was 12, 17 or 22 °C. The following parameters were measured: serum cortisol, serum glucose, liver glycogen, muscle glycogen, serum protein, serum cholesterol.
Result: The treatment led to increased serum cortisol and serum glucose and to decreased liver glycogen, muscle glycogen, serum protein and serum cholesterol.
Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
11-OCT-2002 (95)

Type: static
Species: Lebistes reticulatus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
SG : = 500

Method: other: DIN 38412 T.15
Year: 1973
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: SG = Schaedlichkeitsgrenze (limit of toxicity)
Test substance: Methanol, no further data.
Reliability: (4) not assignable
Secondary quotation

11-OCT-2002 (96)

Type: static
Species: Leuciscus idus melanotus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC0: = 7900
LC50: > 10000
LC100: > 10000

Method: other: not indicated
GLP: no data
Test substance: other TS

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions

11-OCT-2002 (97)

Type: static
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 19000

Method: other: not indicated
GLP: no data
Test substance: other TS

Test substance: Methanol, no further data.
Reliability: (4) not assignable
Secondary quotation

11-OCT-2002 (98)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 28200

Method: other: 5 concentrations and 4 fish/concentration were used
GLP: no data
Test substance: other TS

Remark: 11 alcohols with increasing chain length were tested to create a structure activity relationship with LC50 and octanol-water partition coefficient.

Result: A bilinear relationship was found between log LC50 and log P with increasing toxicity with increasing log P.

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
 11-OCT-2002 (99)

Type: static
Species: Semolitus atromaculatus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
NOEC: < 8000
EC : >= 8000 - 17000

Method: other: the "critical range of toxicity" was measured
GLP: no data
Test substance: other TS

Remark: The test result is described as the critical range of toxicity, but the signs of toxicity examined are not reported in detail.

Test substance: Methanol, no further data.
Reliability: (3) invalid
 11-OCT-2002 (100)

Species: Cyprinus auratus
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
TLm : = 1659

Method: other: no details indicated
GLP: no data
Test substance: other TS

Remark: The test substance concentration is given as 0.21 %, the test result is described as median tolerance limit without further details.

Test substance: Methanol, no further data.
Reliability: (4) not assignable
 11-OCT-2002 (101)

Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
TLM : = 28440

Method: other: no details indicated
GLP: no data
Test substance: other TS

Remark: The test substance concentration is given as 3.6 % and the test result is described as median tolerance limit (no detailed definition).
Test substance: Methanol, no further data.
Reliability: (4) not assignable
11-OCT-2002 (101)

Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC0: > 1000

Method: other: DIN 38412 Teil 15
GLP: no data
Test substance: other TS

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
11-OCT-2002 (102)

Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 10800

Method: other: not indicated
GLP: no data
Test substance: other TS

Remark: 13680 ppm (original data)
Test substance: Methanol, no further data.
Reliability: (4) not assignable
11-OCT-2002 (103)

Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
NOEC: = 8530

Method: other: not indicated
GLP: no data
Test substance: other TS

Remark: 10800 ppm (original data)
Test substance: Methanol, no further data.
Reliability: (4) not assignable
11-OCT-2002 (103)

Species: Oryzias latipes (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
TLM : = 41080

Method: other: no details indicated
GLP: no data
Test substance: other TS

Remark: The test substance concentration is given as 5.2 %, the result is described as median tolerance limit without further details.
Test substance: Methanol, no further data.
Reliability: (4) not assignable
11-OCT-2002 (101)

Species: Semolitus atromaculatus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
: = 6320

Method: other: not indicated
GLP: no data
Test substance: other TS

Remark: River (water) is indicated as test environment. The concentration indicated is given as toxic threshold.8000 ppm (original data).
Test substance: Methanol, no further data.
Reliability: (4) not assignable
11-OCT-2002 (103)

Species: other: Armed Bullhead
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: 7900 - 27700

Method: other: not indicated
GLP: no data
Test substance: other TS

Remark: 10000 - 35000 ppm (original data)
Test substance: Methanol, no further data.
Reliability: (4) not assignable
 11-OCT-2002 (103)

Type: other
Species: other
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: 10630

Remark: QSAR Estimation of acute toxicity of methanol to fish
 conducted with ECOSAR v.099.
 ECOSAR Class: Neutral Organics
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 09-DEC-2003 (59)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
EC0: > 10000
EC50: > 10000

Method: other: acute Toxicity to daphnia magna
Test substance: as prescribed by 1.1 - 1.4

Method: DIN 38412 Teil 11, Bestimmung der Wirkung von
 Wasserinhaltsstoffen auf Kleinkrebse

Remark: Test procedure:
 From the solution with the pollutant, dilution series were
 prepared using dilution water of the specified composition.
 The concentration steps of the test solution were selected
 so as to give 3-4 EC values in a range between EC0 and
 EC100, of which at least one value was below and one above
 EC50.
 The test vessel were 50 ml beakers.
 Two parallel preparations were made for each concentration
 step. Loading amounted to one test animal per 2 ml test

medium as ten 6-24 h old daphnids were placed in each test and control vessel, i.e. 20 animal per concentration test. After 24 h and again after 48 h the number of animals in the control and test solutions that could swim was counted. After 48 h the oxygen content were determined in order to ensure that it had not fallen below a minimum oxygen concentration of 2 mg/l. Furthermore the ph value was determined at the end of the test.

The test was considered as valid when fewer than 10% of the animals in the control solutions were unable to swim, when the pH value was not below 7.0 and the oxygen concentration was not below 4.0 mg/l.

The EC0 and EC100 values were taken from the results obtained for the test solution, the 24 and 48 h EC 50s calculated arithmetically from the concentration/effect ratio.

Result: EC0 (24 h) > 10000 mg/l
EC50 (24 h) > 10000 mg/l

Test condition: 6-24 h old daphnids were used as test animals.

Specific composition of dilution water used:

acid capacity: 0.8 mmol/l
total hardness: 2.4 mmol/l
Calcium to magnesium ratio: 4:1
Sodium to potassium ratio: 10:1
initial pH value: 8.0 +/- 0.2

The test solutions were kept at 20 °C in an incubator. The test period lasted 24 and 48 h. No feed was given during the test period.

Reliability: (1) valid without restriction
Test procedure in according with generally accepted standard methods

Flag: Critical study for SIDS endpoint
05-DEC-2003 (104)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: > 10000

Method: other: inhibition of swimming ability
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
13-FEB-2001 (105)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: > 10000
EC100: > 10000

Method: other: DIN 38412 Teil 11, Bestimmung der Wirkung von Wasserinhaltsstoffen auf Kleinkrebse
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions (106)
 13-FEB-2001

Species: Nitocra spinipes (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC(I)50 : = 12000

Method: other: acute toxicity
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions (93)
 11-OCT-2002

Species: Daphnia magna (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50 : > 100

Test substance: as prescribed by 1.1 - 1.4

Remark: nominal concentration
Reliability: (2) valid with restrictions (107)
 13-FEB-2001

Species: Artemia salina (Crustacea)
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:**
EC50: = 43.6

Result: = 1360 mM
Reliability: (2) valid with restrictions (108)
 14-JAN-2000

Species: Artemia salina (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50 : > 10000

Method: Endpoint evaluated was death.
Remark: Concentrations tested: 100, 1000 and 10000 mg/l
Reliability: (2) valid with restrictions
 14-OCT-2002 (62)

Species: Asellus intermedius (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50 : > 100

Remark: nominal concentration
Reliability: (2) valid with restrictions
 11-OCT-2002 (107)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:**
EC50: = 21.4

Result: = 668 mM
Test substance: methanol, purity > 97 %
Reliability: (2) valid with restrictions
 11-OCT-2002 (108)

Type: other
Species: Daphnia sp. (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: 9374

Remark: QSAR Estimation of acute toxicity of methanol to daphnid
 conducted with ECOSAR v.099.
 ECOSAR Class: Neutral Organics
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 09-DEC-2003 (59)

Species: Gammarus fasciatus (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50 : > 100

Test substance: as prescribed by 1.1 - 1.4

Remark: nominal concentration
Reliability: (2) valid with restrictions
13-FEB-2001 (107)

Species: other aquatic mollusc: Helisoma trivolvis
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50 : > 100

Remark: nominal concentration
Reliability: (2) valid with restrictions
11-OCT-2002 (107)

Species: other aquatic worm: Dugesia tigrina
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50 : > 100

Remark: nominal concentration
Reliability: (2) valid with restrictions
11-OCT-2002 (107)

Species: other aquatic worm: Lumbriculus variegatus
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50 : > 100

Remark: nominal concentration
Reliability: (2) valid with restrictions
11-OCT-2002 (107)

Species: other: Anodonta imbecilis
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50 : = 37.02

Reliability: (3) invalid
inconsisten results
14-OCT-2002 (109)

Species: other: Brachionus clyciflorus
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:**
EC50: = 35.9

Result: = 1120 mM
Reliability: (2) valid with restrictions
 14-JAN-2000 (108)

Species: other: Ceriodaphnia dubia
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50 : = 11

Reliability: (3) invalid
 inconsisten results
 14-OCT-2002 (109)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus quadricauda (Algae)
Endpoint: biomass
Exposure period: 8 day(s)
Unit: mg/l **Analytical monitoring:**
TGK : = 8000

Method: other: growth inhibition
Test substance: as prescribed by 1.1 - 1.4

Remark: The toxicity threshold of methanol for the green alga Scenedesmus quadricauda was determined in the cell multiplication inhibition test. Culture media and conditions of culturing were standardized already for stock and preliminary cultures because a standardized history of the test organisms is essential insofar as history and resistance to pollutants show a close physiological relationship. Composition of culture media and conditions of culturing are described in the paper. Prior of preparations of test cultures, the pollutant solution of known content was neutralized. Dilutions at different volume ratios with the factor 2 were prepared in flasks from the pollutant solution and from bidistillated water under sterile conditions. In the dilution series to be inoculated an addition of 5 ml of each of the stock nutrient solution and of the algal suspension from the preliminary culture having a known adjusted extinction value was made to obtain the rated value of 50 ml. The quantity of the cell material to be used for inoculation was determined turbidimetrically and was standardized.

The flasks of the non-inoculated dilution series were completed to the rated value of 50 ml by addition of 5 ml each of the stock nutrient solution and bidistilled water. While shaking the contents, 10 ml each were taken from each flask of the inoculated dilution series and transferred to 3 culture tubes each and also from each flask of the non-inoculated dilution series, however transferred to one culture tube only.

Test cultures and control cultures were kept then under standardized conditions for a period of 8 days, exposed to constant lighting at 27 °C and a relative umidity of 50%. At the end of the test the concentration of the algal suspension was measured turbidimetrically and expressed as the extinction of the primary light of the monocromatic radiation at 578 nm for a 10 mm layer.

For graphical evaluation of the toxicological findings after termination of the test period, the median value (A) of the extinction of all test cultures not under toxic influence nor stimulated (provided that their extinction values were within the range of a <3% standard deviation) and the median value (B) of the extinction of the three test cultures having the lowest toxic pollutant concentration were determined.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
08-DEC-2003 (110)

Species: other algae: Plancton algae
Endpoint: other: photosynthetic oxygen production
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
EC10: = 1600
EC50: = 12000

Method: other: assimilation test
Test substance: as prescribed by 1.1 - 1.4

Remark: graphic evaluation (probability distribution)
nominal concentration

Reliability: (2) valid with restrictions
13-FEB-2001 (111)

Species: Chlorella pyrenoidosa (Algae)
Endpoint: growth rate
Unit: g/l **Analytical monitoring:**
EC50: = 28.44

Remark: The green alga Chlorella pyrenoidosa was obtained from the National Research Council of Canada, Atlantic Research Laboratory, Halifax, Nova Scotia, Canada. Stock cultures were grown in 250-ml erlenmeyer flasks containing 150 ml of an inorganic, nitrogen-free medium supplemented with 1.5 mg/l of NaNO₃. Flasks were incubated at 25 +/-1 °C and a light intensity of 7000 lux on a 12 hour light-dark cycle. During solvent bioassay experiment test organisms were cultured in test tubes (150 x 25 mm O.D) in both presence and absence of solvent. Growth was monitored by following the increase in optical density over time for 10 to 14 days by means of a spectrophotometer. The most suitable wavelength was 660 nm. Cell concentrations were determined microscopically using a haemocytometer. A total of 5-10 concentrations between 2% and 6% methanol by volume were tested (15.8-47.4 g/l). Each concentration was replicated five to ten times. Appropriate control systems containing no solvent were included in each experiment. Bioassay systems contained 9.5 ml of growth medium, an appropriate volume of solvent and 0.5 ml of inoculum standardized to yield an initial cell concentration of 6.5 to 7.8 E+04 cells/ml. In each experiment percent inhibition values, relative to growth in control systems, were calculated daily using spectrophotometric data. The EC50 values (the concentrations causing 50% reduction in algal growth) were calculated using linear regression analysis of percentage inhibition values against solvent concentration data. All correlations coefficients were >0.960. The EC50 value calculated was 3.6% v/v methanol (28.44 g/l).

Test condition: Exposure period: 10 - 14 days
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

05-DEC-2003 (112)

Species: other algae: green algae
Endpoint: other
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
EC10: calculated
EC50: = 4982

Remark: QSAR Estimation of acute toxicity methanol to green algae
 conducted with ECOSAR v.099
 ECOSAR Class: Neutral Organics

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

14-APR-2004

(59)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Pseudomonas fluorescens (Bacteria)
Exposure period: 16 hour(s)
Unit: mg/l **Analytical monitoring:**
TGK : = 6600

Method: other: Vereinfachte Modifikation des DEV L8 (1960); Messung
 der beginnenden Hemmung der Glucose-Assimilation;
 turbidometrische Zellzahlbestimmung

Test substance: as prescribed by 1.1 - 1.4

Remark: The toxicity threshold of methanol for Pseudomonas
 fluorescens (bacteria) was determined in the
 glucose-assimilation inhibition test.
 Pure cultures of single-cell model organism were used in the
 test. Culture media and conditions for the preparations of
 stock cultures, preliminary cultures and test cultures of
 the model organism were standardized and described in the
 paper.
 Prior of preparations of test cultures, the pollutant
 solution of known content was neutralized (pH: 7.0).
 From the pollutant solution four parallel dilution series
 were prepared in 300 ml Erlenmeyer-flasks stoppered with
 plastic caps.
 The dilution series were prepared as follows: the first
 flask of each series contained 160 ml of pollutant solution.
 Starting from this flask the subsequent dilution steps were
 prepared at a constant dilution ratio by consistently mixing
 80 ml of preliminary pollutant dilution and 80 ml
 bidistilled water.
 Consequently each flask contained 80 ml of culture liquid at
 the start. Each flask of the three dilution series to be
 inoculated was completed to 100 ml by adding 5 ml of each of
 the stock solutions and of 10 ml each of the prepared
 bacterial suspension from the preliminary culture having a

known adjusted extinction.
 The quantity of the cell material to be used for inoculation was determined turbidimetrically and was standardized. The flasks of the non-inoculated dilution series were completed to 100 ml by adding 5 ml each of the stock solutions and 10 ml saline. Both inoculated and non-inoculated dilution series were kept at 25 °C for 16 h. After termination of the test period the concentration of the bacterial suspension was measured turbidimetrically: it is expressed by the extinction of the monochromatic radiation Hg 436 nm. The concentration at which the inhibitory action of a pollutant starts will be present in that step of a dilution series of the pollutant having an extinction value at the end of the test that is >3% below the mean value of the extinction for non-toxic dilutions of the test cultures.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 08-DEC-2003 (113)

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 16 hour(s)
Unit: mg/l **Analytical monitoring:**
TGK : = 6600

Method: other: growth inhibition test
Test substance: as prescribed by 1.1 - 1.4

Remark: The toxicity threshold of methanol for Pseudomonas putida (bacteria) was determined in the cell multiplication inhibition test. Pure cultures of single-cell model organism were used in the test. Culture media and conditions for the preparations of stock cultures, preliminary cultures and test cultures of the model organism were standardized and described in the paper. Prior of preparations of test cultures, the pollutant solution of known content was neutralized. From the pollutant solution four parallel dilution series were prepared in 300 ml Erlenmeyer-flasks stoppered with plastic caps. The dilution series were prepared as follows: the first flask of each series contained 160 ml of pollutant solution. Starting from this flask the subsequent dilution steps were prepared at a constant dilution ratio by consistently mixing 80 ml of preliminary pollutant dilution and 80 ml bidistilled water. Consequently each flask contained 80 ml of culture liquid at the start. Each flask of the three dilution series to be inoculated was completed to 100 ml by adding 5 ml of each of the stock solutions and of 10 ml each of the prepared

bacterial suspension from the preliminary culture having a known adjusted extinction. The quantity of the cell material to be used for inoculation was determined turbidimetrically and was standardized.

The flasks of the non-inoculated dilution series were completed to 100 ml by adding 5 ml each of the stock solutions and 10 ml saline.

Both inoculated and non-inoculated dilution series were kept at 25 °C for 16 h.

After termination of the test period the concentration of the bacterial suspension was measured turbidimetrically: it is expressed by the extinction of the primary light of the monochromatic radiation at 436 nm for a layer of 10 mm thickness.

The concentration at which the inhibitory action of a pollutant starts will be present in that step of a dilution series of the pollutant having an extinction value at the end of the test that is $\geq 3\%$ below the mean value of the extinction for non-toxic dilutions of the test cultures.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
08-DEC-2003 (114)

Type: aquatic
Species: activated sludge of a predominantly domestic sewage
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
SG : = 5000

Method: ETAD Fermentation tube method "Determination of damage to effluent bacteria by the Fermentation Tube Method"
Year: 1973
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: SG = toxicity limit
Reliability: (4) not assignable
secondary quotation
13-FEB-2001 (67)

Type: aquatic
Species: Entosiphon sulcatum (Protozoa)
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:**
TGK : > 10000

Method: other: growth inhibition test
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
13-FEB-2001 (115)

Type: aquatic
Species: Microcystis aeruginosa (Bacteria)
Exposure period: 192 hour(s)
Unit: mg/l **Analytical monitoring:**
TGK : = 530

Method: other: growth inhibition test
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
13-FEB-2001 (110)

Type: aquatic
Species: Tetrahymena pyriformis (Protozoa)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
IC50 : = 18756.34

Method: other: Population Growth Inhibition Test
Test substance: other TS

Test substance: purchased from Aldrich Chemical Co., Milwaukee, Wisconsin,
USA with a purity of 95%

Reliability: (2) valid with restrictions
13-FEB-2001 (116)

Type: aquatic
Species: Uronema parduzci (Protozoa)
Exposure period: 20 hour(s)
Unit: mg/l **Analytical monitoring:**
TGK : > 10000

Method: other: growth inhibition test
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
13-FEB-2001 (117)

Type: aquatic
Species: Vibrio fisheri (Bacteria)
Exposure period: 5 minute(s)
Unit: mg/l **Analytical monitoring:** no
EC50: = 44400 - 88500

Method: other: ToxAlert 10, Microtox and LumiStox standard assays using the appropriate manufacturers's luminometer (see respective manual)
GLP: no
Test substance: no data

Result: EC50 (5 min): 83600 mg/l (ToxAlert10), 44400 mg/l (Microtox), and 88500 mg/l (Lumistox); data related to nominal concentrations;
Test condition: temperature: 15 degree C, pH 7
reference standard: 440 mg/l Zinc sulphate in 2% NaCl

The same stock chemical solution was used to prepare the dilution series for each assay (ToxAlert 10, Microtox and Lumistox, respectively) and all tests were carried out by the same person on the same day.

test criterion: inhibition of light emitted by the bioluminescent bacteria Vibrio fischeri after 5 min compared with zero dose expressed as EC (effect concentration);

Reliability: (2) valid with restrictions
scientifically acceptable, commercially used assay systems
03-JUL-2003 (118)

Type: aquatic
Species: other bacteria: Bakterienmischpopulationen
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
EC10: = 13000
EC50: = 30000

Method: other: oxygen consumption
Test substance: as prescribed by 1.1 - 1.4

Remark: graphicevaluation
nominal concentration

Reliability: (2) valid with restrictions
13-FEB-2001 (111)

Type: aquatic
Species: other bacteria: Nitrosomonas sp./ Nitrobacter sp.
Exposure period: 2 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: ca. 160

Method: other: inhibition
Test substance: as prescribed by 1.1 - 1.4

Result: Hooper and terry showed methanol to be completely inhibitory to ammonia oxidation by Nitrosomonas bacteria at a concentration of 5*E-3 M (about 160 mg/l).

Reliability: (2) valid with restrictions
15-FEB-2001 (119)

Type: aquatic
Species: other bacteria: activated sludge from a municipal sewage treatment plant
Unit: g/l **Analytical monitoring:**
EC50: ca. 71.21

Method: other: Activated Sludge Respiration Inhibition Test

Reliability: (4) not assignable
secondary quotation
13-FEB-2001 (120)

Type: aquatic
Species: other bacteria: cyanobacteria
Unit: g/l **Analytical monitoring:**
EC50: = 20.3 - 43.29

Method: other
Test substance: as prescribed by 1.1 - 1.4

Remark: A minimum of 10 concentrations were tested for each species, ranging from 0.6% to 10% methanol by volume (4.74-79.00 g/l). The EC50 values were calculated from percentage inhibition values measured daily relative to growth in the controls.
Anabaena cylindrica: EC50=2.57% v/v (20.3 g/l); Anabaena inaequalis: EC50=2.68% v/v (21.17 g/l); Anabaena sp.: EC50=3.13% v/v (24.73 g/l); Nostoc sp.: EC50=5.48% v/v (43.29 g/l).

Reliability: (2) valid with restrictions
13-FEB-2001 (121)

Species: Nitrosomonas sp. (Bacteria)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
IC50 : = 880

Remark: Inhibition in ammonia consumption
Reliability: (2) valid with restrictions
 13-FEB-2001 (122)

Species: Paramecium caudatum (Protozoa)
Exposure period: 4 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50 : = 7690

Remark: Rajini et al. (1989) exposed the ciliated protozoan Paramecium caudatum to methanol. The 4 hour LC50 value was quoted as 1.0% (v/v) methanol or 0.24 M, which corresponds to a concentration of 7690 mg/l (calculated from the molar concentration reported). The 10 minute LC50 value was quoted as 6.0% (v/v) methanol or 1.4 M, i.e. 44860 mg/l calculated from the molar concentration quoted.
Reliability: (4) not assignable
 secondary quotation
 13-FEB-2001 (123)

Species: Photobacterium phosphoreum (Bacteria)
Exposure period: 5 minute(s)
Unit: g/l **Analytical monitoring:**
EC50: = 157

Remark: EC50 (5/15/30 minutes) = 157.00/285.56/320.40 g/l.
Reliability: (4) not assignable
 Secondary quotation
 11-OCT-2002 (124)

Species: Photobacterium phosphoreum (Bacteria)
Exposure period: 15 minute(s)
Unit: g/l **Analytical monitoring:**
EC50: = 14.7

Reliability: (4) not assignable
 13-FEB-2001 (125)

Species: Photobacterium phosphoreum (Bacteria)
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:**
EC50: = 29.3

Result: = 916 mM
Reliability: (2) valid with restrictions
 14-JAN-2000 (108)

Species: other bacteria: Steptocephalus proboscideus
Exposure period: 24 hour(s)
Unit: g/l **Analytical monitoring:**
EC50: = 1020

Result: = 1020 mM
Reliability: (2) valid with restrictions
 14-JAN-2000 (108)

Species: other bacteria: aerobic heterotrophs
Exposure period: 15 hour(s)
Unit: g/l **Analytical monitoring:**
IC50 : = 20

Remark: Inhibition of oxygen uptake
Reliability: (2) valid with restrictions
 11-OCT-2002 (122)

Species: other bacteria: methanogenes
Exposure period: 48 hour(s)
Unit: g/l **Analytical monitoring:**
IC50 : = 22

Remark: Inhibition in gas production
Reliability: (2) valid with restrictions
 11-OCT-2002 (122)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

-

4.5.2 Chronic Toxicity to Aquatic Invertebrates

-

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

-

4.6.2 Toxicity to Terrestrial Plants

Species: other terrestrial plant
Expos. period: 14 day(s)

Remark: Plants such as Phaseolus vulgaris, Raphanus sativus radricula, Lepidum sativum, Trifolium pratense, Saintpaulia and Petunia hybrids were exposed to air containing methanol at concentrations between 0.4 and 2.5 mg/m³ for 14 days. Retarded growth, measured as the effect on both wet and dry weight of the plants, was reported for each species.

Reliability: (4) not assignable
secondary quotation

13-FEB-2001

(126)

Species: other terrestrial plant

Remark: US EPA (1976) reported that methanol has not been implicated in vegetation damage as other pollutants, eg. ethylene, nitrogene oxides, sulphur dioxide and ozone, have. However, a Russian study has indicated that plants may be sensitive to methanol vapour at concentrations >0.15 ppm (>0.2 mg/m³). Branches from eight different species of tree were studied and the permissible standard (0.15 ppm or 0.2 mg/m³) was taken as the concentration which did not produce a decrease in photosynthesis after 5 minutes exposure.

Reliability: (4) not assignable
secondary quotation

13-FEB-2001

(127) (128)

4.6.3 Toxicity to Soil Dwelling Organisms

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4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

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4.7 Biological Effects Monitoring

-

4.8 Biotransformation and Kinetics

-

4.9 Additional Remarks

Memo: more information see IPCS report No. 196

13-FEB-2001

(84)

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: Sprague-Dawley
Value: < 790 mg/kg bw

Year: 1971
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Neonatal rats, 24 - 48 h old, weight 5 - 8 g:
The LD50 could not be exactly determined, but was below 790 mg/kg.

Test substance: Methanol, no further data.
15-AUG-2002 (129)

Type: LD50
Species: rat
Strain: Sprague-Dawley
Value: = 5800 mg/kg bw

Test substance: as prescribed by 1.1 - 1.4

Remark: The LD50 was determined in rats in 14-d old rats, weight 16-50 g, not yet sexually mature.

Test substance: Methanol, no further data.
15-AUG-2002 (129)

Type: LD50
Species: rat
Strain: Sprague-Dawley
Value: = 10300 mg/kg bw

Year: 1971
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: The LD50 was determined in young adult male rats (80-160 g).

Test substance: Methanol, no further data.
Reliability: (4) not assignable
Secondary source
Flag: Critical study for SIDS endpoint
05-FEB-2003 (129)

Type: LD50
Species: rat
Strain: Sprague-Dawley
Value: = 7000 mg/kg bw

Year: 1971
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: The LD50 was determined in adult male, old rats (300-470 g).
Test substance: Methanol, no further data.
Reliability: (4) not assignable
Secondary source
Flag: Critical study for SIDS endpoint
05-FEB-2003 (129)

Type: LD50
Species: rat
Value: = 6200 mg/kg bw

Test substance: no data

Test substance: Methanol, no further data.
15-AUG-2002 (103)

Type: LD50
Species: rat
Value: = 5628 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (103)

Type: LD50
Species: rat
Value: = 9100 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
15-AUG-2002 (130)

Type: LD50
Species: rat
Value: = 12900 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
15-AUG-2002 (131)

Type: LD50
Species: rat
Strain: Wistar
Sex: male/female
No. of Animals: 10
Vehicle: water
Doses: applied in log scale
Value: = 13000 mg/kg bw

Method: other
Year: 1941
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
 Comparable to guideline study, however, documentation limited.

Flag: Critical study for SIDS endpoint
 03-FEB-2003 (132)

Type: LD50
Species: rat
Strain: Sprague-Dawley
Sex: male/female
No. of Animals: 10
Vehicle: other: as 15 - 35-% aqueous solution
Doses: 7914; 5390 and 3672 mg/kg
Value: > 7914 mg/kg bw

Method: other: BASF-Test
Year: 1975
GLP: no
Test substance: other TS

Remark: No mortality was observed in any dose group.
 No pathological findings after post-observation period of 7 days.

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
 Comparable to guideline study, limited documentation, acceptable for assessment

Flag: Critical study for SIDS endpoint
 18-FEB-2003 (133)

Type: LD0
Species: rat
No. of Animals: 10
Vehicle: water
Doses: limit test: 6.4 ml/kg
Value: >= 5065 mg/kg bw

Method: other: BASF-Test
Year: 1961
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Oral application of 5065 mg/kg (6.4 ml/kg) did not lead to mortality in any of 10 animals used. Only clinical symptoms including staggering, narcotic effects.
Postobservation period: 7 days

Test condition: Methanol was administered as 50-% aqueous solution.
Test substance: Methanol, containing 1.5 % water, no other contamination
Reliability: (2) valid with restrictions
Comparable to guideline study, limited documentation, acceptable for assessment

09-AUG-2002

(134)

Type: other: LD values
Species: rat
Strain: no data
Vehicle: water
Doses: no data

Year: 1955
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Methanol was administered as 50-% aqueous solution by gavage.

Result: Severe narcosis was produced without latency, however, no acidosis and no changes of the eye fundus.

Mean LD10 was at 9000 mg/kg
mean LD70 was at 9500 mg/kg
mean LD100 was at 10000 mg/kg.

Test substance: Methanol, no further data.
Reliability: (4) not assignable
Secondary source

Flag: Critical study for SIDS endpoint

18-FEB-2003

(135)

5. Toxicity

date: 21-JUL-2004
Substance ID: 67-56-1

Type: LD50
Species: mouse
Strain: C57BL
Value: = 7300 - 10000 mg/kg bw

Method: other: no details reported
GLP: no data
Test substance: other TS

Test substance: Methanol, no further data.
03-FEB-2003 (84) (136)

Type: LDLo
Species: mouse
Value: = 420 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (103)

Type: LD50
Species: rabbit
Value: = 14400 mg/kg bw

Method: other: no details inidicated
GLP: no data
Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (137)

Type: LD50
Species: rabbit
Value: = 14200 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (138)

Type: LDLo
Species: rabbit
Value: = 7500 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (103)

Type: other: ND50
Species: rabbit
Value: = 6000 mg/kg bw

Test substance: other TS

Remark: ND50: narcotic dose in 50 % of the animals used.
Test substance: Methanol, no further data.
04-MAR-1997 (137)

Type: other: lethal dose
Species: rabbit
Value: = 7000 mg/kg bw

Method: other: no details indicated
Year: 1955
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: 7 g Methanol/kg bw per gavage led to mortality within 1 - 3 days. All animals showed ataxia, nystagmus and coma but no acidosis and no ophthalmological changes.
Test substance: Methanol, 99.5 % pure from Merck, no further data.
Reliability: (4) not assignable
Secondary source
Flag: Critical study for SIDS endpoint
18-FEB-2003 (135)

Type: LDLo
Species: dog
Value: = 7500 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (103)

Type: other: lethal dose
Species: dog
Value: >= 4000 - 7000 mg/kg bw

Method: other: only single animals were used
Year: 1955
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Methanol was applied orally together with each 20 g Saccharose to prevent irritant effects. The animals receiving 9000 mg/kg body weight died within 46 hours, one after 4000 mg/kg, while 7 other animals receiving between 2500 and 8000 mg/kg bodyweight survived. All animals showed signs of an influence on the central nervous system like ataxia, nystagmus, coma, narcosis, euphoria and vomiting. No ophthalmological changes occurred and mostly no acidosis.

Test substance: Methanol, 99.5 % pure from Merck, Germany, no further data.
Reliability: (4) not assignable
Secondary source

Flag: Critical study for SIDS endpoint

03-FEB-2003 (135)

Type: other: acute intoxication
Species: miniature swine
Strain: other: minipig YU, CR
Sex: female
No. of Animals: 3
Vehicle: water
Doses: 1000, 2500, or 5000 mg/kg (20 % solution)

GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Three animals were used per dose group and treated by gavage.
Control tests were conducted with formate administration.

Result: Dose-dependent signs of acute intoxication including mild CNS depression, tremor, ataxia, and recumbency, developed within 1.5 to 2 h, and resolved by 52 h. Methanol- and formate-dosed animals did not develop optic nerve lesions, toxicologically relevant formate accumulation, and metabolic acidosis.

Average maximum methanol levels in blood:
3100 +-700, 6200 +-2300, and 15200 +-900 ug/ml,
respectively, within 0.5 to 4 h. T/2 ranged from about 9
(low dose) to about 19 and 22 h for the higher doses.

Formate plasma levels: transiently 1.74 - 3.4 mEq/L vs. 0.5
+-0.3 mEq/L (control) within 4 to 30 h post-treatment.

Conclusion: The minipig, maintained on a normal folate-diet, does not appear to be a satisfactory model for human methanol poisoning.

Reliability: (2) valid with restrictions
03-FEB-2003 (139)

Type: LD50
Species: monkey
Strain: other: Rhesus macaca
Sex: no data
No. of Animals: 12
Vehicle: water
Doses: 500-4000, 5000, 6000, 7000, 8000, and 9000 mg/kg
Value: ca. 7000 - 9000 mg/kg bw

Year: 1961
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: There was no evidence of marked acidosis after high doses (no hyperventilation, no increase in urinary excretion of organic acids, or shift in serum bicarbonate). Signs of acidosis in only one monkey receiving 6 g/kg were not accompanied by the characteristic "Kussmaul respiration" (Cooper and Felig, 1961).

Blindness was not generally noted, but only 4 days after exposure in one very high-dosed monkey (9 g/kg) who survived. This effect apparently was transient, because the animal showed again normal responses to visual stimuli and normal behaviour by the following day (Cooper and Felig, 1961).

Characterisation of typical symptomatology:
- Ataxia, weakness, and lethargy: 1 to 2 h after exposure and disappeared with 24 h.
- Transient coma: Usually 0.5 h after exposure intoxication evident: progressive ataxia, weakness, and lethargy resulting in coma 1 to 4 h later for 5 to 12 h, but appeared to fully recover after 24 h.
- Death: previous narcosis then death within 20 to 30 h. (Cooper and Felig, 1961).

Test condition: Methanol was administered orally by catheter, diluted to 20 - 30 % in water.
12 animals received doses from 0.5 to 9 g/kg, with 7 and 8 monkeys at sublethal doses and with 6 to 2 monkey at lethal doses. Some animals were re-used after a time of recovery, on the whole 28 applications with 12 animals.

Test substance: Methanol, 99.9 % pure, Fisher Scientific Comp.
Conclusion: The lethal dose in this study (Cooper and Felig, 1961) was significantly higher than published elsewhere for monkeys (minimal lethal dose at approx. 3000 mg/kg from Potts, 1955; Gilger et al., 1956) and also above reported fatal doses in humans (approx. 3000 mg/kg). In this study 6000 mg/kg were sublethal, while Gilger et al.(1959) found a mortality of 78%.

The clinical picture was always one of intoxication progressing to a narcotic state which in fatal cases was irreversible. The characteristic features of human poisoning, such as a latent period, rapid and deep breathing, vomiting, and blindness, were almost never be found, but may temporarily occur. Therefore, acute methanol intoxication in monkeys appears to resemble that in rodents and other lower animals (Cooper and Felig, 1961).

Reliability: (2) valid with restrictions
Test procedures in accordance with accepted standard methods, limited, but sufficiently documented

Flag: Critical study for SIDS endpoint
18-FEB-2003 (140) (141) (142) (143)

Type: other: intoxication and lethality
Species: monkey
Strain: other: Rhesus
No. of Animals: 8
Doses: 6000 mg/kg

Year: 1955
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Eight young adult rhesus monkeys received 6000 mg/kg methanol by gavage.

After 1 h: narcosis at maximum after 4 h.
after 15 h: normal appearance without apparent anomalies;
after 20 - 24 h: marked decrease in alkaline blood capacity.
Following bicarbonate supplementation, 4/8 animals survived.

Clinical observations:
Eye fundus: In case of mild intoxication symptoms, no or only transitory alterations of the eye fundus were observed. In severe cases, an extended edema of the retina and the optic papilla as well as wide, non-responsive pupils.

Histological changes: 6/8 animals exhibited cystic degeneration of the outer retinal granular layer, in 1 animal there was evidence of significant demyelination of the optic nerve.

In the elektroretinogramme, a-wave signal was negative, while the b-wave was extinguished.

Neurological symptoms were clinically expressed as rigor and tremor, histological lesions were seen in the putamen and nucleus caudatus (3/8 animals).

Test substance: methanol, no further data
Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint
03-FEB-2003 (144) (143)

Type: other: lethal dose
Species: monkey
Value: >= 3000 - 8000 mg/kg bw

Method: other: only single animals were used
Year: 1955
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Methanol was applied orally to male rhesus monkeys. 1 to 2 g/kg bodyweight did not lead to mortality while animals receiving 3 to 8 g/kg bodyweight died within two days. Treated animals showed acidosis and partly semicoma and ophthalmological changes. 20 g Saccharose was applied concurrently to prevent irritant effects (Gilger and Potts, 1955).

[Compare also Cooper and Felig, 1961 (other entry this Chapter) who found no mortality in this dose range.]

Test substance: Methanol, 99.5 % pure from Merck, no further data.
Reliability: (4) not assignable
Secondary source

Flag: Critical study for SIDS endpoint
18-FEB-2003 (140) (135)

5.1.2 Acute Inhalation Toxicity

Type: LC50
Species: rat
Exposure time: 4 hour(s)
Value: = 83.2 mg/l

Method: other: no details reported
GLP: no data
Test substance: no data

Remark: Substance concentration is given as 64000 ppm.
Test substance: Methanol, no further data.
15-AUG-2002 (145)

Type: LC50
Species: rat
Sex: male/female
No. of Animals: 100
Exposure time: 4 hour(s)
Value: 128.2 mg/l

Method: other: BASF-Test
Year: 1980
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: 10 male and 10 female Sprague-Dawley rats (185 +-15 g bw) were tested per dose group. Following analytical / nominal methanol vapor concentrations were tested in a head-nose inhalation system:
87.6 / 127 mg/l
115.9 / 159 mg/l
139.0 / 168 mg/l
150.9 / 193 mg/l
151.1 / 242 mg/l.

Mainly in the high concentrations, where also mortality occurred following clinical symptoms were described: aqueous secretion of eyes and nose, closed eyes, laboring breathing, stagger, apathy, narcosis, prone position, opacity of the cornea. Animals died within 5 days after substance administration. Necropsy showed cardiac dilatation and hyperaemic congestion as well as pulmonary edema with extended or scattered hyperaemia.

Some animals, surviving, still showed some effect after the 14-day post observation period.

Test substance: Methanol, purity 99.8 % (Merck)
Reliability: (2) valid with restrictions
Comparable to guideline study, meeting current standards, acceptable for assessment
Flag: Critical study for SIDS endpoint
05-FEB-2003 (146)

Type: LC50
Species: rat
Sex: male/female
No. of Animals: 160
Exposure time: 6 hour(s)
Value: 87.5 mg/l

Method: other: BASF-Test
Year: 1980
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: 10 male and 10 female Sprague-Dawley rats were tested per

dose group. Following analytical / nominal methanol vapor concentrations were tested in a head-nose inhalation system:
31.1 / 36.1 mg/l
64.2 / 93.3 mg/l
65.22 / 81.3 mg/l
66.5 / 103.0 mg/l
83.2 / 104.0 mg/l
88.36 / 121.9 mg/l
94.4 / 113.3 mg/l
160.0 / 197.6 mg/l.

Mainly in the high concentrations following clinical symptoms were described: aqueous secretion of eyes and nose, closed eyes, laboring breathing, stagger, apathy, narcosis, prone position, opacity of the cornea. Animals were dying within 7 days after substance administration. Necropsy showed cardiac dilatation and hyperaemic congestion as well as pulmonary edema and scattered hyperaemia. Some animals of high concentration groups, surviving, still showed some effect after the 14 day post observation period. No effect was observed in the lowest concentration group.

Test substance:
Reliability:

methanol, purity 99.8 %
(2) valid with restrictions
Comparable to guideline study, meeting current standards, acceptable for assessment
Critical study for SIDS endpoint

Flag:

05-FEB-2003

(147)

Type:

other: Hormone status

Species:

rat

Strain:

Long-Evans

Sex:

male

No. of Animals:

10

Doses:

200, 5000 or 10000 ppm (0.26, 6.5 or 13 mg/l)

Exposure time:

6 hour(s)

Method:

other

GLP:

no data

Test substance:

as prescribed by 1.1 - 1.4

Method:

Two experiments were conducted to evaluate the acute effects of inhaled methanol on serum hormones associated with reproductive function in the male rat:
1. 10 rats per group (60 d old) exposed to methanol (0, 200, 5000 and 10,000 ppm) for 6 hours were killed at the end of the exposure period or the following morning. The effect of the handling associated with placing the rat in the exposure chamber was also evaluated by comparing hormonal changes in sham- and methanol-exposed groups acclimated for two weeks with groups that were not acclimated. [note: Prior handling resulted in an increase in serum lutenizing hormone greater in the non-acclimated groups than in the acclimated group.]

2. Groups of acclimated and non-acclimated rats were exposed to 0 or 5000 ppm methanol for 1, 2 and 6 hours and killed immediately after removal from the chamber.

Result: Serum luteinizing hormone, testosterone and follicle stimulating hormone values were not different in sham- vs methanol-exposed rats at any time point. An effect of prior handling was noted: In general, the concentrations of these hormones and serum prolactin in the non-acclimated rats were greater than those observed for acclimated rats. However, methanol exposure appeared to result in increased prolactin concentrations under both handling conditions.

Conclusion: Effects on serum hormone levels (luteinizing hormone, follicle stimulating hormone, testosterone, prolactin) were inconsistent with partly no effects and partly contradictory results. According to the author methanol treatment led to increased prolactin levels in serum.

Reliability: (2) valid with restrictions
Test procedures based on scientific principles, sufficiently documented, acceptable for assessment

15-DEC-2003

(148)

Type: other: IRT

Species: rat

Exposure time: 7 hour(s)

Method: other: based on H.F. Smyth et al. Am. Ind. Hyg. Ass. J. 23,95-107, (1962)

Year: 1980

GLP: no

Test substance: as prescribed by 1.1 - 1.4

Remark: No mortality was observed when rats were exposed for 7 hours to an atmosphere that has been saturated at 20 degrees celsius with the volatile part of the compound. Irritant effects on the respiratory tract were observed.

Test substance: Methanol, no further data.

06-FEB-2003

(149)

Type: other: IRT
Species: rat
No. of Animals: 6
Doses: approx. 150 ml/l (estimated from vapour saturation properties and measured decrease in substance during vapour generation)
Exposure time: 4 hour(s)

Method: other: BASF-Test
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Mortality was observed when rats were exposed for more than 1 hour to an atmosphere that had been saturated at room temperature with the volatile part of the compound (11 - 13 Vol%): 3/6 dead after 2 h; 6/6 dead after 4 h.
 No mortality occurred up to 1 hour.

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
 Screening test
Flag: Critical study for SIDS endpoint
 18-FEB-2003 (150)

Type: other: Inhalation Risk Test
Species: rat
Sex: male/female
Exposure time: 8 hour(s)

Method: other: BASF-Test
Year: 1975
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: No mortality was observed when 12 rats were exposed for 3 hours to an atmosphere that had been saturated with the volatile part of the compound. The inhalation of this atmosphere over 8 hours caused mortality of all 6 rats tested.
 Clinical symptoms were strong irritation of mucosa, opacity of the cornea and narcosis.

Test substance: methanol
 10-FEB-2003 (151)

Type: other: lethal dose
Species: rat
Exposure time: 18 hour(s)
Value: >= 41 mg/l

Method: other
GLP: no
Test substance: no data

Remark: Six concentrations were tested at different exposure times: Rats were exposed to Methanol concentrations of 2.6; 11.5; 30 mg/l 8 hours, 17 mg/l 0.33 hours, 65.5 mg/l 2.5 hours and 41 mg/l 18-20 hours. Only 41 mg/l for 18-20 hours was lethal.

Test substance: Methanol, no further data.
15-AUG-2002 (152)

Type: other: lethal dose
Species: rat
Exposure time: 6 hour(s)
Value: >= 41 - 91 mg/l

Method: other: various concentrations and exposure times were used
Test substance: no data

Remark: Exposure to 65-100 mg/l for 1.5 hours and 51-70 mg/l for 3 hours did not lead to mortality. Exposure to 70-111 mg/l for 3 hours or 30-120 mg/l for 6 hours led to a dose related increase in mortality. Clinical signs indicating effects on the central nervous system occurred in treated animals.

Test substance: Methanol, no further data.
Reliability: (4) not assignable
Secondary source
Flag: Critical study for SIDS endpoint
18-FEB-2003 (153)

Type: LC50
Species: mouse
Exposure time: 134 minute(s)
Value: = 79.43 mg/l

Method: other: no details reported
GLP: no data
Test substance: other TS

Remark: The substance concentration is given as 61100 ppm.
Test substance: Methanol, no further data.
24-MAR-1997 (145)

Type: other: effective dose
Species: mouse
Exposure time: 3.2 hour(s)
Value: = 40 mg/l

Test substance: other TS

Remark: 40 mg/l for 3.2 hours led to narcosis.
Test substance: Methanol, no further data.
04-MAR-1997 (154)

Type: other: narcotic dose
Species: mouse
Exposure time: 7 hour(s)
Value: = 55 mg/l

Test substance: other TS

Remark: Exposure to 55 mg/l for 7 hours is reported as narcotic dose.
Test substance: Methanol, no further data.
15-AUG-2002 (155)

Type: LC50
Species: cat
Exposure time: 6 hour(s)
Value: = 26 - 48 mg/l

Method: other
Test substance: no data

Remark: Cats were exposed for 6 hours to concentrations of 26; 48; 97; 210 mg/l or for 3.5 hours to 380 mg/l. Concentrations of 26 and 48 mg/l led to 50 % mortality which increased with higher concentrations. The number of animals used is not indicated.
Test substance: Methanol, no further data.
15-AUG-2002 (156)

Type: LC50
Species: cat
Exposure time: 6 hour(s)
Value: = 43.68 mg/l

Method: other: no details reported
GLP: no data
Test substance: no data

Remark: The substance concentration is given as 33600 ppm.
Test substance: Methanol, no further data.
15-AUG-2002 (145)

Type: LC50
Species: cat
Exposure time: 4.5 hour(s)
Value: = 85.41 mg/l

Method: other: no details reported
GLP: no data
Test substance: other TS

Remark: The substance concentration is given as 65700 ppm.
Test substance: Methanol, no further data.
24-MAR-1997 (145)

Type: LCLo
Species: cat
Exposure time: 6 hour(s)
Value: = 44 mg/l

Test substance: no data

Test substance: Methanol, no further data.
15-AUG-2002 (103)

Type: LC0
Species: dog
Exposure time: 1 hour(s)
Value: = 65.5 mg/l

Test substance: no data

Remark: Dogs were exposed for 24 hours to 2.6 mg/l, for 4 hours to 18 mg/l, for 8 hours to 48 mg/l or for 1 hour to 65.5 mg/l. At 48 or 65.5 mg/l signs of effects on the central nervous system occurred.
Test substance: Methanol, no further data.
15-AUG-2002 (157)

Type: other: lethal dose
Species: monkey
Strain: other: Rhesus
Sex: male/female
No. of Animals: 11
Exposure time: 18 hour(s)
Value: >= 13 mg/l

Method: other
Year: 1931
GLP: no
Test substance: other TS

Remark: Exposure to 1.3 mg/l (1000 ppm) for 41 hours, to 10000 ppm (13 mg/l) for 18 h or to 52 mg/l (40000 ppm) for 1-4 hours led to mortality.

Blindness based on ophthalmology associated with optic atrophy is reported (conditions not specified). Recovery from this lesion is observed.

Test substance: Methanol, "synthetic", >95 % pure, no further data.

Reliability: (2) valid with restrictions
Test procedures based on scientific principles, limited documentation, acceptable for assessment

Flag: Critical study for SIDS endpoint
05-FEB-2003 (158)

5.1.3 Acute Dermal Toxicity

Type: other: lethal dose
Species: rat
Value: >= 45000 mg/kg bw

Method: other
Test substance: no data

Remark: Occusive application of up to 35000 mg/kg bodyweight to rats did not lead to mortality while 45000 mg/kg was lethal.

Test substance: Methanol, no further data.

Reliability: (4) not assignable
Secondary source

Flag: Critical study for SIDS endpoint
18-FEB-2003 (153)

Type: LD50
Species: rabbit
Value: = 20000 mg/kg bw

Test substance: no data

Test substance: Methanol, no further data.
15-AUG-2002 (103)

Type: LD50
Species: rabbit
Value: = 17100 mg/kg bw

Year: 1981
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: No detailed data: LD50 given as 20 ml/kg.

Test substance: Methanol, no further data.

Reliability: (4) not assignable
Secondary source

Flag: Critical study for SIDS endpoint
05-FEB-2003 (159)

Type: LD50
Species: rabbit
Value: = 15800 mg/kg bw

Test substance: no data

Test substance: Methanol, no further data.
15-AUG-2002 (138)

Type: LDLo
Species: monkey
Value: = 393 mg/kg bw

Test substance: no data

Test substance: Methanol, no further data.
15-AUG-2002 (138)

Type: other: lethal dose
Species: monkey
Strain: other: Rhesus
Sex: male/female
No. of Animals: 8
Doses: 4x 0.5 and 1.3 ml/d under occlusive condition
Value: 1600 - 4000 mg/kg bw

Method: other
Year: 1931
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Four doses of 400 mg/kg daily caused sickness within 24 h, and eventually death. At 1000 mg/kg (4 doses daily), severe illness takes place, followed by death the second day.

Test substance: Methanol, "synthetic", >95 % pure, no further data.
Reliability: (2) valid with restrictions
Test procedures based on scientific principles, limited documentation, acceptable for assessment

Flag: Critical study for SIDS endpoint
10-FEB-2003 (158)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: rat
Route of admin.: i.p.
Value: = 9540 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (103)

Type: LD50
Species: rat
Route of admin.: i.p.
Value: = 7529 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (138)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: 10500 - 11500 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (160)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: = 10765 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (138)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: 7914 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Test substance: Methanol, no further data.
04-MAR-1997 (151)

Type: LD50
Species: rabbit
Route of admin.: i.p.
Value: = 1826 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (138)

Type: LD50
Species: guinea pig
Route of admin.: i.p.
Value: = 3556 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (138)

Type: LD50
Species: hamster
Route of admin.: i.p.
Value: = 8555 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (138)

Type: LD0
Species: rat
Sex: male
No. of Animals: 25
Vehicle: physiol. saline
Route of admin.: i.p.
Value: >= 3000 mg/kg bw

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: Methanol was applied to male F344 rats i. p. at dose levels of 25; 125; 600 und 3000 mg/kg. After 1; 3; 10; 24; 30 und 48 hours a range of clinical chemical parameters were determined (formate level, organic acids, folic acid, pH, pCO₂, glucose, urea, Na, K, Cl). The highest dose level led to a slight increased formate level in blood, but no decrease in the blood pH. No other parameters were changed.

Test substance: Methanol, reagent special grade of Junsei Chemicals Co., no further data.

Reliability: (2) valid with restrictions
08-AUG-2002 (161)

Type: LDLo
Species: mouse
Route of admin.: i.p.
Value: = 120 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (103)

Type: other: effective dose
Species: rat
Route of admin.: i.p.
Value: >= 6000 mg/kg bw

Year: 1975
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Rats were given 6 g/kg bw Methanol i. p.. The treatment led to depressionen of the central nervous system for 4-6 hours.

Test substance: Methanol, reagent grade, no further data.
15-AUG-2002 (162)

Type: other: lethal dose
Species: rat
Route of admin.: i.p.
Value: = 5065 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: The application of 2530 mg/kg did not lead to mortality in 5 animals but 5065 mg/kg led to mortality in 10 of 10 rats.
Test substance: Methanol, no further data.
04-MAR-1997 (163)

Type: other: lethal dose
Species: mouse
Route of admin.: i.p.
Value: >= 5065 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: The application of 1270 mg/kg did not lead to mortality in 5 animals but 5065 mg/kg led to mortality in 1 of 5 animals and 7910 mg/kg in 6 of 10 animals.
Test substance: Methanol, no further data.
04-MAR-1997 (163)

Type: other: lethal dose
Species: monkey
Strain: other: Macaca fascicularis
Sex: male
No. of Animals: 5
Vehicle: physiol. saline
Doses: 0; 25; 125; 600 and 3000 mg/kg.
Route of admin.: i.p.
Value: = 3000 mg/kg bw

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: After 1; 3; 10; 24; 30 and 48 hours the following clinical chemical parameters were examined: pH; pCO₂; formate, organ. acids, folate, glucose, urea, K, Na, Cl.

No abnormalities were seen up to 600 mg/kg bw. All monkeys exposed to 3000 mg/kg fell into a coma immediately after administration and remained recumbent for 3 h to 2 d. Additionally, anorexia was observed. Autopsy and pathological examination revealed no lesions in the eye, optic nerve or central nervous system except for slight

effects in the liver (p. 65). During another part of the study (p. 66), 3 animal died after injection of 3000 mg/kg within 24 to 48 h.

The highest dose level led to increased formate level, decreased pH in blood as well as delayed excretion of methanol and secondary products.

Test condition: Results created within a metabolism study (NEDO, 1987, p. 61): Methanol was diluted in water and applied to male monkeys (*Macaca fascicularis*) i.p. A total of 5 monkeys were repeatedly used.

Test substance: Methanol, reagent special grade of Junsei Chemicals Co., no further data.

Reliability: (2) valid with restrictions
09-AUG-2002 (161)

Type: other: lethal dose / acidosis
Species: monkey
Strain: other: adult rhesus *Macaca mulatta* (females) and *Macaca nemestrina* (pigtail monkey) (females + 1 male)
Sex: male/female
No. of Animals: 10
Vehicle: physiol. saline
Doses: 2000, 3000, and 4000 mg/kg bw (as 20- or 25 % solution)
Route of admin.: i.p.
Value: = 4000 mg/kg bw

Year: 1975
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Objective of the study was
1. to find the suitable model animal to represent methanol intoxication in humans (acidose model) and
2. to identify those organic acids which are responsible for development of acidosis.

Blood buffer status was measured: pH, pCO₂, CO₂, bicarbonate.
Further blood parameters: ion status, glucose, urea.
Blood formate was measured by colorimetric analysis. Formate and formate-borne metabolites were analysed in blood, urine and exhaled air by using ¹⁴C-formate. Blood controls were from the same test animals 12 h prior to methanol exposure.

Organic acids were measured using GC-MS.

Remark: Mortality occurred at the highest dose level in one monkey, all treated animals showed depression of the central nervous system and acidosis
: The pigtail monkey was tested for effects of

toxic methanol exposure to establish an animal model for human methanol toxicity. Blood acidosis was measured as major toxic effect of methanol and the responsible acid was identified. In pigtail monkeys but not in rhesus monkeys significant blood acidosis was produced by a single i.p. dose of 4 g/kg bodyweight methanol. Blood concentration of formate was correlated with acidosis, no other organic acid was found

Result:

Test substance: Methanol, reagent grade, no further data.
1. Symptomatology and changes in various blood parameters

After 4000 mg/kg bw, i.p., 3/4 rhesus macacques exhibited no apparent signs of toxicity within 24 h and later, although a mild acidosis was produced: pH at 7.22 - 7.24, pCO₂ at 30 - 34 mmHg vs pH 7.40 and 37 mmHg for the control.

1/4 rhesus macaque became progressively weaker after treatment and developed a severe metabolic acidosis which led to coma and death: His blood values were pH = 7.03 and pCO₂ = 14.

After 4000 mg/kg bw, i.p., 4/4 pigtail monkeys exhibited distinct signs of methanol intoxication: depression of the CNS within 30 min, thereafter apparently normal appearance, followed by weakness and apathy at 15 - 18 h later and eventually coma and death.

All displayed a sharp decrease in blood pH (7.03 - 7.10) and in pCO₂ (15 to 20, and 33 mmHg): 1/4 died after 4-h coma and 22-h post treatment, 2/4 recovered from coma, 1/4 in coma after 32 h and sacrificed after 36 h. The acidosis developed within about 10 - 12 h accompanied and followed by a marked decrease in blood bicarbonate, total CO₂ and blood pH over about 20 h post-treatment.

The two pigtail monkeys given 2000 and 3000 mg/kg showed no apparent signs of toxicity, but displayed a mild acidosis (pH 7.21 and 7.29; pCO₂ 30 and 32 mmHg).

Inhibition of ADH prior to methanol treatment (4-methylpyrazole, 50 mg/kg i.v.) prevented the occurrence of acidosis in the pigtail monkey at a dose which otherwise routinely produced acidosis.

Blood analysis identified only bicarbonate as the sole ion to change during development of acidosis. Additionally, an time-dependent increase in blood glucose was evident (<=106 mg/100 ml vs. 73 mg/100 ml of the control).

2. Blood organic acids
Bood formate increased steadily in pigtail monkeys given 4000 mg/kg methanol and reached >800 mg/l (note: In SD rats given 6000 mg/kg, no increase occured. Concomitantly, bicarbonate declined. Other metabolic organic acids measured (lactate, alpha-OH-butyrate, beta-OH-butyrate, alpha-ketobutyrate, etc.) showed a significant increase versus control values, but the excess sum in these anions accounted for only about 0.5 mEq./L anf therefore, for not more than 2 - 3% of the observed decrease in bicarbonate (p. 54).

3. Formate elimination
The calcaulted half-lifes for NaFormate (50 mg - 470 mg/kg bw, i.v.) ranged from 31 min (50 mg/kg) to 51 min (470 mg/kg) in monkey (nor specfied which, and no comparisao between both strains).
[note in rat: half-lifes were significant shorter from 12 min (100 mg/kg) to 23 min (670 mg/kg) (p.57)]

Test substance: Methanol, reagent grade, no further data.
Conclusion: The pigtail macacque appeared to serve as a suitable experimental animal in which to study human methanol acidosis: Biochemical and clinical parameters as well as the parallelism of the time course for the development of symptoms and acidosis observed in human cases of acute intoxication are comparable to those found in this monkey.

Results support the view that formic acid is the major agent to produce acidosis and methanol intoxication in monkeys and humans: I appears that formate, a fixed base, replaces bicarbonate, a volatile base, in the blood and that the resultant decrease in blood buffer capacity renders the organismen incapable of mantaining blood pH within normal limits (p. 58).

Reliability: (2) valid with restrictions
lc: Test procedures in accordance with accepted standard methods, sufficiently documented, acceptable for assessment

Type: LD50
Species: mouse
Route of admin.: s.c.
Value: ca. 4160 mg/kg bw

Test substance: other TS

Remark: LD50 in newborn animals.
Test substance: Methanol, no further data.
04-MAR-1997 (164)

Type: LD50
Species: mouse
Route of admin.: s.c.
Value: = 4100 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (103)

Type: LD50
Species: mouse
Route of admin.: s.c.
Value: = 9800 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (103)

Type: LD50
Species: rat
Route of admin.: i.v.
Value: = 2131 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (138)

Type: LD50
Species: mouse
Route of admin.: i.v.
Value: = 5673 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (103)

Type: LD50
Species: mouse
Route of admin.: i.v.
Value: = 5660 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (165)

Type: LD50
Species: mouse
Route of admin.: i.v.
Value: = 4710 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (138)

Type: LD50
Species: mouse
Route of admin.: i.v.
Value: = 4710 mg/kg bw

Method: other: no details reported
GLP: no data
Test substance: other TS

Test substance: Methanol, no further data.
24-MAR-1997 (166)

Type: LD50
Species: rabbit
Route of admin.: i.v.
Value: = 8907 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (138)

Type: LD0
Species: dog
Route of admin.: i.v.
Value: = 4700 mg/kg bw

Test substance: other TS

Remark: Methanol was applied i. v. to 4 female dogs at dose levels of 2.5 - 4.7 mg/kg bodyweight. No mortality occurred but ataxia and severe inflammation of the eyes with blindness for some days.

Test substance: Methanol, no further data.
04-MAR-1997 (167)

Type: LDLo
Species: cat
Route of admin.: i.v.
Value: = 118 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (103)

Type: LDLo
Species: cat
Route of admin.: i.v.
Value: = 4641 mg/kg bw

Test substance: other TS

Test substance: Methanol, no further data.
04-MAR-1997 (138)

Type: other: lethal dose
Species: rabbit
Route of admin.: i.v.
Value: = 4200 mg/kg bw

Test substance: other TS

Remark: 4200 mg/kg Methanol applied as 50 % solution led to mortality after 25 min..

Test substance: Methanol, no further data.
04-MAR-1997 (135)

5.2 Corrosiveness and Irritation**5.2.1 Skin Irritation**

Species: rabbit
Concentration: 500 mg
Result: moderately irritating

Method: other: no data
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Test substance: Methanol, no further data.
Reliability: (4) not assignable
 Secondary source

06-FEB-2003

(168)

Species: rabbit
Concentration: 100 %
Exposure: Occlusive
Exposure Time: 20 hour(s)
No. of Animals: 5
Result: not irritating
EC classificat.: not irritating

Method: other: BASF-Test
Year: 1975
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The irritation potential of methanol was examined after several exposure periods of 1, 5, and 15 min and 20 h. Effects were assessed 24 h and 8 days after treatment according to Draize scoring.

20-h exposure was conducted on the skin of the back as well as the ear.

Result: After 1, 5, 15 min and 20 h of exposure, no signs of irritation were apparent after 24 h and 8 d.

Test substance: Methanol, not further specified

Reliability: (2) valid with restrictions

Test procedures in accordance with accepted standard methods, sufficiently documented, acceptable for assessment

Flag: Critical study for SIDS endpoint

18-FEB-2003

(133)

5.2.2 Eye Irritation

Species: rabbit
Result: irritating
EC classificat.: irritating

Method: other: Smyth Carpenter
Test substance: other TS

Test substance: Methanol, no further data.
Reliability: (4) not assignable
 Secondary source

06-FEB-2003 (169)

Species: rabbit
Concentration: 100 %
Dose: .05 ml
Comment: not rinsed
No. of Animals: 2
Result: slightly irritating

Method: other: BASF-Test
Year: 1975
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: After 1 h, mild erythema and cornea opacity as well as moderate edema associated with secretion were observed. After 24 h, the effects were assessed as mild. After 8 days the animals were without symptoms.

Test substance: methanol, not further specified
Conclusion: This screening test gives evidence of mild to moderate irritation of the mucous membrane on contact with pure methanol, but which is reversible.

Reliability: (2) valid with restrictions
 Comparable to guideline study and current standards, limited documentation

Flag: Critical study for SIDS endpoint
 18-FEB-2003 (133)

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Comment: not rinsed
No. of Animals: 6
Result: slightly irritating

Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 1990
GLP: no data
Test substance: no data

Result: New Zealand White rabbits treated according to OECD guidelines and assessed based on Draize scoring criteria exhibited the following mean scores for irritation effects:

	Time after application [h]				
	1	4	24	48	72

	Mean scores				
conjunctivitis	0.89	2.00	1.67	2.28	2.22
chemosis	2.00	2.00	0.67	1.00	0.50
iritis	0.33	1.00	1.00	1.00	0.33
corneal opacity	0.00	0.00	0.50	0.50	0.67

Conclusion: Eight- and 14-d findings not shown.
The mean scores of the single symptoms did not exceed the respective limits for classification: Therefore, this result can be interpreted in terms of a mild irritation potential not requiring classification as eye irritating.

Reliability: (2) valid with restrictions
Guideline study, limited documentation, only abstract

Flag: Critical study for SIDS endpoint

06-FEB-2003

(170)

5.3 Sensitization

Type: Guinea pig maximization test
Species: guinea pig
Concentration 1st: Induction 50 other: % active substance with and without Freund's Adjuvans (see Method) intracutaneous
2nd: Induction 50 % active substance open epicutaneous
3rd: Challenge 50 % active substance occlusive epicutaneous
No. of Animals: 34
Vehicle: water
Result: not sensitizing

Method: other: modified Magnusson-Kligman test
Year: 1979
GLP: no

Test substance: as prescribed by 1.1 - 1.4

Method: Two studies were conducted using 10 test animals (1st study) and 24 test animals (2nd study) with each 5 control animals. During challenge in the first study, a formaldehyde solution (25 %) was applied in order to identify possible sensitisation effect that might be caused by metabolism-related transformation of methanol to formaldehyde.

The induction scheme was modified as follows (1 wk between each treatment):

- 1st intradermal induction (including 6 parallel injections with Freund's Adjuvans solely, with methanol solely and both combined)
- 1st epidermal induction
- 2nd intradermal induction (including 4 parallel injections with methanol solely and combined with Freund's Adjuvans)
- 2nd epidermal induction.

Result: In the first study, no contact sensitisation was observed, in the parallel test with formaldehyde, also a negative result was seen.

In the second study using 24 female animals (two tests à 12 animals), 1/12 (test 1) and 2/12 (test 2) exhibited a slight skin response (score 1) after 24 and 48 h which can be interpreted as a weak sensitising potential.

The intracutane induction produced necroses and some open ulcerations.

Conclusion: In summary, the low number of 3/24 animals with score 1 gives no evidence of a notable sensitisation potential of methanol.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

5.4 Repeated Dose Toxicity

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: 90 days
Frequency of treatment: daily
Doses: 0, 100, 500 or 2500 mg/kg bodyweight
Control Group: yes, concurrent vehicle
LOAEL: = 2500 mg/kg bw
NOEL : = 500 mg/kg bw

Method: other: EPA Test protocol
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: 30 animals per sex and group were used. After 6 weeks, 10 rats per sex and dose group were subjected to interim sacrifice.
 The following parameters were examined: bodyweight, food consumption, clinical signs, ophthalmological evaluations, mortality, blood and urine chemistry, gross and microscopic evaluations.

Result: Elevated levels of SGPT, SAP and increased (not statistically significant) liver weights in males and females of the high dose group. Brain weights of both high dose males and females were significantly less than those of controls. There were no histopathological effects. The NOAEL was determined at 500 mg/kg/day, based on increased liver enzyme levels and reduced brain weights.

Test substance: Methanol, no further data.
Conclusion: The subchronic NOAEL was determined at 500 mg/kg/day, based on increased liver enzyme levels and reduced brain weights. Due to the low pathological relevance of effects, this value is considered to be a NOEL.

Reliability: (4) not assignable
 Guideline study, only abstract available
Flag: Critical study for SIDS endpoint

18-FEB-2003

(173) (174)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 1; 2; 4; or 6 Wk
Frequency of treatment: 6 h/d, 5 d/Wk
Post exposure period: 16 h after the last exposure. One group exposed for 6 wks: recovery 2 wks
Doses: 200, 2,000 or 10,000 ppm (0.265; 2.65; 13.3 mg/l)
Control Group: yes, concurrent no treatment
NOAEL: 13.3 mg/l

Year: 1982
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Limited study: Only biochemical/physiological and cytological parameters of the lung were examined (tissue-specific: lung weight, tissue DNA and protein contents, RNase, Protease; in the filtered lavage fluid: surfactant, number of cells, PMN, protein, surface LDH and N-acetyl-beta-glucosaminidase). Histologic analyses of lung tissue were not conducted. Four rats per group were used. Chamber concentration was analysed by GC.

Result: Even the highest concentration caused no signs of lung inflammation or irritation.

Test substance: Methanol, no further data.

Reliability: (2) valid with restrictions
Special study design, meets generally accepted scientific principles, restrictions in assessment

Flag: Critical study for SIDS endpoint

18-FEB-2003

(175)

Type: Sub-acute
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 4 weeks
Frequency of treatment: 6 h/d, 5 d/Wk
Doses: 0, 520, 1980 and 5010 ppm (0.663; 2.65; 6.63 mg/l)
Control Group: yes, concurrent no treatment
NOAEL: 6.63 mg/l

GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: The study used 5 rats/sex/group. Parameters evaluated included, ophthalmoscopic exam, body weight, clinical signs, organ weights, histopathology and survival.

Remark: A good screening study. Daily exposure was 6 hours per day, the normal daily exposure length for inhalation studies evaluating potential workplace exposure.

Result: All animals survived. In rats nasal and eye discharge (mucoid, nasal, red nasal, lacrimation) was noted in the treatment groups. Only mucoid nasal discharge appeared to be dose related. There were no treatment-related effects on body weight. No ocular abnormalities were noted at the terminal ophthalmoscopic exam. No treatment related histopathological effects (35 tissues) were noted, but spleen weights were increased in female rats exposed at 2,000 ppm (not at 5,000 ppm). No other organ weight effects were noted.

Test substance: Methanol, 99.85 % pure from Celanese Corp. NY, no further data.

Conclusion: Rats were exposed to up to 5,000 ppm (6 hr/d, 5d/wk for 4 weeks) showed no treatment related histopathological effects. Inhalation exposure had resulted in some slight treatment-related signs.

Reliability: (2) valid with restrictions
 Test procedures in accordance with accepted standard methods, well documented, acceptable for assessment

Flag: Critical study for SIDS endpoint

18-FEB-2003 (176)

Type: Sub-acute
Species: monkey **Sex:**
Strain: Macaca Fascicularis
Route of administration: inhalation
Exposure period: >= 15 d
Frequency of treatment: 21 h/d
Post exposure period: none
Doses: 10000; 7000; 5000; 3000 ppm (13.27; 9.29; 6.63; 3.98 mg/l)
Control Group: yes, concurrent no treatment

GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive study programme on three species (rat, mouse, monkey) including metabolic, pharmacokinetic and short-, long-term and reproduction studies and carcinogenicity studies: Four animals were used per test and control group. Exposure period was variable, but was expected to be at least 15 days (p. 12 ff).

Result: Blood levels (p. 51):

	3000 ml/m3	5000 ml/m3
	=====	=====
Methanol:	80 mg/l	5250 mg/l
Formate:	30 mg/l	1210 mg/l
	=====	=====

The animals at the top dose showed lethargy, and after 3 treatment fell into coma and died.
 The animals of the 7000-ppm group had to be killed because of suffering.
 The animals at 5000 and 3000 ppm exhibited weakness and uncoordinated movement. Food consumption was reduced at 5000 ppm. The body weight was normal.

Haematological findings displayed no changes, except for a somewhat higher leukocyte count at the two highest exposure levels.
 Blood pH in one animal of the 5000-ppm and at the highest dosed groups were significantly decreased showing acidoses. No changes were found in other clinical-chemical blood and liver values, but in the 7000-ppm group alkaline phosphatase increased.

At 5000 ppm, necrosis of the basal ganglion of the cerebrum is reported, associated with marked cerebral edemas.

These effects were not seen at 3000 ppm.

Effects (mild atrophy) on the visual system (retina, optic nerve, state of myelinated fibres) were hardly noticeable and considered as insignificant at 3000 ppm and below, but

pronounced at 5000 ppm.

Fatty degeneration in the liver occurred at 3000 ppm and more pronounced at the higher exposures, with necrosis after 5000 ppm. Also at this exposure level, kidneys were partly affected, showing vacuolated degeneration.

Test condition: Exposure time 21 hours per day, significantly longer than according to guideline (6 hrs). This does not represent normal industrial and consumer use.

Test substance: Methanol of first grade by Junsei Chemicals, <1 ppm vinyl chloride, <3 ppm formaldehyde

Reliability: (2) valid with restrictions
Test procedures in accordance with accepted standard methods, limited, but sufficiently documented

Flag: Critical study for SIDS endpoint

27-NOV-2003

(161)

Type: Chronic
Species: monkey **Sex:** no data
Strain: Macaca Fascicularis
Route of administration: inhalation
Exposure period: a) 7 months; b) 1 year + 7 months; c) 2 years + 5 months
Frequency of treatment: 21 h/d
Post exposure period: none
Doses: 1000; 100; 10 ppm (1.3; 0.13; 0.013 mg/l)
Control Group: yes, concurrent no treatment
NOAEL: 1000 ppm

Method: other: see Method
Year: 1985
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive study programme on three species (rat, mouse, monkey) including metabolic, pharmacokinetic and short-, long-term studies, reproductive assays and carcinogenicity studies:
Whole-body exposure: 4 monkeys were housed in one inhalation chamber.

8 animals per exposure group were used. Sacrifices were conducted 7 months (2/group), 19 months (3/group), and at 29 months (3/group) (Tab. 3, p. 29). A further group at 1000 ppm was included for recovery (30 d and 6 months) (p. 31 f).

Clinical, clinical-chemical, hematological, ophthalmological, and pathological/histopathological examination were done. Furthermore, endocrine status was tested after 1 year and 7 months as well as after 2 years

and 5 months.

Analytical control of chamber concentration: Analytical values close to nominal ones. (p. 20 ff)

Remark:

The blood levels of methanol and formate were not measured. Note: Exposure per day was 20 h, which implies that prolonged steady-state blood levels were reached which even may have been higher than in studies using the same exposure concentration, but shorter exposure times.

Relevance of observations limited by the low number of animals available at each time interval.

Result:

The animals showed normal body-weight gain, food and water consumption throughout. The monkeys of the 1000-ppm group showed abnormal scratching of the skin and clinical symptoms such as frequent yawning and nasal discharge (see also recovery test, p. 31 f).

In both upper dose groups, the ECG of 1/8 of the 100-ppm exposed monkey and 3/8 of the 1000-ppm exposed monkeys showed abnormalities (negative T- or Q-wave changes and flattening of T-wave) which indicated slight myocardial disorder (p. 21).

Examinations of the eye fundus revealed no changes in any group.

Haematological examinations revealed no deviations from normal in Hb, Ht, red and white blood-cell count, or percentage of white blood cells in any group.

No significant differences in serum values were seen, but one monkey in the 100-ppm group for most liver parameters (total protein, GOT, GPT, total cholesterol, free cholesterol, thymol and glucose). This was not considered treatment-related.

In both upper exposure groups, the number of responsive stellate cells containing increased endoplasmic reticulum (minimal hypertrophy of astroglia) appeared in the basal ganglions after 1 year and 7 months of exposure. In the cerebral white substance, slight hyperplasia of the astroglia was noted in the 100 and 1000-ppm groups, in particular in the 100-ppm group after 7 months (NEDO, 1987, p.53). Yet, this was not characteristic of a degenerative process and was unrelated to dosing, because no such phenomena were noted after 2 years and 5 months.

In the cerebral white substance, diffuse increases of responsive stellate cells centered in the subcortical white substance and the semioval center of the frontal and

parietal lobes was noted at the higher dose groups, but also emerged in 3/8 monkeys of the 10-ppm group after 29 months (p. 23). This was apparently a reversible effect, as shown from monkeys after recovery.

There were a few cases of degeneration of the optic nerve and the corpus geniculatum after 7 months (ophthalmencephalon): In one or two cases in the groups of 8 animals exposed for 2 years and 5 months, including the 10-ppm group, slight degeneration of the optic nerve was suspected, but not considered as significant (p. 23).

There were mild degenerations of the livers and kidneys which showed no clear correlation with exposure levels and exposure time. There was some evidence of an increase in fatty granules in liver parenchyma at 1000 ppm along with signs of fibrosis in the hepatic cord more pronounced than at the lower concentration (NEDO, 1987, p. 26/27).

There was an increase in the Sudan-positive granules in heart tissue at 1000 ppm, but not manifested in any morphological lesion of the heart muscle.

There were no cases of pneumonia in any of the exposed monkeys. In the trachea, atrophy of the epithelium of the mucous membranes and reduction of goblet cells were observed in a total of 4 cases of exposed animals, but not correlated with the amount of methanol inhaled. This was not seen in the controls (p. 28).

Test condition: Exposure time 21 hours per day, significantly longer than according to guideline (6 hrs) (see p. 5). This does not represent normal industrial and consumer use.

Test substance: Methanol, reagent special grade from Junsei Chemicals Co., <1 ppm vinyl chloride, <3 ppm formaldehyde

Conclusion: At 1000 ppm, no necrotic effects occurred in the nervous tissue. Hyperplasia of astroglia, not considered as degenerative, might be a transient methanol-dependent effect which appears to subside upon cessation of long-term methanol exposure (p. 52/53). But there was no clear correlation to the exposure concentration and time. Therefore, the biological relevance is questionable.

There was evidence of an increase in mild fatty changes in the liver and kidney upon exposure to 1000 ppm, which were associated with signs of fibrosis. This effect was still present after recovery (see other entry). Given the small histological effect (p. 27), the pathological relevance appears to be low, but indicates that long-term exposure to 1000 ppm methanol is on the borderline to toxicologically relevant, histological manifestations (authors statement, p.

27).

There were histological signs of diffuse responsiveness of the astroglia in some parts of the cerebral white substance at all exposure levels, at high exposure after shorter time period, after 10 ppm after 29 months in a few animals. The relevance was not clearly commented by the authors. However, under test conditions (continuous exposure), there was no evidence of irreversible effects arising from long-term exposure to up to 1000 ppm methanol.

Therefore, the NOAEL for continuous exposure may be established at 1000 ppm (1300 mg/m³), but the low number of animals and limited description do not allow to draw firm conclusions on the apparent neural effect.

Note: This study served as basis for risk assessment by Vyskocil and Viau (2000): A 1-h reference concentration (RfC) was derived to be at about 100 mg/m³, taking into account sensitive people. A very conservative chronic RfC was obtained and proposed at 0.38 mg/m³, based on the assumption that 10 ppm has to be used as NOAEL and 100 ppm as the LOAEL for "neurotoxic effects". This appears to be not in compliance with the authors' observations and is not suggested in the report.

Reliability:

(2) valid with restrictions

Comparable to guideline study, also largely meeting current standards, however, documentation and number of animals limited.

Flag:

18-FEB-2003

Critical study for SIDS endpoint

(161) (177)

Species:

monkey

Sex: no data**Strain:**

Macaca Fascicularis

Route of administration:

inhalation

Exposure period:1000 ppm: 7 months; 2000 ppm + 3000 ppm 20 d; 5000 ppm
6 d + 4000 ppm 6 d**Frequency of treatment:**

21 h/d

Post exposure period:

30 d; 6 months; 10 or 4 months (see Method)

Doses:1000; 2000; 3000; 5000+4000 ppm (1.3; 2.6; 3.9; 6.5+5.2
mg/l)**Control Group:**

yes, concurrent no treatment

Method:

other: Special acute to chronic inhalation test programme including recovery phases

Year:

1985

GLP:

no data

Test substance:

as prescribed by 1.1 - 1.4

Method:

Comprehensive study programme on three species (rat, mouse, monkey) including metabolic, pharmacokinetic and short-, long-term studies, reproductive assays and carcinogenicity

studies:
Whole-body exposure: 4 monkeys were housed in one inhalation chamber.

Here recovery study with specific focus on neurological effects apart from organ toxicology:

1000 ppm: 7 months exposure + 30 d or 6 months recovery
2000 and 3000 ppm: 20 d exposure + 30 d, 6 or 10 months recovery
5000 ppm: 6 d exposure + 6 d 4000 ppm exposure with 30 d or 4 months recovery.

Clinical, clinical-chemical, hematological, ophthalmological, and pathological/histopathological examination were done.

Analytical control of chamber concentration: Analytical values close to nominal ones.

Result:

At the concentration of 4000 and 5000 ppm, irreversible lesions/degenerations appeared to be likely in the basal ganglions of the cerebrum, because they did not subside within the recovery period, while after 3000 ppm (20 d exposure) the slight necrotic degenerations were not progressive as evidenced after recovery.

The increase of responsive astroglia seen in the cerebral white substance was still present after recovery from 3000-ppm exposure.

Degenerative changes in the visual system were evident after exposure to 3000 ppm and higher: atrophy of the optic nerve and reduction in myelinated fibers. Recovery was unequivocal at exposures below at 1000 and 2000 ppm, but at 3000 ppm, there appeared to be a trend of these lesions to progress, although -due to the low number of animals- this could also reflect individual variability acc. to authors.

Lesions of the liver occurred in all dose groups with round-cell infiltration and fibrosis noted in dose-related manner. They were still present histologically after the recovery phase.

Changes of the kidneys were observed in all groups: hyalinisation of glomeruli, cell infiltrations into the renal tube stroma. These effects were no longer noted after recovery.

Test substance:

Methanol, reagent special grade from Junsei Chemicals Co., <1 ppm vinyl chloride; <3 ppm formaldehyde

Conclusion:

At 1000 ppm (7 months exposure and higher; see chronic exposure other entry), some irreversibility of degenerative

changes (mild fibrosis presumably following fatty degeneration) unrelated to the recovery period was noted in the liver (p.54)[see also p. 27: chronic study]. These effects were histologically small and, therefore, of little pathological relevance.

All other effects -if there were any at all- were not persistent and not considered pathologically relevant at that level, but were significantly more pronounced at 3000 ppm and higher after shorter exposure (20 d), associated with eventual lack of recovery. Between 2000 and 3000 ppm appears to be a threshold concentration where methanol-induced neurotoxic effects may become biological relevant (p. 35).

Reliability: (2) valid with restrictions
Comparable to guideline study, also largely meeting current standards, however, documentation and number of animals limited.

Flag: Critical study for SIDS endpoint
18-FEB-2003 (161)

Type: Chronic
Species: rat **Sex:** male/female
Strain: Fischer 344/DuCrj
Route of administration: inhalation
Exposure period: 12 months
Frequency of treatment: continuous, average about 20 h/d, total exposure time 7318 - 7341 h (males); 7474 - 7496 h (females)
Post exposure period: none
Doses: 10; 100; 1000 ppm (0.013; 0.13; 1.3 mg/l)
Control Group: yes, concurrent vehicle
NOAEL: 1000 ppm
NOEL : 100 ppm

Method: other: in accordance with OECD Guideline Oct. 1980
Year: 1983
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive study programme on three species (rat, mouse, monkey) including metabolic, pharmacokinetic and short-, long-term and reproduction studies and carcinogenicity studies (NEDO, 1987):
Whole-body exposure: The animals were housed individually in wire-mash cages attached to the inhalation chamber.

20 animals per sex and dose group were tested. Clinical, ophthalmological, clinical-chemical, hematological and pathological/histopathological examination were done. The latter included among others the optic nerve, the eye, and reproductive organs.

Analytical control of chamber concentration: Analytical values close to nominal ones.

Remark: The blood levels of methanol and formate were not measured.
Result: One female rat of the 1000 ppm dose group and one male rat of the 10 ppm dose group died or were sacrificed in extremis.
This was considered spontaneous on the basis of the absence of a consistent dose relationship.

A very slight transient suppression of body-weight gain was observed for males and females of the 1000 ppm dose group from week 27 through 44 (less than 5 %). This has to be attributed to temporary occurrence of slight diarrhea in these groups during this period. The otherwise addressed significant decreases in body weight at the end of the study are not noticeable in Fig. 3 representing the time-course of body weight development. The high-dose males showed a minimal, but significant decrease in food consumption from week 30 to the end. However, this was not more than about -5 % from the control.

Data of the organ/body weight ratio revealed a dose-related upward tendency in the liver and spleen for females which remained within a 5-% range.

Urinalysis showed no changes suggesting effects from exposure to methanol.
Hematologic examinations revealed no clear changes which could be attributed to exposure to methanol.
Serum biochemical examination showed a small tendency to decrease for alkaline phosphatase, the enzymatic activities of GOT, GPT, LDH and gamma-GTP did not show any differences. Free fatty acid showed lower values in all exposure groups without dose-response relationship. Cholesterol and triglyceride remained unchanged.
Changes of other clinical-chemical parameters showed no correlation with the methanol exposure.

Histopathological examinations showed a variety of non-tumoral changes in various organs, many of them were infrequent findings which were possibly accidental. For tumoral changes, similarly, almost all the findings observed were considered spontaneous ones due to aging.

Test condition: Exposure time 20 hours per day, significantly longer than according to guideline (6 hrs). This does not represent normal industrial and consumer use.

Test substance: Methanol of first grade by Junsei Chemicals, <1 ppm vinyl chloride, <3 ppm formaldehyde

Conclusion: According to the authors, only in the 1000 ppm dose-group

changes could be considered treatment-related. But based on the minor degree and severity, these changes have no pathological meaning and are considered as toxicologically irrelevant.

Levels of 100 ppm or less (at 20 h/d) did not produce any effect (= NOEL). 1000 ppm can be established as NOAEL, while it is the LOEL for non-pathological, apparently treatment-related effects.

(see also IPCS/WHO, 1997)

Reliability:

(2) valid with restrictions

Comparable to guideline study, also largely meeting current standards, documentation limited.

Flag:

18-FEB-2003

Critical study for SIDS endpoint

(84) (161)

Type: Chronic
Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of administration: inhalation
Exposure period: 12 months
Frequency of treatment: continuous, average ca. 20 h, total 7202 - 7225 h (males); 7352 - 7373 h (females)
Post exposure period: none
Doses: 10; 100; 1000 ppm (0.013; 0.13; 1.3 mg/l)
Control Group: yes, concurrent no treatment
NOAEL: 1000 ppm
NOEL : 100 ppm

Method: other: in accordance with OECD Guideline Oct. 1980

Year: 1983

GLP: no data

Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive study programme on three species (rat, mouse, monkey) including metabolic, pharmacokinetic and short-, long-term and reproduction studies and carcinogenicity studies (NEDO, 1987):
 Whole-body exposure: The animals were housed individually in wire-mash cages attached to the inhalation chamber.

30 animals per sex and dose group were tested. Clinical, ophthalmological, clinical-chemical, hematological and pathological/histopathological examination were done. The latter included among others the optic nerve, the eye, and reproductive organs.

For interim examination, 10 animals each were sacrificed after 6 months.

Analytical control of chamber concentration: Analytical

values close to nominal ones.

Remark: The blood levels of methanol and formate were not measured. Note: Exposure per day was 20 h, which implies that prolonged steady-state blood levels were reached which even may have been higher than in studies using the same exposure concentration, but shorter exposure times.

Result: One female animal of the 100-ppm group died on day 174, another one had to be killed in moribund state on day 178.

During the whole study, there were no changes in the general appearance that could suggest any relation to treatment.

The body-weights of both sexes of the treated groups tended to be higher than the controls in the second half of the study (NEDO, Fig. 3, p. 168).

There was a decreased food consumption in high-dose females during the first 2 months and after 7 months without effect on body-weight gain.

All clinical and blood test failed to reveal any methanol-related alteration in parameters from normal.

In the group of high-dose females, an increase in organ weight with significant differences was noted for the liver after 6 months and for the kidney and spleen after 12 months, however, the latter without statistical significance and a clear dose-response.

After 12 months, the incidence of more severe fatty degeneration of hepatocytes was pronounced in high-dose males: This was graded as moderate in 8/20, mild in 8/20 and negative in 4/20 surviving males vs. 1/20 (moderate), 9/20 (mild) and 10 negative in the male control group. On the other hand, this effect was common and found to similar extent in untreated/external male mice. The female mice showed now difference among the groups.

Test condition: Exposure time 20 hours per day, significantly longer than according to guideline (6 hrs). This does not represent normal industrial and consumer use.

Test substance: Methanol, reagent special grade from Junsei Chemicals Co., <1 ppm vinyl chloride, <3 ppm formaldehyde

Conclusion: According to the authors, only in the 1000 ppm dose-group changes could be considered treatment-related. But based on the minor degree and severity, these changes have no pathological meaning and are considered as toxicologically irrelevant.

Levels of 100 ppm or less did not produce any effect (= NOEL).
1000 ppm can be established as NOAEL, while it is the LOEL for non-pathological, apparently treatment-related effects.

(see also IPCS/WHO, 1997)

Reliability: (2) valid with restrictions
Comparable to guideline study, also largely meeting current standards, however, documentation limited.

Flag: Critical study for SIDS endpoint
18-FEB-2003 (84) (161) (178)

Type: Sub-acute
Species: monkey **Sex:** male
Strain: other: rhesus *Macaca mulatta*
Route of administration: gavage
Exposure period: 1.5 to 6 d
Frequency of treatment: variable
Doses: initial 2000 mg/kg, thereafter 500 or 1000 and 2000 mg/kg (variable frequency)
Control Group: other: internal / same animal prior to treatment

Method: other: (see Method)
Year: 1978
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Test model in monkeys for methanol-induced ocular toxicity to characterise the toxicity syndrome and histological manifestations. Methanol was applied as 20% solution by nasogastric tube (see: McMartin et al., 1975).

Ophthalmoscopic examinations and ultrastructural histopathology (electronmicroscopy) were performed.

Result:

- Under methanol treatment acc. to this test design, formate levels were between min. 7.2 and max. 14.4 mEq/L in blood and cerebrospinal fluid, blood bicarbonate min. 4.0 and max. 10.2, and blood pH min. 7.13 and max. 7.28. Methanol levels ranged from 1540 to 2840 mg/L (Martin-Amat et al., 1977).
- All six animals developed fundus changes at the head of the optic nerve (optic disc) within 43 to 171 h after methanol ingestion, expressed as intraaxonal swellings (Hayreh et al, 1977). Electronmicroscopic studies revealed swelling of the nerve fibers with an accumulation/clustering of swollen mitochondria in the optic nerve head being maximally in the lamina cribrosa region. Furthermore, in the retrolaminar and intraorbital optic nerve, swelling of astrocytes was prominent as well as swelling of the cytoplasm of the oligodendroglial cytoplasm in contact with

the axons (Baumbach et al., 1977).
Alterations were not observed in the retina itself: The ganglion cells of the retina were intact with only minimal swellings of the mitochondria and loss of cristae. But these findings were also present in the control tissue (Baumbach et al., 1977).

Test condition: A total of six monkeys was treated such as to maintain a state of metabolic acidosis at attenuated toxicity (arterial blood pH 7.1 to 7.3 and bicarbonate at 10 mEq/L or above) for a prolonged time: A high initial dose was followed by lower doses depending on the animal's acidotic response in blood.
[note: Experience had told (McMartin et al., 1975) that after a single dose of 3000 mg/kg bw., in general, the animals died within 20 to 30 h without demonstrating ocular abnormalities.] (Martin-Amat et al., 1977).

Test substance: Methanol, highest purity available, no further data (see: McMartin et al., 1975)

Conclusion: 1. While acute methanol toxicity in monkeys (after a single dose) does not yield ocular signs, repeated dosing succeeded in producing ocular lesions (Martin-Amat et al, 1977).

The only detectable ocular change was optic disc edema (of the optic papilla) which was similar to that seen in raised intracranial pressure in humans, but without this pressure after methanol (Hayreh et al, 1977).
The primary sites of ocular injury were the optic nerve heads and the anterior segment of the optic nerve rather than the retinal ganglion cells themselves. It appears that interference with oxidative phosphorylation causes mitochondrial damage, thus disruption of active axoplasmic flow in the retrolaminar optic nerve (Baumbach et al., 1977; Hayreh et al., 1977).

[note: In humans it has been hypothesised that optic atrophy, which often follows acute methanol intoxication, is secondary to injury of the retinal ganglion cells.]

2. Mechanistically, there is a close causal relationship between the prolonged increase in formic acid from methanol and the development of optic edema. Similar effects can be produced by i.p. administration of formate without acidosis (see other entry 5.11: Martin-Amat et al., 1978).

Reliability: (2) valid with restrictions
Test procedures in accordance with accepted standard methods, sufficiently documented

Flag: Critical study for SIDS endpoint
28-APR-2004 (179) (180) (181) (182) (183) (184)

Type: Sub-chronic
Species: rat **Sex:**
Strain: no data
Route of administration: inhalation
Exposure period: 3 months
Frequency of treatment: 12 h/d, 5 d/wk
Doses: a) 1.77; 49 mg/m3; b) 0.57; 5.3 mg/m3
Control Group: yes, concurrent no treatment

GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Special neurophysiological study design: chronaximetry = ratio of the minimum time necessary for a stimulus of twice the absolute threshold intensity to evoke a response measured as muscle contractions in response to an electric current applied to an animal's hind leg. Normally, the flexor chronaxia is shorter than the extensor chronaxia, and their ratio is stated to be relatively stable (NTP-CERHR, 2001, p. 80).

Result: -----
 Low concentrations of methanol inhaled for 3 months by rats are reported to exhibit changes in the average motor chronaxia ratio between antagonistic muscles. No significant effects were noted at 1.77 mg/m3 (Chao, 1959) and 0.57 mg/m3 (Ubaydullayev, 1966). At the other doses the chronaxia returned to normal during the recovery period.

Unspecified histopathological changes in relation to the mucous membranes of the respiratory tract were seen at 450 mg/m3, but not at the lowest doses. Some shifts in liver parameters in blood were also mentioned.

Test substance: Methanol, no further data.
Conclusion: The analysis by Kavet and Naus (1990) indicated that these studies do not provide adequate evidence of an association between neurobehavioral effects and low-level exposure to methanol: limited animal number, insufficient reporting of methods, and unclear statistical analysis. The biological significance of changes in the chronaxia ratio is considered as uncertain. (see also NTP-CERHR, 2001, p. 80/81)

Reliability: -----
 (4) not assignable
 Test methods and performance uncertain, limited documentation available

Type: Sub-acute
Species: rat **Sex:** male
Route of administration: inhalation
Exposure period: 1; 2; 4 or 6 weeks
Frequency of treatment: 8 h/d 5 d/Wk
Doses: 200, 2000, and 10000 ppm (0.265; 2.65; 13.3 mg/l)
Control Group: yes, concurrent no treatment

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Study design was to elucidate sexual hormone status in mature male rats (Sprague-Dawley): determination in blood after exposure of circulating free testosterone, luteinizing hormone and follicle stimulating hormone.

Remark: Conflicting results as compared to findings of others (see also other entry 5.1.2: Cooper et al., 1992; and 5.4: Lee et al., 1991).

Result: Significantly decreased levels of circulating free testosterone were observed among rats exposed at 200 ppm for 2 and 6 weeks (32 % of control) and 6 weeks at 2,000 ppm. The high dose group (10,000-ppm) showed no change. The authors interpreted this as evidence that methanol exposure had lowered testicular production of testosterone. In addition, significant increases in circulating LH were observed after six weeks of exposure to 10,000 ppm. No changes in follicle stimulating hormone levels were observed.

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
Special study design, sufficiently documented, meets generally accepted scientific principles, restrictions in assessment (see Remark)

Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 1 d or 1 wk
Frequency of treatment: 6 h/d
Post exposure period: none or 18 h
Doses: 200 ppm (0.265 mg/l)
Control Group: yes, concurrent no treatment

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Study design was to elucidate sexual hormone status in mature male rats (Sprague-Dawley): determination in blood after exposure of circulating free testosterone, luteinizing hormone and follicle stimulating hormone.

Remark: Conflicting results as compared to findings of others (see also other entry 5.1.2: Cooper et al., 1992; and 5.4: Lee et al., 1991).

Result: SD rats exposed to 200 ppm methanol for 6 h for 1 day or 1x/d for one week showed a significant depression of serum testosterone (59 %) immediately after the exposure, but not after one-week daily 6-h exposure or after 18-h recovery. There was no evidence of an effect on LH.

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
Special study design, sufficiently documented, meets generally accepted scientific principles, restrictions in assessment (see Remark)

15-DEC-2003

(190)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: a) 1, 2, 4, and 6 wk; b) 13 wk
Frequency of treatment: a) 8 hours/day, 5 days/week; b) 20 hours/day, 7 days/week
Doses: a) 200 ppm (260 mg/m³); b) 50, 200, 800 ppm (1040 mg/m³)
NOAEL: = 800 ppm

GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Special study design: The potential toxic effects of methanol vapors on testicular production of testosterone (a) and the morphology of testes (b) were investigated using normal or methanol-sensitive folate-reduced rats.

(a) Nine rats per group were used.

(b) 11 to 12 animals (10 months old) for three exposure levels, and 8 animals (18 months old) for a 800-ppm group were used.

Result: Methanol inhalation did not reduce serum testosterone levels in normal rats. Testes isolated from methanol-exposed (200 ppm) rats had the same capability as those from air-exposed rats in synthesizing testosterone, whether testes were incubated in the absence or presence of hCG.

The testes-to-body weight ratio and testicular morphology of rats exposed up to 800 ppm methanol for up to 13 weeks (20 hours/day, 7 days/week) were not different from control animals, irrespective of the nutritional state, normally fed or folate deficient.

A greater incidence of testicular degeneration was seen only in 18 month old, folate-deficient rats exposed to 800 ppm methanol for 13 weeks (20 hours/day, 7 days/week). The results are equivocal.

800 ppm, the top level, can be established as subchronic NOAEL based on testicular histopathology.

Test substance: Methanol, no further data
Reliability: (2) valid with restrictions
Test procedures in accordance with accepted standard methods, sufficiently documented, acceptable for assessment

15-AUG-2002

(191)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Long-Evans
Route of administration: gavage
Exposure period: 21 d
Frequency of treatment: 2 x/d
Post exposure period: none
Doses: 800; 1600 mg/kg/d
Control Group: yes, concurrent no treatment

Method: other: special test on endocrin function and male reproduction parameters

GLP: no data

Test substance: as prescribed by 1.1 - 1.4

Result: Treatment with the highest dose produced a transient, but significant decrease in serum testosterone. On day 21 of treatment, the testosterone level was normal again, but a significant increase in the luteinising hormone was analysed. The testis weights were reduced as well as the number of morphologically normal sperms in the epididymis.

The acute treatment with high doses of 800, 1600, and 3200

mg/kg (2x 12 h apart) had no effect on testis weight, and on sperm size. At day 1 after treatment, a reduced testosterone value was found in serum and in the interstitial fluid. After 7, 14, and 21 d post-application, testosterone in both compartments were normal without significant difference from the control. LH was distinctly higher on day 21.

(see also other entry 5.1.2: Cooper et al., 1992)

Test substance: Methanol, no further data.
Reliability: (4) not assignable
Only abstract
15-AUG-2002 (192)

Type: Sub-acute
Species: rabbit **Sex:** male
Route of administration: gavage
Exposure period: 5-7 d
Frequency of treatment: daily
Doses: 4.68 g/kg as 30-% solution

Result: Lethal dose for all 14 rabbits within the following 5 to 7 days.

Test substance: Methanol, no further data.
29-JAN-2003 (193)

Type: Chronic
Species: dog **Sex:**
Route of administration: inhalation
Exposure period: 379 d
Frequency of treatment: 8 h/d, 7 d/wk
Doses: 0.585 - 0.65 mg/l

GLP: no

Result: Four dogs were tested for a broad spectrum of haematological parameters and ophthalmological alterations. There was no evidence of a treatment-related effect.

Test substance: Methanol, no further data.
Reliability: (4) not assignable
Secondary source
29-JAN-2003 (194)

Type: Sub-acute
Species: monkey **Sex:** male/female
Strain: Macaca Fascicularis
Route of administration: inhalation
Exposure period: 4 wk
Frequency of treatment: 6 h/d, 5 d/wk
Post exposure period: none
Doses: 500, 2000, and 5000 ppm (0.663; 2.65; 6.63 mg/l)
Control Group: yes, concurrent no treatment
NOAEL: 6.63 mg/l

Year: 1987
Test substance: as prescribed by 1.1 - 1.4

Method: Three monkeys per sex and dose were used. Chamber methanol concentration was measured regularly by IR. Histopathological examinations included ophthalmoscopy at the end of exposure, but apparently no microscopical inspection of brain and neural tissue.

Result: There were no clinical signs of intoxication. There were no methanol-related abnormalities.

Test substance: Methanol, purity 99,85 % (from Celanese Corp., N.Y.)

Reliability: (4) not assignable
 Test procedures in accordance with accepted standard methods, insufficiently documented, limitations in tissue examination (see Method).

02-FEB-2003

(195)

Species: monkey **Sex:** male
Route of administration: gavage
Exposure period: 3 d
Frequency of treatment: daily
Doses: 2340 mg/kg as 30-% solution

Test substance: as prescribed by 1.1 - 1.4

Result: Lethal dose for all 7 animals under test after 3 days.

Test substance: Methanol, no further data.

Reliability: (2) valid with restrictions

14-AUG-2002

(193)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100 (plate incorporation standard test)
Concentration: <= 2.5 mg/plate
Cytotoxic Concentration: non-toxic within the range of testing
Metabolic activation: with and without
Result: negative

Method: other: acc. to Ames et al., 1975
Year: 1981
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Test condition: S9 mix was prepared from Arochlor-induced SD rat livers.
Test substance: Methanol, from Carlo Erba, no further data.
Reliability: (2) valid with restrictions
 Comprehensive test programme using test procedures in accordance with accepted standard methods, but limited documentation

Flag: Critical study for SIDS endpoint
 18-FEB-2003 (196)

Type: Ames test
System of testing: Salmonella typhimurium TA97, TA102 (plate incorporation standard system)
Concentration: <= 7.5 mg/plate
Metabolic activation: with
Result: negative

Method: other: acc. to Maron and Ames, 1983
Year: 1984
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: In TA102, a slightly positive trend was indicated [(+-) ambiguous].
Test condition: S9 mix was prepared from Arochlor-induced SD rat livers.
Test substance: Methanol, from Carlo Erba, no further data.
Reliability: (2) valid with restrictions
 Comprehensive test programme using test procedures in accordance with accepted standard methods, but limited documentation

Flag: Critical study for SIDS endpoint
 18-FEB-2003 (197) (198)

Type: Ames test
System of testing: Salmonella typhimurium TA1538, TA98, TA1537, TA1535
Concentration: <= 3.6 mg/plate
Metabolic activation: with and without
Result: negative

Method: other: acc. to Ames
Year: 1981
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Metabolic activation system was derived from liver S-9 fraction of Aroclor-1254 pretreated rats.

Test substance: Methanol, from Merck, Germany, no further data.

Reliability: (2) valid with restrictions
Comprehensive test programme using test procedures in accordance with accepted standard methods, limited documented

Flag: Critical study for SIDS endpoint

18-FEB-2003

(199)

Type: Ames test
System of testing: Salmonella typhimurium TA100, TA98, TA1537, TA1538, TA1535 Escherichiacoli WP2uvrA
Concentration: 5, 10, 50, 100, 500, 1000, and 5000 ug/plate
Metabolic activation: with and without
Result: negative

Year: 1985
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: Metabolic activation system was derived from liver S-9 fraction of KC500(polychlorinated biphenyl)-pretreated rats.

Test substance: Methanol, 99.6 % pure, no further data.

Reliability: (2) valid with restrictions
Comprehensive test programme using test procedures in accordance with accepted standard methods, sufficiently documented

Flag: Critical study for SIDS endpoint

18-FEB-2003

(200)

Type: other: Micronucleus Test
System of testing: V79 Chinese hamster cells
Concentration: 50 ul/ml (approx. 40 mg/ml)
Metabolic activation: without
Result: negative

Year: 1984
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: Comparative study including alcohols, acetone, and various alkylating agents. Exposure was 48 h for alcohols. 4000 - 7000 interphase cells were scored per treatment.

Result: No MN increases induced by any alcohol or acetone, whereas the alkylating agents produced significant MN frequencies above medium controls.

Test substance: Methanol, 95 % pure from Prolabo, no further data.

Reliability: (2) valid with restrictions
 Test procedures in accordance with accepted standard methods, sufficiently documented, acceptable for assessment

Flag: Critical study for SIDS endpoint

18-FEB-2003

(201)

Type: Sister chromatid exchange assay
System of testing: CHO-Zellen
Concentration: up to 0.1%, 1x/d for 8 days
Metabolic activation: without
Result: negative

Method: other
Year: 1977
GLP: no
Test substance: no data

Method: Repeated exposure design: ethanol, propanol, butanol, and acetaldehyde were also tested. No standard positive control substance was included. Test medium was change every second day, but test substances appeared to be added every day "up to a final concentration of 1 %".

Result: The alcohols produced no increases in the SCE rate compared to the control, but acetaldehyd induced a dose-related, significant increase.

Test substance: Methanol, no further data.

Reliability: (2) valid with restrictions
 Only short communication. Test procedure largely accordance with current standards and based on scientific principles. Positive result of acetaldehyde raises the level of reliability for the proper functionality of the test system.

Flag: Critical study for SIDS endpoint

18-FEB-2003

(202)

Type: other: Cell transformation assay
System of testing: C3H/10T1/2 Cl 8 cells (mouse embryo fibroblasts cells)
Concentration: 5, 10, 20, 50, 100 mg/ml
Cytotoxic Concentration: 50 mg/ml (> 30 % inhibition of growth)
Metabolic activation: with and without
Result: negative

Year: 1981
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark:

Result: 12-O-Tetradecanoyl-phorbol-13-acetat T
Methanol and formic acid induced no foci type I or III
either in the presence or absence of TPA
(12-O-Tetradecanoyl-phorbol-13-acetate) as promoting agent.

[note: Formaldehyde, on the other hand, caused a high number
of transformed cells in the presence of TPA, but failed to
in its absence.]

Test substance: Methanol, HPLC grade from Fisher, no further data.

Reliability: (2) valid with restrictions
Test procedures in accordance with accepted standard
methods, sufficiently documented

Flag: Critical study for SIDS endpoint

18-FEB-2003

(203)

Type: other: Cell transformation assay
System of testing: BHK 21 C 13 (Baby Syrian Hamster Kidney Cells)
Concentration: 10, 50, and 100 ul/ml (approx. 8, 40, and 80 mg/ml)
Cytotoxic Concentration: LC50 approx. 60 mg/ml
Metabolic activation: with and without
Result: negative

Year: 1981
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark:

Metabolic activation system was derived from liver S-9
fraction of Aroclor-1254 pretreated male NMRI mice (500
mg/kg, i.p.).

Result: No increase in transformants, while mitomycin, MMN,
cyclophosphamide and dichromate were highly active.

Test substance: Methanol from Merck, Germany, p.a., no further data.

Reliability: (2) valid with restrictions
Test procedures in accordance with accepted standard
methods, sufficiently documented

Flag: Critical study for SIDS endpoint

18-FEB-2003

(204)

Type: Mammalian cell gene mutation assay
System of testing: Chinese Hamster V79 Zellen 8-Azaguanin-, 6-Thioguanin-,
 Ouabain-Resistenz
Concentration: 15.8, 31.7, 47.4, and 63.3 mg/ml
Cytotoxic Concentration: 63.3 mg/ml (approx. 70-% inhibition of colony
 formation)
Metabolic activation: with and without
Result: negative

Year: 1987
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: No increases in mutant frequency in gene mutation to drug
 resistance vs. neg. control, whereas the pos. control DMN
 produced increases in dose-related manner.

Test substance: Methanol, reagent special grade from Junsei Chemicals Co.,
 <1 ppm vinyl chloride, <3 ppm formaldehyde

Reliability: (2) valid with restrictions
 Comparable to guideline study, also largely meeting current
 standards, sufficient documentation, acceptable for
 assessment

Flag: Critical study for SIDS endpoint

18-FEB-2003

(161)

Type: Chromosomal aberration test
System of testing: Chinese hamster lung cell
Concentration: 7.1, 14.3, and 28.5 mg/ml
Cytotoxic Concentration: 28.5 mg/ml (approx. 50-% inhibition)
Metabolic activation: with and without
Result: negative

Year: 1987
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: MNNG was used as pos. control (0.5 and 1.0 ug/ml) in the
 absence of S9 and 2-aminoanthracene (2 and 4 ug/ml) in the
 presence of S), along with two negative control (untreated
 and solvent = medium). 200 metaphases were scored after 6,
 24, and 48-h exposure (p. 242).

Result: No (dose-dependent) increases in any type of chromosome and
 chromatid breaks and gaps were noted at any time interval,
 while MNNG and 2-AA produced clear dose-related increases in
 breaks and gaps (Tables, p. 250 - 255).

Test substance: Methanol, reagent special grade from Junsei Chemicals Co.,
 <1 ppm vinyl chloride, <3 ppm formaldehyde

Reliability: (2) valid with restrictions
 Comparable to guideline study, also largely meeting current

standards, sufficient documentation, acceptable for assessment

Flag: Critical study for SIDS endpoint
18-FEB-2003 (161)

Type: Mouse lymphoma assay
System of testing: mouse lymphoma cells L5178Y
Concentration: 7.9 mg/ml
Metabolic activation: with
Result: positive

Method: other: acc. to Clive and Spector. Mut. Res., 31, 17-29, 1975
Year: 1985
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Objective: Optimisation of the amount of the S9-mix to be used to obtain maximum yield of mutations. Comparative study including typical mutagens.
Exposure time 4 h.

Result: In the presence of 10 - 15 ul/ml S9 and methanol (7.9 mg/ml), there was a significant increase in mutation frequency. No detailed results presented.

Test substance: Methanol, no further data.
Reliability: (4) not assignable
Only abstract, no detailed data.

Flag: Critical study for SIDS endpoint
18-FEB-2003 (205)

Type: Mitotic recombination in *Saccharomyces cerevisiae*
System of testing: *Aspergillus nidulans* diploid strain P1
Concentration: 6 % (v/v)
Cytotoxic Concentration: no data
Metabolic activation: without
Result: positive

Method: other: no data
Year: 1989
GLP: no data
Test substance: no data

Remark: Approx. 3 % chromosomal malsegregation was induced.

The result was statistically significant at two concentrations and a dose-response relationship was evident (IPSC/WHO 1997).

Test substance: Methanol, not further specified
Flag: Critical study for SIDS endpoint
29-APR-2004 (206) (84)

Type: Yeast gene mutation assay
System of testing: Schizosaccharomyces pombe (ade 6 locus)
Concentration: <= 5 %
Cytotoxic Concentration: 10 %
Metabolic activation: with and without
Result: negative

Year: 1980
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: There was no increase in mutant frequency as compared to the negative controls (buffer and DMSO) at a the maximum conc. of 5 % with or without post-mitochondrial fraction from phenobarbital-induced mouse liver.

Test substance: Methanol, from Carlo Erba, Milano, no further data.
10-AUG-2002 (207)

Type: Ames test
System of testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537 (spot test)
Concentration: 3 umol/plate
Metabolic activation: with and without
Result: negative

Year: 1979
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Metabolic activation system was derived from liver S-9 fraction of Aroclor-1254 and 3-methylcholanthrene-pretreated rats.

Test substance: Methanol, no further data.
Reliability: (3) invalid
Significant methodological deficiency: Test concentration may have been too low, no dose-response relationship given
10-AUG-2002 (208)

Type: Ames test
System of testing: Salmonella typimurium TA1535, TA1537, TA1538, TA98, TA100
Concentration: <= 5 mg/plate
Metabolic activation: with and without
Result: negative

Method: other: acc. to Ames
Year: 1977
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Metabolic activation system S9 mix was prepared and applied according to Ames

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
 Comprehensive test programme using test procedures in accordance with accepted standard methods, limited documented

10-AUG-2002 (209)

Type: other: Cell transformation assay
System of testing: Syrian Hamster Embryo Cells
Result: negative

Year: 1983
GLP: no data
Test substance: no data

Test substance: Methanol, no further data.
Reliability: (4) not assignable
 Secondary source

17-SEP-2002 (210) (211)

Type: Bacterial reverse mutation assay
System of testing: Salmonella typhimurium TA1535, TA 1537, TA1538, TA100, TA98 + E. coli WP2 uvvA (trp dependent)
Concentration: 10, 50, 100, 500, 1000, and 5000 ug/plate
Metabolic activation: with and without
Result: negative

Method: other: largely in compliance with OECD Guide-lines
Year: 1987
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: Metabolic activation system was derived from liver S-9 fraction of PCB-pretreated rats (500 mg/kg, single i.p.) (p. 241).

Test substance: Methanol, reagent special grade from Junsei Chemicals Co., <1 ppm vinyl chloride, <3 ppm formaldehyde

Reliability: (4) not assignable
 Comprehensive test programme using test procedures in accordance with accepted standard methods, only short communication.

10-AUG-2002 (161)

Type: other: Micronucleustest in vitro
System of testing: Syrian hamster embryo cells
Concentration: not indicated
Metabolic activation: without
Result: negative

Method: other: according to Schmuck et al. 1988
Year: 1988
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comparative study including a broad spectrum of chemicals. Exposure time was 18 hours, no further details of method are reported.

Test substance: Methanol, analytical grade

Reliability: (4) not assignable

Test procedures apparently in accordance with accepted standard methods, limited documented

17-SEP-2002

(212)

Type: other: mutagenic inactivation of a bacteriophage repressor
System of testing: E. coli RK+ (lysogenic)
Concentration: up to 23 % (0.23 ml/ml = 0.18 g/ml)
Metabolic activation: without
Result: positive

Method: other
GLP: no data
Test substance: no data

Method: The mutagenic inactivation of a bacteriophage repressor gene leading to subsequent replicative killing of the bacteria was used as mutagenicity screening model. Bacteria were exposed for 10 min. at 30 °C.

Result: Methanol was toxic and mutagenic at concentrations of 23 % (v/v) and more (extreme high concentrations necessary to produce the "mutagenic" effect).

Test substance: Methanol, no further data.

Reliability: (3) invalid

Questionable, not validated test system, extreme high concentrations necessary to produce the "mutagenic" effect.

17-SEP-2002

(213)

Type: DNA damage and repair assay
System of testing: Escherichia coli WP67, WP2, CM871 (trp-dependent, repair deficient)
Concentration: not indicated
Metabolic activation: with and without
Result: ambiguous

Method: other: no details indicated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: No further details are reported.
Result: Reported to be negative in the presence of S9, but ambiguous (Flora et al., 1984) or positive in the absence of S9 (Flora et al., 1990).

Test substance: Methanol, no further data.
Reliability: (4) not assignable
 Test method without clear evaluation criteria, limited documentation

17-SEP-2002 (214) (198)

5.6 Genetic Toxicity 'in Vivo'

Type: other: Cytogenetic assays including chromosomal aberration, SCE, and micronuclei
Species: mouse **Sex:** no data
Strain: no data
Route of admin.: oral unspecified
Exposure period: no data
Doses: 1000 mg/kg
Result: positive

Method: other: no data
Year: 1982
GLP: no data
Test substance: no data

Result: The incidences of chromosomal aberrations, particularly aneuploidy and SCE in bone marrow cells as well as micronuclei in polychromatic erythrocytes are reported to be increased. No detailed results presented.

Reliability: (4) not assignable
 Only abstract, no detailed data.
Flag: Critical study for SIDS endpoint

18-FEB-2003 (215)

Type: Sister chromatid exchange assay
Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: 6 h/day, 5 days
Doses: 800 or 4000 ppm (1.04 or 5.2 mg/l)
Result: negative

Method: other: see Method
Year: 1991
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive test programme (see also other entries this chapter): Primary cultures of lung cells of treated mice and controls were established and 50 second-division metaphases were scored for SCE induction. Replication indices were determined of 200 metaphases and mitotic indices of 1000 cells.

Result: No treatment related SCE induction was seen: SCEs ranged from about 11 - 12 per metaphase irrespective of exposure and control.

The replicative and mitotic indices were not significantly different from the control.

Test substance: Methanol, 99.9 % pure from Fisher Scientific Corporation, no further data.

Reliability: (2) valid with restrictions
 Comparable to guideline study and current standards, sufficient documentation, acceptable for assessment, however, missing positive control

Flag: Critical study for SIDS endpoint
 18-FEB-2003 (216)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: ICR
Route of admin.: gavage
Exposure period: single, 24 h
Doses: 1050; 2110; 4210; 8410 mg/kg
Result: negative

Method: other: see Method
Year: 1987
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Six animals were used per group. A positive control group was enclosed (TEM, 0.5 mg/kg). Bone-marrow was sampled 24 h post-treatment; 1000 polychromatic erys were scored per mouse for the frequency of micronuclei.

Result: There was no methanol-related increase in the induction of micronuclei: In treated groups, the mean MN rate varied from 0.5 to 2.2/1000 vs. 1.5/1000 in the neg. control and 45/1000 in the pos. control group.

Test substance: Methanol, reagent special grade from Junsei Chemicals Co., no further data.

Reliability: (2) valid with restrictions
Comparable to guideline study and current standards, limited documentation, restriction: only one sampling time

Flag: Critical study for SIDS endpoint
18-FEB-2003 (161)

Type: Micronucleus assay

Species: mouse **Sex:** male/female

Strain: NMRI

Route of admin.: i.p.

Exposure period: single

Doses: 1920; 3200; 4480 mg/kg

Result: negative

Method: other: see Method

Year: 1981

GLP: no data

Test substance: as prescribed by 1.1 - 1.4

Method: Comparative study including a broad spectrum of 30 h after a single dose, bone-marrow cells were isolated from 2 male and 2 female mice per dose, and 1000 polychromatic erythrocytes per mouse scored.

Result: There was no significant effect on the frequency of micronuclei in treated as compared to control animals: 1.3/1000 (control and low dose), 2.0/1000 (2nd dose), and 3.7/1000 (top dose).

Test substance: Methanol, from Merck, Germany, no further data.

Reliability: (2) valid with restrictions
Comparable to guideline study and current standards, limited documentation, restriction: only one sampling time

Flag: Critical study for SIDS endpoint
18-FEB-2003 (199)

Type: Micronucleus assay
Species: mouse **Sex:** female
Strain: CD-1
Route of admin.: gavage
Exposure period: gd 6 through 10 during pregnancy
Doses: 2500 mg/kg/d
Result: negative

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Study performed during a developmental investigation under folate-deficient and -sufficient conditions (see other entry 5.8.2).

Maternal peripheral blood was collected 48 h after the final exposure to methanol (still during pregnancy), and fetal blood was taken on gd 18. The frequency of MN was scored in 1000 reticulocytes.

Result: The MN rate in maternal and fetal reticulocytes was similar among the groups. There was no association between either maternal or fetal hepatic folate concentrations. Furthermore, the comparison of MN frequencies in malformed and normal litters gave no evidence of an increase of MN.

Reliability: (2) valid with restrictions
 Test procedures in accordance with accepted standard methods, sufficiently documented

Flag: Critical study for SIDS endpoint

18-FEB-2003

(217)

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: Swiss Webster
Route of admin.: i.p.
Exposure period: 4 days
Doses: 300, 600, 1200, 2500 mg/kg bw
Result: negative

Method: other: ASTM 1988
Year: 1990
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Objective of the study: possible effects of methanol were to be evaluated as function of folate status, because folate deficiency is known to increase the spontaneous incidence of micronucleated erythrocytes and strongly enhance the incidence of micronuclei induced by certain chemicals, and because methanol oxidation via formate to CO₂ depends on tetrahydrofolate.

Two positive control groups were included, one receiving urethane known to be responsive irrespective of the folate status, and caffeine which is active under the conditions of folate deficiency.

Remark: Objective of the study: possible effects of methanol were to be evaluated as function of folate status, because folate deficiency is known to increase the spontaneous incidence of micronucleated erythrocytes and strongly enhance the incidence of micronuclei induced by certain chemicals, and because methanol oxidation via formate to CO₂ depends on tetrahydrofolate.

Result: 4/10 folate-deficient animals receiving 2500 mg/kg died between days 2 and 3.

No difference in micronucleus frequency (MN in PCEs) and in RNA-positive blood erythrocytes was seen between treated groups and controls while animals treated with the positive control substances responded as expected:

+folate: MN 0.17 - 0.23 % vs. 0.23 % in saline control
-folate: MN 0.31 - 0.37 % vs. 0.38 % in saline control.

Caffeine (+folate): MN 0.34 %
Caffeine (-folate): MN 2.42 %

Urethane (+folate): MN 2.52 %.

Test condition: Mice were made folate-deficient by maintaining on a diet with no folic acid and 1 % succinyl sulfathiazole, inhibitor of the catalase, and another group received folate-supplemented diet containing 5 mg/kg folic acid. Blood samples were taken at 24 hours after the last dosing.
1
10 mice per dose group were used.

Folate limitation resulted in an about 15-20 times lower folate blood level than in the high-folate groups.

Test substance: Methanol 99.9 % pure, no further data.
Conclusion: The results indicate that methanol is nonclastogenic to the developing erythroblast in bone marrow and does not inhibit red blood cell production in either normal or folate-deficient mice.

Reliability: (2) valid with restrictions
Comparable to guideline study and current standards,
sufficiently documented, acceptable for assessment
Flag: Critical study for SIDS endpoint

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: 6 h/day, 5 days
Doses: 800 or 4000 ppm (1.04 or 5.2 mg/l)
Result: negative

Method: other: see Method
Year: 1991
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive test programme (see also other entries this chapter): Five animals per dose were used.

- Peripheral blood MN: Blood smears from tail vein blood were used to score for 2000 polychromatic and 2000 normochromatic erythrocytes per animal.
- Primary cultures of lung cells: Lung MN were analyzed in 1000 binucleated cells per animal after cell isolation from exsanguinated and perfused lung.

Result: No treatment related effect on micronuclei frequency or cell kinetics and no toxicity was seen at any dose level:

Blood erys: Mean MN rates were 3.1/1000 and 5.2/1000 in PCE, and 3.4/1000 and 3.6/1000 in NCE at either dose, respectively, vs. 5.0/1000 and 3.7/1000 of respective controls.

Lung cells: Mean MN rates were about 20/1000 to 24/1000 irrespective of a dose or control group. The ratio of bi- to mononucleated cells was not influenced by the treatment, neither was there evidence of a treatment-related increase in the incidence of multi-nucleated cells.

Test substance: Methanol, 99.9 % pure from Fisher Scientific Corporation, no further data.

Reliability: (2) valid with restrictions
 Comparable to guideline study and current standards, sufficiently documented, acceptable for assessment, however, missing positive control

Flag: Critical study for SIDS endpoint

18-FEB-2003 (216)

Type: Cytogenetic assay
Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: 6 h/day; 5 days
Doses: 800 or 4000 ppm (1.04 or 5.2 mg/l)
Result: negative

Method: other: lung-cell analysis
Year: 1991
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive test programme (see also other entries this chapter): Primary cultures of lung cells): Chromosome aberrations were scored in primary culture lung cells of treated mice. Primary cultures of lung cells were established from 5 mice per group and 100 first-division metaphases per animal were examined for chromosome aberrations.

No positive control group was concomitantly examined.

Result: No increase in the frequency of aberrations per cell and percent aberrant cells and no toxic effects were induced by the treatment:
Aberration rate per cell (including chromatid and chromosome breakage as well as rearrangement events) was from 0.04 to 0.07 in treated animals vs. 0.09 in the negative control. The percentage of aberrant cells ranged from 3.5 to 6.2 % vs. 6.9 in the neg. control.

The replicative and mitotic indices were not significantly different from the control.

Test substance: Methanol, 99.9 % pure from Fisher Scientific Corporation, no further data.

Reliability: (2) valid with restrictions
Comparable to guideline study and current standards, sufficiently documented, acceptable for assessment, however, missing positive control

Flag: Critical study for SIDS endpoint

18-FEB-2003 (216)

Type: Cytogenetic assay
Species: mouse **Sex:** male
Strain: C57BL
Route of admin.: inhalation
Exposure period: 6 h/d; 5 d
Doses: 1.06; 5.3 mg/l (800; 4000 ppm)
Result: negative

Method: other: spermatocyte analysis
Year: 1991
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive test programme (see also other entries this chapter): Spermatocytes of the pachytene substage of the meiotic prophase after 5 days of last exposure were isolated, and isolates fixed for electronmicroscopy: Several morphological parameters concerning the meiotic chromosome structure were examined (synaptonemal complex analysis). The so-called "synaptonemal complex" is the attachment site of homologues chromosomes during meiotic prophase. An increase in the frequency of synaptic lesions may be an indicator for exposure to mutagens.

No positive control group was concomitantly examined.

Result: Methanol exposure gave no evidence of a dose-related increase in structural abnormalities of the synaptonemal complex.

Spermatocytes of the pachytene substage of the meiotic prophase after 5 days of last exposure were isolated, and isolates fixed for electronmicroscopy: Several morphological parameters concerning the meiotic chromosome structure were examined (synaptonemal complex analysis). The so-called "synaptonemal complex" is the attachment site of homologues chromosomes during meiotic prophase. An increase in the frequency of synaptic lesions may be an indicator for exposure to mutagens.

Test substance: Methanol, purity 99 %
Reliability: (2) valid with restrictions
 Test procedures based on scientific principles and standards, sufficiently documented, acceptable for assessment, however missing pos. control.

Flag: Critical study for SIDS endpoint

18-FEB-2003 (216)

Type: Cytogenetic assay
Species: mouse **Sex:**
Strain: no data
Route of admin.: i.p.
Doses: 75 - 300 mg/kg (3x/d)
Result: positive

Method: other: no data
Year: 1983
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: There is some evidence that bone marrow cytogenetic analysis indicated a dose-related response for structural aberrations, especially centric fusions in mice treated with three daily doses of methanol (IPCS/WHO 1997).

Flag: Critical study for SIDS endpoint
 29-APR-2004 (219) (84)

Type: Drosophila SLRL test
Species: Drosophila melanogaster **Sex:**
Route of admin.: oral feed
Doses: LD50

Test substance: as prescribed by 1.1 - 1.4

Result: Methanol was not mutagenic in the "Basc-Test in Drosophila".
Test substance: Methanol from Merck, Germany, no further data.
 11-AUG-2002 (199)

Type: Micronucleus assay
Species: mouse **Sex:** no data
Strain: Swiss
Route of admin.: unspecified
Exposure period: 4 days
Doses: 300, 600, 1200, 2500 mg/kg bw
Result: negative

Method: other
Year: 1991
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Groups of folate deficient and folate supplemented mice were compared to clarify the role of tetrahydrofolate mediated formate metabolism in the genotoxic activity of methanol.

 Ten mice per dose level, no further details indicated.

Result: No genotoxic effects were observed in any treated group.
Test substance: Methanol, no further data.
Reliability: (4) not assignable
 Only short communication, abstract: But see Amer. Petrol.

23-JAN-2003

Inst.: probably the same study.

(220)

5.7 Carcinogenicity

Species: rat **Sex:** male/female
Strain: Fischer 344/DuCrj
Route of administration: inhalation
Exposure period: 24 months
Frequency of treatment: continous, 20 h/d
Post exposure period: none
Doses: 10; 100; 1000 ppm (0.013; 0.13; 1.3 mg/l): 14255 -
 14323 h (males); 14407 - 14468 h (females)
Result: negative
Control Group: yes, concurrent no treatment

Method: other: acc. to OECD Guide-line for Chronic toxicity, Oct. 1980
Year: 1985
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive study programme on three species (rat, mouse, monkey) including metabolic, pharmacokinetic and short-, long-term and reproduction studies and carcinogenicity studies:
 Whole-body exposure: The animals were housed individually in wire-mash cages attached to the inhalation chamber.

52 animals per sex and dose group were tested (initial age 6 weeks). Clinical, ophthalmological, clinical-chemical, hematological and pathological/histopathological examination were done.

Analytical control of chamber concentration: Analytical values close to nominal ones.

Remark: -----
 The blood levels of methanol are said to be measured, but not documented (reported to be similar to those found in the two-generation study: see there). Formate was not measured.

Note: Exposure per day was 20 h, which implies that prolonged steady-state blood levels were reached which even may have been higher than in studies using the same exposure concentration, but shorter exposure times.

Result: -----
 19.2 to 34.6 % of males and 32.7 to 40.4 % of females died or had to sacrificed in extremis. There were no significant differences from the control groups.

No abnormalities of the general state could be observed in treated animals. Body weight was somewhat but not significantly retarded between weeks 51 through 72 in the

highest female dose-group (1000 ppm).
Urinalysis revealed a positive trend in glucose and a decrease in pH in males of the 1000-ppm group, but values were within normal range. In 1000-ppm females, higher bilirubin values were found, yet within normal range. No changes were evident in haematological and biochemical parameters.

Non-neoplastic findings were similar to those commonly found in the control animals of 24 months (p. 282).

Overall, tumor frquencies showed no significant differences between all groups. There was no evidence of an increase in liver tumors. Specific tumours appeared at a somewhat higher incidence in high-dose groups of both sexes:

- papillary lung adenomas in males (6/52 vs. 1/52 in the control), but not in females (Tab. 8, p. 149);
- adrenal phaeochromocytomas in females (7/52 vs. 2/52), but not in males;
- 3/52 and 5/52 metastatic (transition) tumors of uncertain origin in males and females, respectively.

Test condition: Exposure time 20 hours per day, significantly longer than according to guideline (6 hrs). This does not represent normal industrial and consumer use.

Test substance: Methanol, reagent special grade from Junsei Chemicals Co., <1 ppm vinyl chloride; <3 ppm formaldehyde

Conclusion: The results gave no evidence of a cancerogenic potential of methanol in rats.

Reliability: (2) valid with restrictions
Comparable to guideline study, also largely meeting current standards, documentation limited, acceptable for assessment.

Flag: Critical study for SIDS endpoint

Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of administration: inhalation
Exposure period: 18 months
Frequency of treatment: continuous, ca. 19 h/d
Post exposure period: none
Doses: 10; 100; 1000 ppm (0.013; 0.13; 1.3 mg/l: 10436 - 10550 h (males); 10573 - 10642 h (females)
Control Group: yes, concurrent no treatment

Method: other: according to OECD Guide-line for chronic toxicity, Oct. 1980
Year: 1985
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive study programme on three species (rat, mouse, monkey) including metabolic, pharmacokinetic and short-, long-term and reproduction studies and carcinogenicity studies:
Whole-body exposure: The animals were housed individually in wire-mash cages installed in the inhalation chamber.

52 animals per sex and dose group were tested (initial age 6 weeks). Clinical, ophthalmological, clinical-chemical, hematological and pathological/histopathological examination were done.

Analytical control of chamber concentration: Analytical values close to nominal ones.

Remark: The blood levels of methanol and formate were not measured.
Note: Exposure per day was 20 h, which implies that prolonged steady-state blood levels were reached which even may have been higher than in studies using the same exposure concentration, but shorter exposure times.

Result: Survival was high without significant differences as compared to the control, mortality ranging between no deaths and 7.5% deaths by the end of the study: 0% (1000 ppm males, 10 ppm females), 2% (controls), 6% (10 and 100 ppm males) and 7.5% (100 and 1000 pm females).

No abnormalities of the general state could be observed in treated animals. Body weight was somewhat increased in treated group, significantly in 1000 ppm males, between months 6 through 12 in males and months 9 through 12 in females.
There was no evidence of a treatment-related effect in clinical/blood parameters.

Non-neoplastic organ findings were similar to those commonly found in the control animals of 12 months.

Individual tumor frequencies showed no significant differences between all groups; there was no difference in the severity of tumors between the groups.

The tumors exclusively observed in the 1000-ppm group were all within the spontaneous range and included fatty cell tumor at the submaxillary lymph node (1/52 males), chromophobe adenoma of the pituitary gland (3/52 females), adrenal pheochromocytoma (1/52 females), dermal fibrosarcoma (1/52 males), and meningeal sarcoma (1/52 females) vs. 0/52 in all other groups.

The spontaneous liver-tumors rate naturally high in this species was not increased by methanol treatment (p. 171; Tab. 5, p. 172).

Test condition: Exposure time 19 hours per day, significantly longer than according to guideline (6 hrs). This does not represent normal industrial and consumer use.

Test substance: Methanol, reagent special grade from Junsei Chemicals Co., <1 ppm vinyl chloride; <3 ppm formaldehyde

Conclusion: The result gave no evidence of a cancerogenic potential of methanol in mice.

Reliability: (2) valid with restrictions
Comparable to guideline study, also largely meeting current standards, documentation limited, acceptable for assessment.

Flag: Critical study for SIDS endpoint

17-SEP-2002

(161) (178)

Species: rat **Sex:** no data
Strain: no data
Route of administration: oral unspecified
Exposure period: no data
Post exposure period: no data
Doses: no data
Result: negative

Method: other: no data

GLP: no data

Test substance: no data

Remark: Tabular compilation of BT institute data without details: The author reports that Methanol was tested in a carcinogenicity study with oral application in the rat. The substance was clearly not carcinogenic according to the author. No further details are reported.

Reliability: (4) not assignable
Only short communication

15-AUG-2002

(221)

5.8.1 Toxicity to Fertility

Type: Two generation study
Species: rat
Sex: male/female
Strain: other: Sprague Dawley (Crj:CD)
Route of administration: inhalation
Exposure Period: F0: 103 - 108 d; F1: 61-62 d and 145 - 153 d; F2: 54 - 56 d (see Method)
Frequency of treatment: continuous (19 - 20 h/d)
Premating Exposure Period
 male: 60 d (approx. 1200 h)
 female: 60 d (approx. 1200 h)
No. of generation studies: 2
Doses: 0.013; 0.13; 1.3 mg/l (10; 100; 1000 ppm)
Control Group: yes, concurrent no treatment
NOAEL Parental: = 1000 ppm
other: LOAEL F1/F2 offspring :
 = 1000 ppm
other: NOEL F1/F2 offspring :
 = 100 ppm

Method: other: in accordance with OECD Guidelines, Oct. 1980
Year: 1985
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive study programme on three species (rat, mouse, monkey) including metabolic, pharmacokinetic and short-, long-term and reproduction studies and carcinogenicity studies:
Whole-body exposure: One multi-stage inhalation chamber was used for each group.

The duration of exposure was as follows (p. 184):

F0-Males from age of 8 wk through end of mating (16 wk or after);
F0-females from age of 8 wk through mating period (16 wk or after) to end of lactation.

F1-males from birth through end of mating (at 14 wk or after);
F1-females from birth through end of mating (at 14 wk or after) to weaning of F2 pups (21 d after delivery).

F2-Males and -females from birth to 21 d (but to age of 8 wk for 1/sex/litter).

F0 generation consisted of 30 animals per sex and group. Additionally, 15 animals were reared for a second mating (p. 185).

Mating period was maximally 21 d. The pairs without evidence of insemination within 21 d were again cohabited with untreated animals (2nd mating) to determine the fertility of each animal, in this case without exposure (p.186).

Pathohistology:

After mating all males were necropsied, and testes as well as all accessory reproductive organs preserved. Females either after second mating or not inseminated ones were necropsied and examined for reproductive organs. Likewise, all other females having delivered or not were sacrificed for histopathology at due time.

Litters: Part of the pups of each generation were examined for intra-uterine, another one for neo- and postnatal development/morphological differentiation including behavioral/function testing and histopathology until one week after weaning (p. 187/188).

Gametogenesis: Histological examination of morphology of ovaries and sperms was not included.

In 9-week old F1 pups, blood methanol was measured, but not formate (p. 191).

Analytical exposure control: Analytical concentration values of methanol were close to nominal ones.

Statistical ANALYSIS:

All data obtained were analysed by t-test, Fischer's exact test, U-test of Mann-Whitney or Armitage's chi2-test.

Remark:

Blood levels of methanol measured in the F1 offsprings (age 9 weeks) (NEDO, 1987, p. 191):

controls (baseline): approx. 2 - 3 ug/ml
10 ppm methanol: approx. 3 - 3.5 ug/ml
100 ppm " : approx. 1 - 4.2 ug/ml
1000 ppm " : approx. 53 (males)-100 (females) ug/ml.

There are no data on formate.

Note: Exposure per day was 20 h, which implies that prolonged steady-state blood levels were reached which even may have been higher than in studies using the same exposure concentration, but shorter exposure times.

Result:

F0-Generation: No treatment-related alterations in all reproductive parameters.

F1-Generation: None of the fertility indices including sexual cycle, mating time, fertility and pregnancy rate

showed a significant difference from untreated F1 controls.

In male pups of the 1000-ppm group, post-natal morphological differentiation appeared to be influenced with respect to the descensus testis occurring 0.5 to 1 d earlier (see same effect in F2 generation) (p. 199/200) [not mentioned by Takeda and Katoh, 1988]: This time-dependent parameter was evaluated by relating the completion of downward migration of the testes (final length of the gubernaculum reached) to the post-natal body-weight gain (The more reliable body length was not available):

F1-generation:

In the F1 pups (108 males), this process was completed within 16 through 20 post-natal days with the climax at day 17 and 18 (32 and 39 %, respectively), while in the respective control (113 males), descent was complete from 16 through 21 days with the maximum at day 19 (32 %), but also relatively high percentages on the days before and after: day 18 (22%), day 17 (19%), day 20 (18%).

Absolute and relative brain weights were significantly lowered in the high-dose groups of either sex at an age of 8 and 16 weeks. This was still found in females necropsied after 24 weeks. Also other organs showed slight shifts in weights: thymus, pituitary (lower), heart, lung, liver (higher).

However, there were no histological manifestations. No effects on testes or ovaries reported.

Likewise, there were no significant differences in functional tests (movement, emotion, learning) as compared with the control or the other groups.

F2 generation:

As in F1 males, an apparently dose-related earlier descensus testis was noted: F2 (94 males) on day 16 (42%), day 17 (40%), day 18 (15%) vs. control (91 males) on day 16 (10%), day 17 (39%), day 18 (31%), day 19 (14%) (p. 200).

Organ weights showed similar tendencies as found in the F1-generation without histological changes. No effects on testes or ovaries reported.

Test condition: Exposure time 19-20 hours per day, significantly longer than according to guideline (6 hrs).

Test substance: Methanol, reagent special grade from Junsei Chemicals Co., <1 ppm vinyl chloride; <3 ppm formaldehyde

Conclusion: No firm conclusions can be drawn about fertility of either sex, as the copulation time of 21 d was comfortably long for

successful insemination (but in accordance with guidelines

at that time) and gametogenesis was not considered.

In the F1 and F2 progeny (both sexes), a decrease in brain weights was evident at 1000 ppm methanol, but without noticeable histological changes and functional impairments. This phenomenon is believed to represent a change occurring during the perinatal period (Takeda and Katoh, 1988).

The meaning of an apparent shift of testis descent in male offspring in relation to body-weight development of the pups in the two following generations is unclear and was not directly addressed by Takeda and Katoh (1988), but detailed by NEDO (1987) and considered a significant difference from untreated controls (p. 285). Furthermore, it is obvious that this parameter showed considerable variation also between the control groups of both generations (see p. 199/200).

Although there was no obvious pathological effect in the progeny of 1000-ppm exposed groups, the effects observed may be considered as biologically relevant under these test conditions and, therefore, 1000 ppm is established as LOAEL and 100 ppm as NOEL for post-natal development while for parental effects, the NOAEL is 1000 ppm.

Reliability: (2) valid with restrictions
 Comparable to guideline study, also largely meeting current standards, documentation limited, acceptable for assessment

Flag: Critical study for SIDS endpoint
 26-APR-2004 (161) (178)

Type: Fertility
Species: monkey
Sex: female
Strain: Macaca Fascicularis
Route of administration: inhalation
Exposure Period: prebreeding about 120 days; breeding 70 days;
 pregnancy about 165 days
Frequency of treatment: 2.5 h/d
Premating Exposure Period
male: none
female: prebreeding about 120 days
Duration of test: birth, first 9 months of life of the offsprings
Doses: 200; 600; 1800 ppm (0.262; 0.786; 2.358 mg/l)
Control Group: yes
NOAEL Parental: = 1800 ppm
other: NOAEL(Repro) (mother) :
 = 1800 ppm

GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Study design limited to exposure of female animals (pre-breeding, breeding, and pregnancy) and

qualitative/clinical signs of toxicity. It comprised

- methanol pharmacokinetics (see other entry: 5.11)
- maternal toxicity
- reproductive toxicity (see other entry: 5.8.2)
- developmental neurotoxicity (see other entry: 5.8.2).

Specific aims of the study were to determine whether

- repeated exposure to methanol alters methanol metabolism in adult female monkeys,
- pregnancy alters methanol metabolism,
- long-term exposure to methanol produces overt adult toxicity, or reproductive toxicity, or both,
- long-term exposure to methanol in utero affects offspring development, especially neurobehavioral development.

Groups of 11-12 adult female monkeys were exposed to methanol vapor in the concentrations of 0; 200; 600 or 1800 ppm, daily for 2.5 hour before breeding, during breeding and during pregnancy. The study was performed in 2 cohorts: 24 females/cohort and 2 males/cohort.

For postnatal developmental evaluation, 8 to 9 infants were available per group, in total 34, among them 26 in-utero treated offsprings (see other entry: 5.8.2).

Result:

 Reproductive performance:
 Methanol exposure had no effect on most measures of reproductive performance, including menstrual cycles, conception rate, and live-birth delivery rate. However, all methanol-exposed animals had a decrease of about six to eight days in duration of pregnancy compared to control animals (Tab. 17, p. 45).
 It is not clear whether this decrease was related to methanol exposure, as there was no dose response and no differences among offspring groups in body weight or other physical parameters.
 Prenatal exposure to methanol had no effect on infant growth and physical development for the first 9 months.

Test substance:

Methanol, no further data

Reliability:

(2) valid with restrictions
 Test procedures in accordance with accepted standard methods, well documented, limitation due to low number of animals.

Flag:

Critical study for SIDS endpoint

26-APR-2004

(222)

Type: Fertility
Species: mouse
Sex: male
Strain: B6C3F1
Route of administration: gavage
Exposure Period: 5 d
Frequency of treatment: 1x/d, 5 consecutive days
Premating Exposure Period
male: sperm morphology, no mating
female: no females
Doses: 5 mal 1000 mg/kg

Method: other: sperm morphology
Year: 1984
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Ten male mice (4 months of age) were treated and maintained without exposure for 5 weeks (the time needed for testicular spermatogenesis and migration of spermatides to the epididymidis), spermatozoa were isolated from dissected cauda epididymidis and fixed and mounted on slides, and stained for light microscopy. 500 sperms were evaluated per animal.

Result: In the methanol-treated animals, a slight, but statistically insignificant increase in sperms abnormalities (mean factor about 1.7 above neg. control, with very high variance: 1.86 +-0.91 vs. 1.12 +-0.39 in the vehicle control) was observed, while the treatment with cyclophosphamide (100 mg/kg) resulted in an about 6-fold increase (5.84 +-1.94).

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
Test procedures based on scientific principles and standards, limited documentation, acceptable for assessment
Flag: Critical study for SIDS endpoint

18-FEB-2003

(223)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: gd 1-19, at the top dose: gd 7- 15
Frequency of treatment: 7h/d
Duration of test: 21 d
Doses: 6.63; 13.3; 26.6 mg/l (5000; 10000; 20000 ppm)
Control Group: yes, concurrent no treatment
NOAEL Maternal Toxicity: = 10000 ppm
NOAEL Teratogenicity: = 10000 ppm

Method: other: acc. to standards
Year: 1985
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Whole-body exposure: Mean analytical chamber concentrations corresponded well to the nominal ones. 15 dams per group were used. Methanol blood levels were measured in concurrently exposed non-pregnant groups by GC on day 1, 10, and 19.

Dams were sacrificed on gd 20, one half of the fetuses was subjected to examination for visceral, the other half for skeletal abnormalities.

Remark: -----
The biological relevance of this study for humans has to be questioned for the following caveats:

1. The exposure concentrations of methanol relate to doses which significantly exceed lethal doses in man: lowest dose (5000 ppm, 7 h) in rats about 1800 mg/(kg bw*d) based upon calculations (NTP-CERHR, 2001, Tab. 2-10) [(human lethal dose: 300 - 1000 mg/(kg*d)].

2. This high dose which would be lethal to humans proved to be ineffective in rats.

3. The study was performed in a range where methanol metabolism is totally saturated in rats, not considered as relevant for humans.

4. Consequently, the blood methanol levels in rats exposed to 5000 ppm are estimated to be at a factor of 20-50 higher than in humans exposed e.g. to 1000 ppm (7-8 h).

5. The differences in metabolism (predominance of catalase in rodents vs. alcohol dehydrogenase (ADH) in humans) has to be regarded: rate limiting steps are catalase given rise to methanol, but not formate, in blood of rodents, while in humans rate-limiting is formate degradation giving rise to

increased formate levels in human blood upon kinetic saturation.

Result:

Blood levels (dams):
1.0-2.17 mg/ml (5000 ppm); 1.84-2.24 mg/ml (10000 ppm),
5.25-8.65 mg/ml (20000 ppm).
No background levels available.

Maternal toxicity:
Only in the high-dose group, slightly unsteady gait and significantly reduced food consumption during the first week without adverse effect on body-weight gain. No signs of maternal toxicity in the other groups.

Effects on maternal reproduction:
No influence on the number of corpora lutea and of implantations. No effect on fetal lethality and resorption.

Effects on litters/fetuses:
Statistically significant and dose-related decrease in fetal body weight in the two high-dose groups (p<0.05) [note: The significance is poor for the male/female offsprings of the 10000-ppm group when compared to the control group of other studies (see: ethanol, Nelson et al., Tab. 2, p. 731)].

At the highest concentration, an increased number of litters with skeletal and visceral malformations was noted, these included in particular rudimentary and extra cervical ribs and exencephaly and encephalocele, and, to minor extent, cardiovascular and urinary-tract defects:
Visceral defects: 7/15 litters with 15/96 malformed fetuses vs. 0/15 litters and 0/107 fetuses in the untreated control.

Skeletal defects: 14/15 litters with 72/92 malformed fetuses vs. 0/15 litters and 0/98 fetuses in the untreated control.

Similar abnormalities also appeared in the progeny from the 10000-ppm group, but not statistically significant: visceral and skeletal defects 2/15 litters each with 2/107 and 2/115 fetuses, respectively).

There was no evidence of embryotoxic/teratogenic activity of methanol at 5000 ppm.

Test substance:
Conclusion:

Methanol, 99.1 % purity
(For biological relevance for humans: see Remarks.)

1. The CERHR Expert Panel placed high weight on the reliability of results of this prenatal study (NTP-CERHR, 2001).

2. Based on a logistic quantitative model of Risk Assessment for Developmental Neurotoxicants (Slikker and Gaylor, 1997, 1998) using the inhalation data in SD rats from Nelson et al., 1995, the following conclusions were drawn and estimations made:

The use of continuous foetal weight data was as sensitive as foetal brain malformation data in estimating excess risk to methanol inhalation exposure in developing rat. Excess risk (assumed to be the additional risk that foetal weight is below the first percentile of control animals) was estimated by using a logistic model: 0.1 at 16000 ppm, 0.01 at 5400 ppm, and 0.001 at 980 ppm.

Reliability: (2) valid with restrictions
 Comparable to guideline study and current standards,
 sufficiently documented, acceptable for assessment
Flag: Critical study for SIDS endpoint
 28-APR-2004 (224) (187) (225) (226)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 7 - 17 gd
Frequency of treatment: continuous, ca. 22.7 h/d (NEDO, 1987, p. 215)
Doses: 200; 1000; 5000 ppm (0.26; 1.3; 6.64 mg/l)
Control Group: yes, concurrent no treatment
NOAEL Maternal Toxicity: > 1000 ppm
NOAEL Teratogenicity: > 1000 ppm
LOAEL Maternal Toxicity : = 5000 ppm
LOAEL Teratogenicity : = 5000 ppm
other: NOEL maternal/fetal :
 = 1000 ppm

Method: other: acc. to national standards (see Method)
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Comprehensive study programme on three species (rat, mouse, monkey) including metabolic, pharmacokinetic and short-, long-term and reproduction studies and carcinogenicity studies:
 Whole-body exposure: Pregnant females were housed individually in a cage placed in the inhalation chamber.
 36 dams were used per test and control group, including 12 dams allowed for natural delivery (p. 211).

The study comprised of three segments:
 - intra-uterine development/teratogenicity,
 - post-natal development F1 (lactation, 21 d, and >=5 d after weaning) (without exposure) (p. 212), and
 - reproductive performance of F1 to produce F2.

Analytical exposure control: Analytical concentration values of methanol were close to nominal ones.

Remark: Note: Exposure per day was >20 h, which implies that prolonged steady-state blood levels were reached which even may have been higher than in studies using the same exposure concentration, but shorter exposure times.

Result: The exposure to 200 and 1000 ppm methanol failed to produce any maternal and embryonal/fetal toxicity or fetal malformations.

At 5000 ppm, maternal toxicity was noted as a decrease in body-weight gain. Food and drinking water consumption were reduced during gd 7 through 12. One dam died, another one had to be killed before delivery.

After Cesarean section:
Embryolethality was increased (late resorptions: 10.4 % vs. 0.6 % in control, $p < 0.05$), but the variance between single litters was high (Tab. 2, p. 219). No differences were evident in the number of corpora lutea, of implantations, and preimplantation resorptions (p. 219). Mean body weight of live fetuses after Cesarean section was reduced (about -20 %, $p < 0.001$) (p. 220).

Morphological abnormalities in fetuses:
Only after intra-uterine exposure to 5000 ppm, the incidences of various malformations and variations were increased:
About 50 % of the fetuses with ventricular septal defects (visceral malformation in 16/20 litters or 64/131 fetuses) vs. 0% or near 0% in all other groups, and residual thymus (variation in all 20 litter or 70/131 fetuses) vs. about 2.4 to 2.9 % in 4 litters each of all other groups (p. 22/223). Other changes included increased incidence of skeletal variations: "atresia of cervical arch/vertebra foramen costotransversarium" (45 %), " bifurcated vertebral center" (14 %) and "cervical rib" (65 %) as well as "excessive sublingual neuropore" (50 %), all of which malformations having no or little relevance in the other group (p. 224/225) except of "atresia foramen" with about 25 % in the control and about 4 to 8 % in the other exposure groups.

After delivery, neo-/postnatal findings:
After 5000 ppm, mean gestation time was prolonged by 0.7 days (p. 221). Food and drinking water consumption of dams was reduced also after birth during lactation.

Among descendants from the high-dose group, mortality was prominent during the first 4 d after birth with live fetuses showing poor vitality in this time (ca. 10% = 2/12 pups per litter died as compared with 1 to 2% fatal cases in the

other groups). Survivors showed normal appearance after day 4. Slight retardation of growth was still significant at weaning. Water consumption was slightly reduced, in particular for females.

Early indicators for post-natal development:
In pups from the 5000-ppm group, eruption of upper incisor and opening of eyelid for both sexes and descensus testis for males were significantly earlier than in the controls in relation to term of delivery, but not in relation to the whole gestation time which was prolonged for this group.

There were no differences in behavioral and functional tests as compared to control and other test groups.

At the age of 8 weeks, brain, thyroid (males), thymus and testis (males) weights were lower ($p < 0.01$), and pituitary-gland weight of males was higher ($p < 0.05$). But histological examination revealed no treatment-related changes.

16.5 % of the offsprings (15/91 in 8/12 litters) had hemilateral thyroprivia (missing thyroid lobe, mostly left). There was no histopathological lesion in the tissue. The defect was attributed to an impairment of organogenesis (p. 233).

Reproductive performances of F1 (from 5000 ppm):
No significant effects on sexual cycle, genital function and reproductive performance of the F1 progeny were noted.

Test condition: Exposure time about 22 hours per day, significantly longer than according to guideline (6 hrs). This does not represent normal industrial and consumer use.

Test substance: Methanol, reagent special grade from Junsei Chemicals Co., <1 ppm vinyl chloride; <3 ppm formaldehyde

Conclusion: The exposure to 5000 ppm of pregnant rats during gestation (>20 h/d) produces maternal toxicity, fetal malformation, increased perinatal mortality and developmental delay in surviving progeny. Teratogenic effects occurred only at maternally toxic exposure concentration.
Exposure levels of 1000 ppm or less did not induce toxic symptoms in maternal animals, structural abnormalities or delay in growth or functional development in the F1-generation.
Therefore, the NOEL for maternal and developmental toxicity is considered to be 1000 ppm.

Reliability: (2) valid with restrictions
Comparable to guideline study and current standards, also largely meeting current standards, documentation limited, acceptable for assessment

Flag: Critical study for SIDS endpoint
18-FEB-2003 (161) (178)

Species: mouse **Sex:** female
Strain: CD-1
Route of administration: inhalation
Exposure period: day 6 - 15 of gestation
Frequency of treatment: daily, 7 hours/day
Duration of test: up to day 17 of gestation
Doses: 1000, 2000, 5000, 7500, 10000 or 15000 ppm (1.3, 2.6, 6.5, 9.75, 13 or 19.5 mg/l)
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxicity: = 15000 ppm
NOAEL Teratogenicity: = 1000 ppm

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Whole-body exposure: Mean measured chamber concentrations were close to the nominal ones. An air-exposed and an unhandled control group was included. Furthermore, for comparison, an orally treated group receiving 2x 2000 mg methanol/kg bw/d by gavage (as 12.5-% solution in water) was studied.

Blood concentrations were analysed (gd 6, 10, 15).

60 to 6 litters were produced depending on toxicity of the exposure levels.

The following parameters were examined: maternal body weight and clinical signs, fetal weight, number of implantation sites, live and dead fetuses, resorptions, gross external anomalies, skeletal and visceral malformations, blood methanol concentrations in dams.

The 7500- and 10000-ppm group as well the oral group were only examined for resorption, exencephaly and cleft palate because earlier studies have demonstrated that these were the major effects.

Remark: -----
The biological relevance of this study for humans has to be questioned for the following caveats:

1. The exposure concentrations of methanol relate to doses which significantly exceed lethal doses in man: lowest dose (1000 ppm, 7 h) in mice about 800 mg/(kg bw*d) based upon calculations (NTP-CERHR, 2001, Tab. 2-10) [(human lethal dose: 300 - 1000 mg/(kg*d)].

2. The minimum dose in mice which is likely to be lethal to humans proved to be ineffective in mice. Those

concentrations which unequivocally showed malformations were 5000 ppm and above, clearly lethal to humans.

3. The study was performed in a concentration range where methanol metabolism becomes saturated in mice, not considered as relevant for humans: 2000 ppm which may be considered as marginally effective (LOAEL) caused already saturation (see: increase in blood level of methanol).

4. Consequently, the blood methanol levels in mice exposed to 1000 ppm are estimated to be at a factor of approx. 2 to 4, those of mice exposed to 2000 ppm at a factor of 10 to 20 higher, and those of mice exposed to distinctly teratogenic 5000 ppm at a factor of 30 to 60 higher than in humans exposed e.g. to 1000 ppm (7-8 h).

5. The differences in metabolism (predominance of catalase in rodents vs. alcohol dehydrogenase (ADH) in humans) has to be regarded: rate limiting steps are catalase given rise to methanol, but not formate, in blood of rodents, while in humans rate-limiting is formate degradation, giving rise to increased formate levels in human blood upon kinetic saturation.

Result:

Mean blood levels:
approx. 100 ug/ml (1000 ppm), 540 (2000 ppm), 1650 (5000 ppm), 3180 (7500 ppm), 4200 (10000 ppm), 7300 (15000 ppm) in ug/ml. In the oral group (4000 mg/d), about 4000 ug/ml were found, corresponding about to inhalation exposure of 10000 ppm. The controls had a background level of about 1.6 ug/ml.

Maternal and fetal effects.

1. There were no signs of maternal toxicity.
2. Significant increases in the incidence of exencephaly and

cleft palate were observed at 5000 ppm (approx. 10 % per litter: 14 fetuses/11 litters vs. 0.3 % in the control) and above, increased embryo/fetal death at 7500 ppm and above: At the highest dose, almost complete resorption of embryos in most litters occurred.

Reduced fetal weight was not noted, but at 10000 ppm and above.

A dose related increase in cervical ribs or ossification sites lateral to the seventh cervical vertebra was significant at 2000 ppm (approx. 50 %/litter vs. 26 to 34 % in the controls; at 1000 ppm: approx. 34 %/litter). The NOAEL for developmental toxicity was determined at 1000 ppm with relation to the most sensitive defect, the cervical-rib event.

The calculation of benchmark doses for a 5-% added risk from the lower 95-% confidence limit on the maximum likelihood

estimates were generally consistent with the NOAELs for various developmental defects: the 5-% added risk above background level was at 3667 ppm for combined cleft palate, exencephaly, and resorption, for cervical-rib defect at 305 ppm.
(see also: NTP-CERHR, 2001)

Test substance: Methanol, high purity from Fisher Scientific
Reliability: (2) valid with restrictions
Comparable to guideline study, also largely meeting current standards, with acceptable restrictions (see Method), sufficient documentation, acceptable for assessment
Flag: Critical study for SIDS endpoint
26-NOV-2003 (187) (227) (228)

Species: mouse **Sex:** female
Strain: no data
Route of administration: gavage
Exposure period: single treatment on day 7 of gestation (split into two equal doses)
Frequency of treatment: single
Duration of test: up to day 18 of gestation
Doses: 0, 4 or 5 g/kg bodyweight
Control Group: yes, concurrent vehicle

Method: other: Special prenatal exposure study
GLP: no data
Test substance: no data

Method: Study was to elucidate time-related manifestations and mechanisms of methanol-induced skeletal malformations in mouse. Therefore, one single dose prior to gd 9, the sensitive period of skeletal development. Six and 7 dams were used, representing about 70 to 80 fetuses.

Result: The treatment led to dose-related increased incidences in several skeletal malformations such as additional rib on C7 (approx. 30 % of fetuses in both dose groups vs. 0 % in control) or split, fused or incomplete vertebra C2 (also about 41 % in the high dose fetuses vs. 7.7 in the control), 25 instead of 26 presacral vertebrae (10 % vs. 2.4 % in control).
These effects suggest posterior transformation of cervical vertebrae by altering segment patterning in the developing mouse embryo (posteriorization of cervical vertebrae).

It is assumed that interference of methanol with retinol conversion to retinoic acid may play a crucial role in these events.

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
Special study design, sufficiently documented, meets

generally accepted scientific principles, acceptable for assessment

Flag: Critical study for SIDS endpoint (229) (230)
18-FEB-2003

Species: mouse **Sex:** female
Strain: CD-1
Route of administration: inhalation
Exposure period: day 6 - 15, 7 - 9 or 9 - 11 of gestation
Frequency of treatment: daily, 6 hours/day
Duration of test: up to day 17 of gestation
Doses: 5000, 10000 or 15000 ppm (6.5, 13 or 19.5 mg/l)
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxicity: = 5000 ppm
NOAEL Teratogenicity: ca. 5000 ppm
LOAEL Embryotoxicity : = 5000 ppm

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Whole-body exposure: The analytical chamber concentration was close to the nominal one.

17 - 22 litters per group were examined.
The following parameters were examined: maternal toxicity as clinical signs and bodyweight changes, embryo/fetal toxicity as % resorptions, live fetuses and fetal bodyweight, gross, skeletal and visceral malformations and histopathology (neural tube defects).

Result: Maternal toxicity was seen at 13000 and 19500 mg/m³ as reduced body weight and clinical signs, fetal toxicity occurred as increased resorptions dose-related at all dose levels.

Teratogenic activity was observed in dose-related manner at the two upper dose levels as increased incidences of neural tube defects and ocular defects (significant during gd 7-9), hind limb anomalies (during gd 9-11), cleft palate, renal pelvic dilatation/hydronephrosis and tail anomalies (significant during either gd period).

(gd 7-9)	10000 ppm	15000 ppm
	% litters (% fetuses)	
Neural tube defect	30 (3.6)	65 (14.7)
Cleft palate	50 (14.6)	88 (50.4)

At 5000 ppm no difference from control: no neural tube defect, cleft palate 4 - 9 % litters and 0.3 - 0.7 %

fetuses affected.

At 5000 ppm, the incidence of pelvic dilatation (not hydronephrosis) appeared to be increased although high in the untreated control, too.

Test substance: Methanol, HPLC-grade from J.T. Baker, no further data.
Conclusion: The data suggest that the spectrum of teratogenic effects is dependent on the number of methanol exposures and the stage of embryogenesis.

Major methanol-induced events included malformations of the brain, eyes, and the urinary tract as well as decreased fetal weight. External abnormalities of the calvaria, hard palate, jaw, and tail also suggest a detrimental methanol-related effect on the axial skeleton.

No clear NOAEL can be derived for developmental effects due to apparently increased resorption and renal pelvic cavitation after 5000 ppm methanol.

Reliability: (2) valid with restrictions
Test procedures in accordance with accepted standard methods, sufficiently documented, acceptable for assessment
Flag: Critical study for SIDS endpoint

26-APR-2004 (231)

Species: rat **Sex:** female
Strain: Long-Evans
Route of administration: inhalation
Exposure period: GD 6 through Postnatal Day 21
Frequency of treatment: 6 hours daily
Duration of test: until adult phase
Doses: 4500 ppm
Control Group: yes

Method: other: Neurotoxicological assay after high-dose exposure
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Whole-body exposure design: Four cohorts of pregnant rats (n = 12), each consisting of an exposure and a control group, were exposed to 4500 ppm methanol vapor for 6 hr daily beginning on GD 6 through post-natal day (PND) 21. Average analytical chamber values were close to nominal ones from about 4350 to 4580 ppm.

1. Methanol blood levels were determined in both dams: blood samples immediately collected from dams on gd 7, 13, and 19 after 6-h exposure, and from both dams and pups on PND 7, 14, and 21 during lactation. In two cohorts, neuro-histopathological examinations were carried out in neonates on PND 1 and 21. In selected adults from one cohort

(following long-term behavioral tests, see below), brain histomorphology and molecular examination on NCAM (neural cell adhesion molecule, by immunoblotting) were performed.

In one cohort, post-weaning blood concentrations in relation to increasing age were measured, extended to 52 d of age, including blood sampling (Stern et al., 1996).

Several behavioral procedures were used to assess exposure effects in the offspring (Stern et al., 1997).

Result:

1. Reproductive Performance, Blood Levels, Neurological Tests:

Control and exposed cohort with similar litter sizes, sex distribution, and dam and pup weight gains. Any variations were not related to exposure.

Blood levels in dams fairly constant with averages of 0.55 +- 0.07 and 0.56 +-0.09 mg/ml during gestation and lactation. Blood levels in pups about twice as high as in dams, mean 1.26 +- 0.23 mg/ml. In an extra test, it was confirmed that the dam-pup difference persisted until 48 days of age, i.e. about 4 wk post-weaning (fig. 4, p. 42).

Morphology/light microscopy of brain tissue: There was no detectable evidence of neuropathology. However, NCAM indices revealed a significant treatment difference in the cerebellum of neonates on PND 4, while after 15 months of age previously exposed offsprings, no effects were seen (Stern et al., 1997).

2. Behavioral Tests:

Male-female littermates were studied whenever possible to examine sex differences, with one pair from a litter for each procedure. Exposure to methanol did not affect suckling latency and nipple attachment on PND 5 or performance on a aversive olfactory condition procedure on PND 10. Exposure to methanol did alter performances in motor activity procedure.

Methanol-exposed neonates were less active on PND 18, but more active on PND 25 than the equivalent control pups. In a conditioning procedure (rotate a running wheel a specified number of revolutions to obtain food-pellet) males showed decreases, females increases in the rate of running. In a stochastic spatial discrimination procedure methanol-exposed rats responded less efficiently at asymptotic levels of performance than controls.

Test substance:
Conclusion:

methanol, hplc grade
Blood methanol levels in adult rats was similar to those found by other. The distinctly higher levels in offsprings may be explained by the still immature states of the alcohol dehydrogenase system. This may have implications for

post-natal neuronal development/synaptogenesis and learning profile and behavior. However, no histopathological evidence was provided, although on the molecular level, a certain deficiency of cell-adhesion molecule pattern (NCAM 180 and 140 -10% to -20%) appears to reflect a delay in the cell-cell contact formation associated with synaptogenesis. But this could no longer be demonstrated in adult, previously post-natally exposed rats any more. The biological meaning remains to be elucidated.

According to the authors, methanol-exposure was associated with subtle behavioral changes in both neonates and adults.

Reliability: (2) valid with restrictions
Test procedures in accordance with accepted standard methods, well documented

Flag: Critical study for SIDS endpoint

18-FEB-2003 (232) (233) (234)

Species: monkey **Sex:** female
Strain: Macaca Fascicularis
Route of administration: inhalation
Exposure period: prebreeding about 120 days; breeding 70 days; pregnancy about 165 days
Frequency of treatment: 2.5 hours daily
Duration of test: birth, first 9 months of life of the offsprings
Doses: 200; 600; 1800 ppm (0.262; 0.786; 2.358 mg/l)
Control Group: yes
NOAEL Maternal Toxicity: = 1800 ppm
NOAEL Teratogenicity: = 1800 ppm
NOAEL Fetotoxicity : = 1800 ppm

Method: other
Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Study design limited to exposure of female animals (pre-breeding, breeding, and pregnancy) and qualitative/clinical signs of toxicity. It comprised

- methanol pharmacokinetics (see other entry: 5.11)
- maternal toxicity
- reproductive toxicity
- developmental neurotoxicity

Specific aims of the study were to determine whether

- repeated exposure to methanol alters methanol metabolism in adult female monkeys,
- pregnancy alters methanol metabolism,
- long-term exposure to methanol produces overt adult toxicity, or reproductive toxicity, or both,

- long-term exposure to methanol in utero affects offspring development, especially neurobehavioral development.

Groups of 11-12 adult female monkeys were exposed to methanol vapor in the concentrations of 0; 200; 600 or 1800 ppm, daily for 2.5 hour before breeding, during breeding and during pregnancy. The study was performed in 2 cohorts: 24 females/cohort and 2 males/cohort.

For postnatal developmental evaluation, 8 to 9 infants were available per group, in total 34, among them 26 in-utero treated offsprings.

Remark: Limitation of the study: low number of animals, which precludes weak effects from detection with certainty (see also: NTP-CERHR, 2001).

Result: A. Blood levels (see details below):
Exposure to methanol vapors did not affect the health of the adult monkeys prior to or during pregnancy. Single 2.5-hour exposures to methanol vapors caused short term elevations in blood methanol concentrations of approx. 1- to 2-fold in the 200 ppm exposure group, 3- to 4-fold in the 600 ppm group and 13- to 16-fold in the 1800 ppm group.

After long-term exposure, peak blood methanol concentrations declined slight over the first month and remained constant thereafter.

The concentration of plasma formate remained at baseline levels during the entire course of the study in all exposure groups.

Pregnancy had no effect on methanol disposition. Serum folate concentrations were not affected by pregnancy and methanol exposure.

B. Reproductive performance:
Methanol exposure had no effect on most measures of reproductive performance, including menstrual cycles, conception rate, and live-birth delivery rate. However, all methanol-exposed animals had a decrease of about six to eight days in duration of pregnancy compared to control animals.
It is not clear whether this decrease was related to methanol exposure, as there was no dose response and no differences among offspring groups in body weight or other physical parameters.
Prenatal exposure to methanol had no effect on infant growth and physical development for the first 9 months.

The results or behavioral assessments of offspring did not

indicate methanol-exposure effects on most domains of early behavioral development. No consistent effects due to methanol exposure were observed on early reflex responses, gross motor development, spatial and concept learning and memory, and social behavior.

Methanol exposure was associated with ratings of "low arousal" on the Neonatal Behavioral Scale. The effects were observed when all of the methanol-exposed infants were compared with controls. Further comparisons, however did not indicate that the effect was dose-dependent. In addition, many of the methanol-exposed infants that were the last to receive optimal scores for the arousal items had been delivered via C-section. Thus, this effect may not be directly related to methanol exposure independent of mode of delivery.

Methanol exposure was also associated with a delay in early sensorimotor development of male infants only. The effect was observed after controlling for the shortened gestation length observed for the 3 methanol-exposed groups. The delay was dose-dependent and ranged from 9 days for the 200 ppm exposure group to over 2 weeks for the 600 ppm- and 1800 ppm-exposure groups.

The results of the Fagan-Test of Infant Intelligence indicated a possible effect of methanol exposure on visual recognition memory when complex stimuli (social problems) were used in testing. Although there were no mean group differences in the novelty scores across the 4 exposure groups, only the control group exhibited a significant novelty preferences for social stimuli.

C. Other observations:

Finally, prenatal methanol exposure was associated with the occurrence of a wasting syndrome in 2/7 female offsprings in the 1800 ppm-exposure group after approximately 1 year of age, both living in the same cohort. Those females began to show growth retardation with 12 resp. 17 months of age. They became very weak and had to be euthanized at the age of 20 resp. 36 months. Assay results for viral infection, blood chemistry, complete blood count, and liver, kidney, thyroid, and pancreatic function were within the normal range.

Necropsies showed signs of severe malnutrition and gastroenteritis. This symptom was not observed in any of the offsprings of the other cohort.

D. Blood levels:

Methanol: No significant differences in the blood concentrations between the 4 phases (measured 0.5 h post-exposure, n = 11 or 12 (non-pregnant); n = 9 or 10 (pregnancy) (Fig 7, Tab. 11, Fig. 14, Part I):

Baseline range approx. 2 ug/ml [approx. 0.06 mM]
200 ppm: range approx. 5 ug/ml [approx. 0.15 mM]
600 ppm: range approx. 10 ug/ml [approx. 0.30 mM]
1800 ppm: range approx. 35 -40 ug/ml [1mM-range]

At 1800 ppm, after 5 h elimination, the residual methanol level was near baseline (max. 2-fold higher).

The mean estimated elimination half-lives (for 600 and 1800 ppm) ranged between about 60 to 90 min.

Formate: Irrespective of concentration levels or exposure intervals, there was no evidence of a significant increase above the background range of about 0.15 - 0.30 mM (approx. 7 to 14 ug/ml) (Fig. 8, Fig. 15, Tab. 12, Part I).

Folate in serum: There was no significant shift in the folate levels during pregnancy, independent of exposure (Tab. 13, Part I).

Test substance:

methanol, no further data

Conclusion:

Peer-reviewed conclusion: "The investigators findings suggest that repeated inhalation exposure to methanol vapors as high as 1800 ppm would not result in accumulation of blood formate above baseline levels. With the exception of an unexpected shortening of gestation, methanol exposure had no effect on reproductive performance. The most significant result to emerge from this study was the wasting syndrome observed in two monkeys exposed in utero to 1800 ppm... Overall, the results provide no evidence of a robust effect of prenatal methanol exposure on the neurobehavioral development of nonhuman primate infants during the first nine months of life..."

The conclusions are not unanimous about wasting syndrome, gestation length, and sensomotoric and intelligence testing (see NTP-CERHR, 2001, p. 62-65), mainly because interpretation is limited by the normal variance of and by the low number of animals under test.

Reliability:

(2) valid with restrictions
Test procedures in accordance with accepted standard methods, well documented, limitation for developmental effects (see Remark)

Flag:

28-APR-2004

Critical study for SIDS endpoint

(222) (187)

Species: mouse **Sex:** female
Strain: CD-1
Route of administration: gavage
Exposure period: day 6 - 10 of gestation
Frequency of treatment: twice daily
Duration of test: up to day 18 of gestation
Doses: 2500 mg/kg bodyweight in water (2x/d)
Control Group: yes, concurrent vehicle
other: LOAEL : 5000 mg/kg bw

Method: other: see Method
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Study designed to clarify the role of folate in the developmental toxicity of methanol in folate-deficient and folate-sufficient mice. Additionally to the developmental toxicity parameters, the micronuclei formation in maternal and fetal blood was determined (see other entry 5.6).

The female animals were fed folate-free diet supplemented either with 400 or 1200 nmol folic acid as well as 1 % succinylsulfathiazole (as inhibitor of intestinal folate synthesis) 5 weeks prior to mating and throughout breeding and gestation.

The short dosing interval of gd 6 through 10 was selected because this was the sensitive period for the induction of the observed malformations (Fu et al. 1996, p. 461).

21 - 24 litters were examined per group, representing 215 to 282 fetuses per test and control group.

Folate was determined in maternal and fetal liver and blood plasma and red cells.

Result: A. Folate levels (measured on gd 18, 8 d after the final methanol treatment):
Low-folate diet produced a decline in folate in the maternal liver (total folate approx. -30%), in maternal plasma (approx. -30%), in maternal erys (approx. -30%) vs. normal-folate diet and in the fetal liver (approx. -60 to -70%) (Fu et al., 1996, Tab. 2).

Methanol had no marked influence on maternal and folate levels irrespective of folate supplementation, except in maternal plasma where there was some evidence of a reduction of about 20 % .

Methanol treatment was slightly fetotoxic (reduced mean fetal weight and reduced mean crown-rump length), but had no impact other reproductive parameters. It showed some evidence of a teratogenic effect (increased incidences of

cleft palate and exencephaly) under folate-adequate supply, but this was hardly statistically significant: cleft palate (2/222 vs. 0/282) and exencephaly (5/222 vs. 1/282 in the respective high-folate control).

Likewise, folate deficiency failed to produce significant malformations (in accordance with previous reports: e.g. Heid et al., 1992): cleft palate (5/215 vs. 0/282) and exencephaly (2/215 vs. 1/282 in the respective high-folate control).

However, cleft palate, but not exencephaly was significantly increased in the presence of methanol: 39/235 vs. 5/215 and 8/235 vs. 2/215 of folate-poor control, respectively.

Methanol did not induce micronuclei in maternal or fetal blood.

B. In pregnant CD-1 mice given methanol (5 g/kg/d) from gestation day 6 - 15, one group receiving folate deficient, the other folate supplemented diet, there were no differences in the formate-blood levels between both groups: 5.13 +-0.68 mmol/L (folate-def.) and 3.90 +-0.94 mmol/L (folate-suppl.) vs. 0.36 +-0.13 mmol/L (untreated control). But developmental toxicity was significantly higher in folate-deficient dams (Hong et al. 1997).

The results indicate that increased plasma formate levels in dams do not underlie the increased developmental toxicity of methanol in mice fed low dietary folate (Hong et al. 1997).

Test substance:
Conclusion:
Reliability:
Flag:
26-APR-2004

Methanol, highest HPLC grade, from Fisher Scientific Comp.
Folate deficiency enhanced the teratogenic effects of methanol in mice.
(2) valid with restrictions
Test procedures in accordance with accepted standard methods, sufficiently documented
Critical study for SIDS endpoint

(217) (235) (236) (237)

Species: rat **Sex:** female
Strain: Long-Evans
Route of administration: inhalation
Exposure period: day 7 - 19 of gestation
Frequency of treatment: daily for 7 hours/day
Duration of test: up to postnatal day 160
Doses: 19.5 mg/l (15000 ppm) corresponding to ca. 6100 mg/kg/day
Control Group: yes, concurrent vehicle

Method: other: postnatal offspring development and behaviour
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Whole-body exposure: The analytical chamber concentrations varied by less than 5 % and were fairly close to the nominal ones. Six and 7 dams were used per group.

The following parameters were recorded: maternal bodyweight, maternal blood methanol levels, offspring bodyweight, offspring mortality, motor activity (day31-21, 30, 60), olfactory learning (day18), behavioural thermoregulation (day 20-21), T-maze learning (day 23-24), acoustic startle response (day 24, 60), reflex modification audiometry (day 60), pubertal landmarks (day 31-56), passive avoidance (day 72), visual-evoked potentials (day 160). 5 or 6 dams were used per group with 10 - 11 offspring. Behavioural tests were performed on 4 - 5 offspring animals.

Remark: The number of pups available and tested (mostly, 1/sex/litter) was probably too small to draw firm conclusions about postnatal developmental effects on behavior.

Result: Maternal blood methanol levels were between 3.8 and 3.1 mg/ml. The treatment led to reduced maternal body weight, the only effect noted in dams. No increase in post-implantation loss. Postnatal bodyweight were modestly but statistically lower after treatment. No increase in postnatal mortality. No other test revealed significant effects of methanol treatment.

Test substance: Methanol, 99.9 % purity, Fisher Scientific
Reliability: (3) invalid
Test procedures based on scientific principles and standards, well documented, limited value for neurological/behavioral assessment

Species: mouse **Sex:** female
Strain: CD-1
Route of administration: inhalation
Exposure period: day 7 - 9 of gestation
Frequency of treatment: daily, 6 hours/day
Duration of test: up to day 8.5, 9.5 or 10.5
Doses: 15000 ppm (19.5 mg/l)
Control Group: yes, concurrent vehicle

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Whole-body exposure: The analytical chamber concentration was close to the nominal one.

4 - 9 litters and 26 - 99 live fetuses were examined per group. The following parameters were examined: maternal body weight and clinical signs, gross, skeletal and visceral malformations in fetuses, histopathology and scanning electron microscopy in fetuses.

Result: Maternal toxicity was seen as clinical signs and reduced bodyweight, in fetuses the treatment induced reduced bodyweight and a range of gross neural defects (anencephaly, encephalocele, holoprosencephaly, ocular anomalies) in up to 41 % of live embryos.

Test substance: Methanol, HPLC grade, from Baker
Conclusion: The result suggest that exposure to high concentrations of methanol injures multiple stem cell populations in the neurologating mouse embryo.
Reliability: (2) valid with restrictions
Test procedures in accordance with accepted standard methods, well documented, acceptable for assessment

13-AUG-2002 (239)

Species: mouse **Sex:** female
Strain: CD-1
Route of administration: inhalation
Exposure period: 6 hours
Frequency of treatment: single treatment on day 8 of gestation
Duration of test: up to day 10 of gestation
Doses: 10000 or 15000 ppm (13 mg/l)
Control Group: yes, concurrent vehicle

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: 12 - 14 litters per group were evaluated.

The effects of methanol and its major metabolite formate as well as the influence of a pretreatment with the alcohol-dehydrogenase inhibitor 4-methylpyrazole were compared. The following parameters were examined: crown-rump length, head length, gross malformations and neural tube defects, methanol concentrations in fetal and maternal tissue. The folate status in RBC and decidual tissue was monitored at 15000 ppm methanol.

Remark: Valuable scientific information about mechanisms. However, the biological relevance for humans is poor, because the estimated systemic dose in mice [≥ 4000 mg/(kg*d)] is distinctly above doses lethal to humans (300 - 1000 mg/kg bw).

Result: Methanol treatment (10000 ppm) led to a significant increase in the incidence of open neural tubes (9.65 \pm 3.13% in 12 litters vs. 2.02 to 2.69 \pm 1.09% in controls, $p < 0.05$). After 4-methylpyrazole pretreatment (4-MP), the percentage of this effect was 7.21 \pm 2.65%, but not significant. No such effect was induced by formate given orally (750 mg/kg). The other parameters examined were not affected by the treatment (Dorman et al., 1995).

Methanol concentrations were equal in fetal and maternal tissues with maximum maternal plasma level at 65 to 75 mM, and 62 to 85 mM in decidual swelling, irrespectively of pretreatment with 4-MP. Likewise, mean formate blood and decidual peaks were at 0.5 to 0.6 mM and 1.8 to 2.1 mM, respectively. Furthermore, after 15000 ppm, too, no significant changes of formate were noted in either compartment.

[That means, 4-MP failed to influence methanol and formate levels significantly as well as embryonal dysmorphogenesis.] (Dorman et al., 1995).

Folate status:
15000 ppm methanol resulted in transient, albeit statistically insignificant decreases in total maternal red blood cell and decidua folate levels (Dorman et al., 1995).

Test substance: Methanol HPLC-grade from Sigma Chemical Corporation, no further data.

Conclusion: Formate seems to play no apparent role in the development of methanol-induced exencephaly, and methanol itself is the proximate teratogen in pregnant CD-1 mice exposed to high concentration of methanol vapor, because

1. no formate accumulation in methanol-treated mice,
2. exencephaly (failure of anterior neuropore to close) occurred in the absence of increased formate levels,
3. no exencephaly after oral treatment with high doses of formate,
4. methanol, but not formate, caused neural tube defects in whole-embryo culture.

The results (although only from single dosing) did not provide evidence that folate deficiency gave rise to methanol-related dysmorphogenesis and appears to be irrelevant at least at low methanol burdens (compare also test results in monkeys: Dorman et al., 1994). But based on observations by others (e.g. Sakanashi et al., 1994), an interaction of folate deficiency and methanol cannot be ruled out under certain conditions. (Dorman et al., 1995; Dorman and Welsch, 1996; see also: Medinsky and Dorman, 1995)

Reliability: (2) valid with restrictions (240) (241) (242) (243) (244)

26-NOV-2002

Species: mouse **Sex:** female
Strain: CD-1
Route of administration: inhalation
Exposure period: see method
Frequency of treatment: see method
Duration of test: until GD 17
Doses: 10000 ppm (13 mg/l)
Control Group: yes

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Critical periods for the developmental toxicity of methanol were assessed in pregnant CD-1 mice exposed to 10000 ppm for 7 hours/day on 2 consecutive days during GD 6-13 (GD 6 and 7; GD 7 and 8; GD 8 and 9, GD 9 and 10; GD 10 and 11; GD 11 and 12; GD 12 and 13) or to single day (7 hour) exposures during GD 5 and 9. Controls were exposed in the same way to filtered air. Mice received water but not food during exposure. Maternal blood methanol was determined at times during, at the end of and subsequent to a single 7 hr exposure on GD 7. On GD 17 mice were killed and gravid uteri removed. Live, dead and resorbed fetuses were counted, and live fetuses were examined, weighed and preserved in 70 % ethanol. All fetuses were examined externally and for cleft palate, eviscerated, and stained with Alizarin red for skeletal examination. Test and control groups consisted out of 12 to 17 pregnant mice.

Remark: Valuable scientific information about mechanisms. However, the biological relevance for humans is poor, because the estimated systemic dose in mice [some 4000 mg/(kg*d)] is distinctly above doses lethal to humans (300 - 1000 mg/kg bw).

Result: Peak maternal blood methanol concentration at the end of the exposure was about 4 mg/ml, methanol was cleared from maternal blood within 24 hr. Some fully resorbed litters were observed with 2-day methanol exposure on gd 6-7 (3/12) and gd 7-8 (1/16) or 1-day exposure on gd 7 (3/17). With 1-day methanol exposure on gd 7, the number of live fetuses (4.4 per litter) was lower than with exposure on any other day. Cleft palate, exencephaly and skeletal defects were the fetal anomalies observed:
cleft palate:
occurred with 2-day exposures on gd 6-7 through gd 11-12 (peak on gd 7-8) and with 1-day exposures on gd 5 through 9 (peak on gd 7)

Exencephaly: occurred with 2-day exposures on gd 6-7 through gd 8-9 (peak on gd 6-7) and with 1-day exposure on gd 5 through 8 (peak on gd 7).

Skeletal elements malformed included the

- exoccipital (peak on gd 6-7 (22.5 %); gd 5 (9.9%))
- atlas (peak on gd 6-7 (72.3 %); gd 5, 6 (55.5 %, 55.3 %))
- axis (peak on gd 6-7(22.3 %); gd 7 (28.8 %))
- cervical vertebra 7 with a rib (peak on gd 6-7 (73.7 %); gd 7 (45.4 %))
- lumbar vertebra 1 with a rib (peak on gd 7-8 (68.3 %); gd 7 (39.4 %)).

An increase incidence of fetuses with 25 presacral vertebrae (normal 26) was observed with methanol exposure on gd 5; whereas an increased incidence of fetuses with 27 presacral vertebrae was observed with methanol exposure on gd 7. According to the authors the results of this study indicate that gastrulation and early organogenesis represent a period of increased embryonal sensitivity to methanol.

Test substance:

Methanol, high purity from Fisher Scientific

Reliability:

(2) valid with restrictions

Test procedures in accordance with accepted standard methods, well documented, acceptable for assessment

26-NOV-2002

(245)

Species:	rat	Sex: female
Strain:	Long-Evans	
Route of administration:	drinking water	
Exposure period:	gd 15 -17 or gd 17 - 19	
Frequency of treatment:	continuous	
Doses:	2 % Methanol in drinking water (ca. 2500 mg/kg/d)	
Control Group:	yes, concurrent no treatment	

Method: The study design was to elucidate postnatal neurological effects in rat pups after intrauterin exposure to methanol: Two behavioral test were selected,
1) the suckling behavior test which measures the latency time to nipple attachment (postnatal day 1) and
2) the nest-seeking/homing behavior test (postnatal day 10)

30 primigravid dams were used, 10 per group.

Result: Maternal toxicity was not observed. In both test groups no differences were found in litter sizes, neonatal birth weight as well as lethality of offsprings as compared with the progeny of the untreated control.

Both behavioral tests on post-natal disorders showed significant retardations in learning: Pups from treated rats required a longer time to begin suckling on post-natal day 1 (80 to 90 sec. vs. 60 sec of controls) and to locate nesting

material on post-natal day 10 than control pups (3 time- and distance-related indicators evaluated).

Test substance: Methanol, no further data.
Conclusion: The data suggest that prenatal methanol exposure may induce neurotoxic effects in the offsprings which are not associated with evident or overt toxicity.

Reliability: (2) valid with restrictions
Test procedures based on scientific principles, sufficiently documented, acceptable for assessment

17-FEB-2003 (246)

Species: rat **Sex:** female
Strain: Long-Evans
Route of administration: drinking water
Exposure period: day 6-15 of gestation
Frequency of treatment: daily
Duration of test: not indicated, presumably to the end of gestation
Doses: 350, 700 or 1400 mg/kg bodyweight (0.5, 1 or 2 % in water)
Control Group: yes, concurrent vehicle

Method: other: teratogenicity study with folate deficient animals
GLP: no data

Method: Pilot study/Screening: Rats were rendered folate deficient by supplying a folate-deficient diet. 7 to 9 dams were used per group.

Result: The average blood levels were given as 0.21, 0.26, and 0.67 mg/L, respectively.

At 1 and 2 % methanol in water fetal resorptions/deaths as well as skeletal malformations were significantly increased. In the 0.5 % group minimal respective changes indicate a NOEL for maternal and developmental below 0.5 % which corresponds to a blood level of 0.21 mg/ml.

No further details are reported.

Test substance: Methanol, no further data.
Reliability: (4) not assignable
Only short communication, abstract

14-AUG-2002 (247)

Species: rat **Sex:** female
Strain: Long-Evans
Route of administration: drinking water
Exposure period: days 6 - 15 of gestation
Frequency of treatment: daily
Duration of test: up to day 20 of gestation
Doses: 357.1, 714.3 or 1428.6 mg/kg bodyweight (0.5, 1 or 2 %)
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxicity: = 357.1 mg/kg bw

Method: other: comparison with folate deficient dams
GLP: no data
Test substance: other TS

Method: 5 dams or litters were used per group, all treated groups and one control group were made folate deficient before treatment. The examination for teratogenic effects was not performed at the date of publication.

Result: The highest dose level was highly embryotoxic, of 5 expected litters only one survived. In the mid dose group resorptions were also significantly increased. Maternal toxicity as reduced bodyweight or bodyweight gain was seen in the mid and high dose group. Teratogenic effects were not yet examined at the date of publication.

Test substance: Methanol, no further data.

Reliability: (4) not assignable
Comparable to guideline study, lacking important results, documentation limited

14-AUG-2002

(248)

Species: rat **Sex:** female
Strain: Long-Evans
Route of administration: gavage
Exposure period: single application on day 10 of gestation
Duration of test: not indicated, presumably to the end of gestation
Doses: 1027, 2054 or 4108 mg/kg bodyweight (1.3, 2.6 or 5.2 ml/kg)
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxicity: = 2054 mg/kg bw

Method: other: no details indicated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: 10 to 13 dams per group were treated. Wilson sectioning, gross necropsy and alizarin red staining of fetuses was performed. No further details are reported.

Result: Maternal toxicity was seen only as body weight loss in the highest dose group (> 20 % decrease). Methanol at all doses failed to produce any significant change in standard reproductive indices (e.g.

postimplantation loss). A significant decrease in fetal body weight (11 to 19.5 %), however, was associated with a dose-dependent increase in anomalies (0.6 % in control, 3.7%; 7% and 16.5% in the dose groups). The dose-related anomalies were undescended testes, exophthalmia and anophthalmia.

Test substance: Methanol, HPLC grade
Reliability: (2) valid with restrictions
 Test procedures in accordance with accepted standard methods, test design limited, sufficiently documented
 14-AUG-2002 (249) (250) (251)

Species: rat **Sex:** female
Strain: Wistar
Route of administration: gavage
Exposure period: day 6 - 15 of gestation
Frequency of treatment: daily
Duration of test: up to day 21 of gestation
Doses: 2.5 g/kg bodyweight
Control Group: yes, concurrent vehicle

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Well nourished and malnourished groups of rats were compared. The following parameters were examined: number of live fetuses, resorptions, corpora lutea, implantation sites, fetal weight, gross and skeletal malformations.
 12 - 17 dams and 110 - 177 live fetuses per group were examined.

Result: The treatment induced maternal toxicity. The treatment led to increased incidences of skeletal malformations in well nourished (45.4 % compared to 5.6 % in controls) and malnourished rats (38.8 % compared to 3.8 % in controls). Malformations were primarily found on ribs (extra ribs) and vertebra (irregular shape). Malnutrition did not increase the incidence of malformations, but fetal growth was retarded.

An increase in skeletal malformation, primarily cervical extra ribs was noted in the methanol treated rats when compared to controls. This is only a single dose study and the 2.5 g/kg/day dose is above the lethal dose in humans.

Test substance: Methanol, no further data.

Conclusion: This is only a single dose study and the 2.5 g/kg/day dose is above the lethal dose in humans.

Reliability: (3) invalid
Unsuitable test design (see Conclusions)

14-AUG-2002

(252)

Species: rat **Sex:** female
Strain: other: Holtzman
Route of administration: gavage
Exposure period: GD 1-8
Frequency of treatment: daily
Duration of test: up to day 9, 11 or day 20 of gestation
Doses: 1600; 2400 or 3200 mg/kg bw
Control Group: yes, concurrent vehicle

Method: other: early pregnancy study

GLP: no data

Test substance: as prescribed by 1.1 - 1.4

Method: Early pregnancy study:
Eight dams per group with 11 - 13 fetuses per dam were used. Animals were killed on days 9, 11, or 20 of pregnancy, and maternal, embryonic, or fetal parameters were assessed:

Effects on early pregnancy were determined on day 9 as bodyweight, uterine weight, number of implantation sites, number of atypical sites, mean implantation site weight, ovarian weight and number of corpora lutea per ovary. Decidual cell response was determined on day 9 as uterine weight, uterine function and normal implantation processes.

Effects on mid pregnancy was determined on day 11 as number of live fetuses, presence of heartbeat, yolk sac circulation, axial rotation, closure of neural tube, presence of limb buds and sensory organs, yolk sac diameter, crown-rump length, head length and somite number.

Effects on late pregnancy were determined on day 20 of gestation as number of live fetuses, number of resorptions, gross external abnormalities and fetal weight.

Additionally, decidualisation was assessed in pseudopregnant rats using a DCR(decidual cell response) technique.

Result: The only effect of methanol treatment was seen on early pregnancy (day 9) as reduced uterine and implantation site weight and increased number of atypical, small implantation sites (with extravasated blood) in the high-dose group.

All other parameters examined showed no statistical significant differences between treated and control groups at any gestation day. There was no treatment-related effect

on serum estradiol, progesterone, lutenizing hormone and prolactin on day 9 (1 day after after cessation of exposure).

No effects on embryo/fetal survival or development including external anomalies were noted at any gestation day.

The 3200 mg/kg/day dose of methanol produced a reduction in body weight gain by day 9, but not day 20, which may be considered an indication of non-specific maternal toxicity.

Decidualisation

After methanol treatment of pseudopregnant rats (all 3 doses), a dose-related inhibition of uterine growth was observed (day 9), but not significant at 1600 mg/kg/d.

Test substance:
Conclusion:

methanol, high purity grade, no further data
Results indicated that effects on uterine weight and implantation sites on gd 9 may have resulted from methanol-induced inhibition of the DCR. However, this apparent potential impact on decidua development was not expressed by a depression of day-20 embryo/fetal survival or development, when treatment ended on gd 8.

The biological relevance of these results is unclear, in particular, failing a comprehensive data base on hormone status and DCR (see also: NTP-CERHR, 2001).

Reliability:

(2) valid with restrictions
Special study design, sufficiently documented, meets generally accepted scientific principles, but technical limitations (see Conclusion).

14-AUG-2002

(253)

Species: rat **Sex:** female
Strain: Long-Evans
Route of administration: oral feed
Exposure period: day 8 of gestation up to parturition
Frequency of treatment: daily
Duration of test: up to some days after parturition
Doses: 0.6, 0.9 or 1.6 % (v/v) (278.6, 417.9 or 742.9 mg/kg bodyweight)
Control Group: yes, concurrent vehicle

Method: other: no details reported
GLP: no data
Test substance: other TS

Method: Dams were fed a liquid diet containing methanol. Number of animals not indicated, no detailed description of method.
Result: Methanol did not affect fecundity but reduced maternal weight gain, decreased litter sizes, increased perinatal and postnatal mortality and decreased weights at weaning. Teratogenic effects are not reported.
Test substance: Methanol, no further data.
Reliability: (4) not assignable
 Only short communication, abstract

14-AUG-2002

(254)

Species: mouse **Sex:** female
Strain: C57BL
Route of administration: i.p.
Exposure period: 2 injections 4 hours apart on gestational day 7
Frequency of treatment: 2 treatments
Duration of test: up to day 17 of gestation
Doses: 1.7 or 2.45 g/kg bodyweight
Control Group: no data specified

Method: other: no details reported
GLP: no data
Test substance: other TS

Method: Study design was to elucidate pathogenic mechanisms in methanol intoxication: Ethanol and methanol were compared with respect to their teratogenic activity on the brain. Ethanol was given at a dose level of 2.45 g/kg bodyweight (Rogers, 1995).

In a further series, so-called confocal laser scanning microscopy was used to elucidate the pathogenesis of craniofacial defects (Rogers et al., 1996).

Result: -----
 1.7 g/kg methanol induced 30 % resorptions and midfacial deficiencies and micro/anophthalmia in over 50 % of live fetuses. 2.45 g/kg resulted in 55 % resorptions and 100 % of live fetuses having malformations of the holoprosencephaly

spectrum (micro/anophthalmia, cebocephaly, absence of forebrain, premaxillary agenesis).

Cell death of cells were observed in embryos on gd 8 at the edge s of the neural plate and on gd 9 in the midbrain and visceral arches. On gd 8, sparse mesenchyme and edema were apparent beneath the cranial neuroepithelium. Prosencephalic deficiencies observed included small or telencephalic and optic vesicles (Rogers et al., 1996).

Test substance: Methanol, no further data.
Conclusion: The pathogenesis of methanol-induced craniofacial malformations is similar to that previously observed for ethanol (Rogers et al., 1996)(for the ethanol effect: see also review Rogers and Daston, 1997).

Reliability: (2) valid with restrictions
Special study design, sufficiently documented, meets generally accepted scientific principles, acceptable for assessment

14-AUG-2002 (255) (256) (257)

Species: mouse **Sex:** female
Strain: C57BL
Route of administration: i.p.
Exposure period: gestation day 6, 7, 8, 9 or 10
Frequency of treatment: 2x/d with 4 h apart
Doses: 4900 mg/kg bw., split in 2 equal doses on resp. gestation day
Control Group: yes, concurrent vehicle

Method: other: Screening test for sensitive stages
Year: 1998
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: The results included external and soft tissue defects, still excluded skeletal malformations.

Result: The total incidence of malformations was highest with gestation day 7, indicating that gastrulation is sensitive to methanol similar to ethanol.

The defects included the eye (an- and microphthalmia, coloboma), the brain and face (holoprosencephaly, facial clefts) as well as cardiovascular system (vetricuar septal and great vessel defects).

Contrary to ethanol, no external digit defects were observed.

Reliability: (4) not assignable
Only abstract.

27-JUL-2002 (258)

Species: other: rat and mouse **Sex:** female
Strain: other: Sprague-Dawley rat and CD-1 mouse
Route of administration: other: in vitro whole embryo culture
Exposure period: 24 or 48 hours
Frequency of treatment: single
Duration of test: 48 hours
Doses: 0, 8, 12 or 16 mg/ml (rat), 0, 2, 4 or 8 mg/ml (mouse)
Control Group: yes, concurrent vehicle

Method: other: in vitro whole embryo culture
GLP: no data

Method: Screening test including developmental stages of embryogenesis:
The following parameters were examined: viability, yolk sac circulation, heart beat, crown-rump length, head length, somite number, Feulgen staining to localize cell death and proliferation, scanning electron microscopy and histology (only rat embryos).

Result: In rat embryos methanol affected adversely yolk sac diameter, head length, developmental score, somite number and mortality. At the highest concentration 100 % of embryos were abnormal and cell death was increased in various parts of the nervous system.
In mouse embryos methanol affected adversely crown rump length, head length, developmental score and somite number. At the highest concentration the percentage of abnormal embryos reached 94 %. Cell death was not induced in treated cultures.

Test substance: Methanol, no further data.
14-AUG-2002 (259)

Species: rat **Sex:** no data
Strain: no data
Route of administration: other: whole embryo culture
Exposure period: 24 hours
Frequency of treatment: single
Duration of test: 48 hours
Doses: 0, 4, 6, 8, 12 or 16 mg/ml
Control Group: yes, concurrent vehicle

Method: other: whole embryo culture, no details indicated
GLP: no data

Method: Screening test including developmental stages of embryogenesis:
Embryos were explanted on day 9 of gestation, cultured for 24 hours in methanol supplemented serum and cultured for further 24 hours in pure serum. The following parameters were measured: embryoletality, somite number, crown rump length, developmental score, head length. Number of embryos used not indicated.

Result: 4 and 6 mg/ml was not toxic and did not affect embryonic development. Higher concentrations led to a dose related decrease in somite number and overall development. 12 and 16mg/ml led to increased lethality. Head length and crown rumplength were not affected by the treatment.

Test substance: Methanol, no further data.

14-AUG-2002

(260)

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: other: whole embryo culture
Exposure period: 24 hours
Frequency of treatment: single
Duration of test: 48 hours
Doses: 0, 2, 4, 8, 12 or 16 mg/ml
Control Group: yes, concurrent vehicle

Method: other: in vitro culture of whole embryos
GLP: no data

Method: Screening test including developmental stages of embryogenesis:
The following parameters were examined: somite number, crownrump length, developmental score, yolk sac diameter, head length, incidence of anomalies.

Result: Rat embryos were explanted and cultured in 0, 2, 4, 8, 12 or 16 mg Methanol/ml rat serum for 24 h and then transferred to rat serum alone for 24 h. Embryonic development of the 2 and 4 mg groups was not significantly different from the controls whereas the higher concentrations resulted in a concentration related decrease in somite number, head length and developmental score. The 12 mg/l dose resulted in some embryoletality as well as dysmorphogenesis, while the

highest dose was embryolethal. Methanol was dysmorphogenic in vitro in rat embryos at a methanol concentration comparable to that reported in maternal serum following teratogenic in vivo exposures.

Test substance: Methanol, no further data. (261)
14-AUG-2002

Species: rat **Sex:** no data
Strain: Sprague-Dawley
Route of administration: other: whole embryo culture
Exposure period: 48 hours
Frequency of treatment: single
Duration of test: 48 hours
Doses: 2.08 - 16.67 ul/ml (1.64 - 13.17 mg/ml)
Control Group: yes, concurrent vehicle

Method: other: in vitro culture of whole embryos
GLP: no data

Method: Screening test including developmental stages of embryogenesis:
9 - 17 embryos were used per treatment scheme, the effect of methanol and its major metabolite, formic acid, was compared. The following parameters were recorded: yolk sac circulation, dorsally bending, heart beat, crown-rump length, somite number and protein content.

Result: Crown-rump length, somite number and protein content was dose related decreased firstly at 11.6 ul/ml (9.16 mg/ml), at 8.75 ug/ml (6.91 mg/ml) no treatment related effects were observed.

Test substance: Methanol, no further data. (262)
14-AUG-2002

Species: mouse **Sex:**
Strain: CD-1
Route of administration: other: whole embryo culture
Exposure period: 24 hours
Frequency of treatment: single
Duration of test: 24 hours
Doses: 0, 2, 4, 6, 8 mg/ml
Control Group: yes, concurrent vehicle

Method: other
GLP: no data

Method: Screening test including developmental stages of embryogenesis:
Day-8 mouse embryos were explanted and cultured in 0, 2, 4, 6 or 8 mg Methanol/ml culture medium (75 % rat serum, 25 % Tyrode's salt solution) for 24 h.

Result: CD-1 mice: Embryonic development in the 2-mg/ml group was not significantly different from the controls but all higher concentration groups had a significant decrease in developmental score and crown-rump length. The high concentration group also suffered 80 % embryo lethality.

C57/BL/6J mice: Somite number was not affected. 2 mg/ml and higher produced a concentration-dependent increase in dysmorphology in the absence of embryo-lethality, including open neural tubes, branchial arch hypoplasia, and prosencephalic hypoplasia. Additionally, 4 and 6 mg/ml caused mes- and rhombencephalic hypoplasia.

These results indicate that C57 mice are more sensitive than CD-1 mice, suggesting that differences in strain sensitivity to in-vitro methanol exposure are more likely due to intrinsic differences in the developing embryo rather than maternal factors.

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
14-AUG-2002

(261) (263)

Species: mouse **Sex:** no data
Strain: CD-1
Route of administration: other: whole embryo culture
Exposure period: 12 hours
Frequency of treatment: single
Duration of test: 45 hours
Doses: 62, 125, 187, 250 or 375 mM (ca. 2, 4, 6, 8 or 12 mg/ml)
Control Group: yes, concurrent vehicle

Method: other: in vitro culture of whole embryos
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Screening test including developmental stages of embryogenesis:
The following parameters were examined: percentage of live embryos, cephalic dysraphism, prosencenphalic lesions, branchial arch hypoplasia.
Result: Methanol treatment led to dose related increased incidences of all anomalies examined for.
Test substance: Methanol, HPLC-grade from Sigma Chemical Corporation, no further data.

14-AUG-2002

(242)

Species: other: rat and mouse **Sex:**
Strain: other: Sprague-Dawley rat, CD-1 mouse
Route of administration: other: whole embryo culture
Exposure period: 48 h
Frequency of treatment: single
Duration of test: 48 h
Doses: about 2 to 9 mg/ml (also in combination with formate)
Control Group: yes, concurrent vehicle

Method: other: in vitro culture of whole embryos
GLP: no data

Method: Screening test including developmental stages of embryogenesis:
Follow-up studies based on previous ones: Methanol and formate as well as combination of both substances were examined.
The following parameters were examined: somite number, crownrump length, developmental score, yolk sac diameter, head length, incidence of anomalies.
The developmental scoring system took into account embryonic growth parameters as well as stages of development.
Remark: In a further preliminary study, there was some evidence that methanol may interfere with ADH and thus prevent oxidative conversion of retinol into retinoic acid, a presumed mediator in embryonic development, which may explain part of methanol-induced disorders (Andrews et al., 1998b).

Result: 1. In rats, a less than additive-dose effect was found: the combination of methanol and formate proved to be less toxic than would be predicted from their individual toxicities, suggesting antagonistic interaction (Andrews et al., 1998a).

A standard methanol dose-response line has been constructed, based on the developmental scoring system. Combinations with low formate concentrations (<1 mg/ml), along with various methanol concentrations, generally fell within the 95-% confidence interval of the standard methanol-curve (Andrews et al., 1998a).

2. Formate alone in whole-embry culture, given to the medium of 9-d rat embryos as well as to 8-d mice embryos at 0.2 - 3.0 mg/ml (for 24 or 48 h) exhibited trends towards reduction of growth and development and increases in the number of dead and abnormal embryos. Formate is embryotoxic at concentrations \geq 4fold lower than those for methanol (Ebron-McCoy et al., 1994)

Test substance: Methanol, no further data

14-AUG-2002

(264) (264) (265) (266)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

Endpoint: Behavioural Effects
Species: rat
Strain: Long-Evans **Sex:** male
Route of administration: oral, gavage
No. of animals: 11
Vehicle: water
Frequency of treatment: single dose
Doses: 1000, 2000, and 3000 mg/kg (50-% aqueous solution)
Control Group: other: test animals = control group (water) prior to treatment

Method: other: fixed wheel running ratio test

Year: 1993

GLP: no data

Test substance: as prescribed by 1.1 - 1.4

Result: The rats displayed no signs of overt intoxication such as gait disturbance, but a significant, dose-related reduction in FR20 response was observed at all dose-levels.

A NOAEL for behavioural changes cannot be derived.

Test condition: DOSE selection: 10, 20, and 30 % of the LD50.

TEST ANIMALS: The same animals were re-used for each dose on different days.

BEHAVIORIAL TESTS:
Ten minutes after treatment, the animals were subjected to the fixed wheel running ratio test to assess operant running. The test required the animal to run inside a wheel and rotate it under a fixed ratio of 20 times (FR20) in order to receive a food reward.

STATISTICS:
Data were evaluated by conducting repeated measures analysis of variance (ANOVA), determining linear trend, correcting for degrees of freedom, and performing analysis of residuals to outliers and skewed distribution.

Test substance: Methanol, HPLC grade, purity 99.93 %, Aldrich
Flag: Critical study for SIDS endpoint
29-APR-2004 (187) (267)

5.10 Exposure Experience

Type of experience: other: remark on selection of literature

Remark: Concerning effects in humans several quality reviews have already been conducted. With a focus on health and safety issues comprehensive reviews including those of the World Health Organization (WHO), 1997, Kavet and Nauss, 1990 and the Center for the Evaluation of Risks to Human Reproduction (CERHR), 1988 and single studies cited in these reviews were selected for this robust summary of methanol. Relevant recent publications have also been taken into account.
25-MAR-2002

Type of experience: other: review, general

Remark: Humans (and non-human primates) are uniquely sensitive to methanol poisoning and the toxic effects in these species is characterized by formic acidemia, metabolic acidosis, ocular toxicity, nervous system depression, blindness, coma and death. Nearly all of the available information on methanol toxicity in humans relates to the consequences of acute rather than chronic exposures. A vast majority of poisonings involving methanol have occurred from drinking adulterated beverages and from methanol-containing products. Although ingestion dominates as the most frequent route of poisoning,

inhalation of high concentrations of methanol vapor and percutaneous absorption of methanolic liquids are as effective as the oral route in producing acute toxic effects. The most noted health consequence of longer-term exposure to lower levels of methanol is a broad range of ocular effects.

The toxic properties of methanol are based on factors that govern both the conversion of methanol to formic acid and the subsequent metabolism of formate to carbon dioxide in the folate pathway. The toxicity is manifest if formate generation continues at a rate that exceeds its rate of metabolism.

The lethal dose of methanol for humans is not known for certain. The minimum lethal dose of methanol in the absence of medical treatment is between 0.3 and 1 g/kg. The minimum dose causing permanent visual defects is unknown.

The severity of the metabolic acidosis is variable and may not correlate well with the amount of methanol ingested. The wide interindividual variability of the toxic dose is a prominent feature in acute methanol poisoning.

Two important determinants of human susceptibility to methanol toxicity appear to be (1) concurrent ingestion of ethanol, which slows the entrance of methanol into the metabolic pathway, and (2) hepatic folate status, which governs the rate of formate detoxification.

The symptoms and signs of methanol poisoning, which may not appear until after an asymptomatic period of about 12 to 24 hours, include visual disturbances, nausea, abdominal and muscle pain, dizziness, weakness and disturbances of consciousness ranging from coma to clonic seizures. Visual disturbances generally develop between 12 and 48 h after methanol ingestion and range from mild photophobia and misty or blurred vision to markedly reduced visual acuity and complete blindness. In extreme cases death results. The principal clinical feature is severe metabolic acidosis of anion-gap type. The acidosis is largely attributed to the formic acid produced when methanol is metabolized.

The normal blood concentration of methanol from endogenous sources is less than 0.5 mg/litre (0.02 mmol/litre), but dietary sources may increase blood methanol levels. Generally, CNS effects appear above blood methanol levels of 200 mg/litre (6 mmol/litre), and fatalities have occurred in

untreated patients with initial methanol levels in the range of 1500-2000 mg/litre (47-62 mmol/litre).

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 1500 mg/m³ (1200 ppm) or more.

A widely used occupational exposure limit for methanol is 260 mg/m³ (200 ppm), which is designed to protect workers from any of the effects of methanol-induced formic acid metabolic acidosis and ocular and nervous system toxicity.

No other adverse effects of methanol have been reported in humans except minor skin and eye irritation at exposures well above 260 mg/m³ (200 ppm).

Reliability:

(2) valid with restrictions
see remark on selection of literature

Flag:

26-SEP-2002

Critical study for SIDS endpoint

(84)

Type of experience: other: review, general

Remark:

Methanol poisoning is an uncommon but an extremely hazardous intoxication. Since methanol is a versatile fuel and is having increasing usage in an energy-conscious society, a high index of suspicion and swift laboratory confirmation is essential in managing this poisoning. Methanol poisoning may occur in sporadic or epidemic circumstances. Chronic exposure may occur in the occupational setting. Man is uniquely susceptible to methanol toxicity, perhaps dependent upon folate metabolism. Classic symptoms of methanol toxicity can only occur in laboratory animals who are rendered folate deficient. Folate may be useful in humans enhancing removal of the toxic products of methanol poisoning. The enzyme responsible for metabolism of methanol is alcohol dehydrogenase. Ethanol has a higher affinity for this enzyme and is preferentially metabolized. Simultaneous ethanol and methanol administration may confuse the onset of the intoxication. Pyrazoles may also be used to inhibit alcohol dehydrogenase thus preventing the intoxication. The most important initial symptom of methanol poisoning is visual disturbance. The symptoms may be delayed up to 24 hours after ingestion due to simultaneous alcohol

administration and metabolic processes. Laboratory evidence of severe metabolic acidosis with increased anion and osmolar gaps strongly suggest the clinical diagnosis. There may be an important association between mean corpuscular volume which is significantly higher in cases of severe methanol poisoning than in mild cases. Once the diagnosis is suspected, a blood level from methanol should be returned rapidly. Treatment of methanol toxicity after good supportive care is to diminish the metabolic degradation of methanol with simultaneous ethanol and then to perform hemodialysis and alkalinization to counteract metabolic acidosis. Folate should be administered to enhance metabolic breakdown of formate. Alcoholic patients may especially susceptible to methanol poisoning due to relative folate deficiency.

Reliability: (2) valid with restrictions
see remark on selection of literature

Flag: Critical study for SIDS endpoint
26-SEP-2002 (268)

Type of experience: other: review, general

Remark: During methanol poisoning in man the concentration of formic acid in the blood is quite variable. In 5 lethal cases it ranged from 9 to 68 mg per cent. In three patients who also died it ranged from 5.7 to 19 mg per cent. Furthermore, the methanol concentration in the blood in 23 lethal cases varied between 51 and 274 mg per cent. It becomes obvious that the mere concentrations of these substances are not the only decisive factors in the clinical course of the poisoning.

Reliability: (2) valid with restrictions
see remark on selection of literature

Flag: Critical study for SIDS endpoint
26-SEP-2002 (269)

Type of experience: other: review, general

Remark: Methanol toxicity in humans and monkeys is characterized by a latent period of many hours followed by a metabolic acidosis and ocular toxicity. This is not observed in most lower animals. The metabolic acidosis and blindness is apparently due to formic acid accumulation in humans and monkeys, a feature not seen in lower animals. The accumulation of formate is due to a deficiency in formate metabolism which is, in turn, related, in part, to low hepatic tetrahydrofolate (H4folate). An excellent correlation

between hepatic H4folate and formate oxidation rates has been shown within and across species. Thus, humans and monkeys possess low hepatic H4folate levels, low rates of formate oxidation and accumulation of formate after methanol. Formate, itself, produces blindness in monkeys in the absence of metabolic acidosis. In addition to low hepatic H4folate concentrations, monkeys and humans also have low hepatic 10-formyl H4folate dehydrogenase levels, the enzyme which is the ultimate catalyst for conversion of formate to carbon dioxide.

Reliability: (2) valid with restrictions
see remark on selection of literature

Flag: Critical study for SIDS endpoint
26-SEP-2002 (270)

Type of experience: other: review, general

Remark: Methanol could become a major automotive fuel in the U.S., and its use may result in increased exposure of the public to methanol vapor. Nearly all of the available information on methanol toxicity in humans relates to the consequences of acute, rather than chronic, exposures. Acute methanol toxicity evaloves in a well-understood pattern and consists of an uncompensated metabolic acidosis with superimposed toxicity to the visual system. The toxic properties of methanol are rooted in the factors that govern both the conversion of methanol to formic acid and the subsequent metabolism of formate to carbon dioxide in the folate pathway. In short, the toxic syndrome sets in if formte generation continues at a rate that exceeds its rate of metabolism. Current evidence indicates that formate accumulation will not challenge the metabolic capacity of the folate pathway at the anticipated levels of exposure to automotive methanol vapor.

Reliability: (2) valid with restrictions
see remark on selection of literature

Flag: Critical study for SIDS endpoint
26-SEP-2002 (186)

Type of experience: other: review, general

Remark: Review of toxicity of methyl aclohol following skin absorption and inhalation in animal studies.

Reliability: (2) valid with restrictions
see remark on selection of literature

26-SEP-2002 (158)

Type of experience: other: review, general

Remark: Methyl alcohol is an aliphatic alcohol with use in a few cosmetic formulations as a solvent and denaturant. Concentrations up to 5% are typically used to denature ethyl

alcohol in cosmetic products. Methyl alcohol is readily absorbed through the skin and from the gastrointestinal and respiratory tracts, is distributed throughout all organs and tissues (in direct relation to the body's water distribution), and is eliminated primarily via the lungs. Undiluted Methyl alcohol is an ocular and skin irritant. Inhalation studies showed a no-effect level for maternal damage of 10,000 ppm and for teratogenic effects of 5,000 ppm. Overall, methyl alcohol is not considered mutagenic. Carcinogenicity data were unavailable. The toxicity of methyl alcohol in humans results from the metabolism of the alcohol to formate and formic acid through a formaldehyde intermediate. Formate accumulation causes metabolic acidosis and inhibits cellular respiration. Methyl alcohol toxicity is time and concentration dependent, and its toxic effect is competitively inhibited with ethyl alcohol. Because of the moderating effect of ethyl alcohol, it was concluded that methyl alcohol is safe as used to denature ethyl alcohol used in cosmetic products. No conclusion was reached regarding any other use of methyl alcohol.

Reliability: (2) valid with restrictions
see remark on selection of literature
04-MAR-2003 (271)

Type of experience: other: odour

Remark: Experimental study in two and seven further subjects for detection of odour thresholds in solvents. For methanol the odour threshold was 5,900 ppm and for detection of a distinct odour was 8,800 ppm.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (272)

Type of experience: other: odour

Remark: In a group of 25 subjects odour threshold was 3,4 ppm.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (273)

Type of experience: other: kinetics, oral

Remark: Methanol blood and urine concentrations were investigated in four volunteers who had ingested small amounts of methanol (0.2 ml hourly for six hours). The methanol urine concentration did not exceed 8.0 µg/ml. It was estimated, that at a MAC value of 200 ppm with a total 8 h ventilatory volume of 10 m³ and assuming complete absorption and no

exhalation 2.6 g methanol would be absorbed. The highest urinary concentration attained by oral ingestion of this amount of methanol at a rate of 0.5 ml hourly in one of the subjects was 17.6 µg/ml.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint
26-SEP-2002 (274)

Type of experience: other: kinetics, inhalation

Remark: Relationship between methanol concentration in the blood, urine, and breath of volunteers exposed to methanol vapor at 800 ppm for periods of 0.5, 1, 2, and 8 h. For methanol, concentrations are not proportional to the exposure duration due to metabolic and other elimination processes that occur concurrently with the exposure. The 0.5 to 2-h periods of exposure were used to estimate the half-life of methanol. Blood data gave a half-life of 1.44 ± 0.33 h. Comparable but slightly more variable results were obtained using urine data corrected for the voiding time (1.55 ± 0.67 h) and breath data corrected for mucous membrane desorption (1.40 ± 0.38 h). Methanol concentrations in blood lagged some 15-30 min. behind the termination of exposure, and concentrations in urine were further delayed.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
04-MAR-2003 (275)

Type of experience: other: kinetics, inhalation

Remark: Experimental study of dermal exposure to methanol in human volunteers estimating percutaneous absorption. 12 volunteers were exposed to methanol via one hand for durations of 0 to 16 min in a total of 65 sessions. The pre-exposure methanol conc. in blood was 1.7 mg/l, and subjects had statistically different mean conc. The maximum methanol conc. in blood was reached 1.9 h after exposure; this is comparable to that reached following inhalational exposure at a methanol conc. of 200 ppm. Delivery rate from skin into blood lagged exposure by 0.5 h, and methanol continued to enter the systemic circulation for 4 h following exposure. The mean derived absorption rate was 8.1 mg/cm²/h.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles
05-JUN-2002 (276)

Type of experience: other: kinetics, inhalation

Remark: Accumulation of formate, the putative toxic metabolic of methanol, in the blood methanol and the relationship between pulmonary intake and blood methanol conc. were investigated in six human volunteers following a 6-hr exposure to 200 ppm methanol. At the end of a 6-hr exposure to 200 ppm at rest, the blood methanol conc. was increased from a mean of 1.8 µg/ml to 7.0 µg/ml. Under light exercise, the total amount of methanol inhaled during the 6-hr exposure period was 1.8 times that inhaled at rest. However, no statistically significant increase in blood methanol conc. was observed under exercise: the conc. averaged 8.1 µg/ml. Formate did not accumulate in the blood above its background level following the 6-hr exposure to 200 ppm methanol whether subjects were exposed at rest or during exercise.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (191)

Type of experience: other: kinetics, inhalation

Remark: Five volunteers were exposed to constant and suitable graded concentration between 103 to 284 mg/m³ of methanol vapours for a period of 8 h. Pulmonary retention ranged from 53.4 to 61.3 %. Elimination half-life was calculated to range between 1.5 to 2.0 h.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint

26-SEP-2002 (277)

Type of experience: other: kinetics, inhalation

Remark: Urinary formic acid was studied in fourteen workers exposed to methanol with 10 +/- 5 years in their current occupation. Exposure to methanol vapor at 200 ppm methanol produced 80 mg formic acid/g creatinine.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principle

26-SEP-2002 (278)

Type of experience: other: kinetics, inhalation

Remark: Twenty persons occupationally exposed to methanol were examined according to their methanol levels in blood and urine and their formic acid excretion. An 8-h exposure to a methanol concentration of 93 ml/m³ in the air at the working

area caused average methanol levels in blood and urine of 8.9 +- 14.7 mg/l and 21.8 +- 20.0 mg/l, respectively, and a mean formic-acid level in urine of 29.9 +- 28.6 mg/l. Based on these results a rough estimate shows the corresponding methanol content in urine to be about 40 mg/l for an 8-h exposure at 200 ml/m³.

No correlation between exposure and urinary concentration of formate could be clearly demonstrated, although 15 % of measured values exceeded the norm

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint

04-MAR-2003 (279)

Type of experience: other: kinetics, inhalation

Remark: Four human subjects were exposed to approximately 200 ppm of methanol in a test chamber for six h. Ambient air in the chamber was monitored for methanol, while urine was monitored for formic acid. Mean urinary formic acid was increased from baseline at the end of the exposure session, but had returned to baseline in samples collected 16 h following cessation of exposure.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (280)

Type of experience: other: kinetics, inhalation

Remark: During a randomized double-blind study of the potential neurobehavioral effects of inhaled methanol at 200 ppm for 4 hours methanol analysis was performed. Methanol was rapidly absorbed by inhalation. Serum methanol conc. were increased by more than fourfold at the end of exposure period, as were urinary methanol excretion rates, although formate conc. were not increased over background conc. The overall elimination half-life was 3.2 + 2.3 h.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (281)

Type of experience: other: kinetics, inhalation

Remark: A study was carried out among 20 workers employed in a printing office at three different work places (methanol concentration: 85, 101, and 134 ppm) to determine whether concentration of formic acid in blood or urine and the methanol content of aveolar air permit the estimation of methanol exposure. The concentration of formic acid in blood

increased significantly from 3.2 +- 2.4 mg/ml before to 7.9 +- 3.2 mg/l after the shift in the exposed workers. In 36 non-exposed persons, the blood formate levels ranged from 0 - 20 mg/l.

The corresponding concentration in urine was increased significantly from 13.1 +- 3.9 mg/l to 20.2 +- 7 mg/l, respectively. On the contrary, in the control groups there was a small but significant decrease of formic acid concentration in blood from 5.6 +- 4.5 mg/l in the morning to 4.9 +- 4.2 mg/l in the afternoon.

Reliability:

(2) valid with restrictions

Flag:

acceptable study, meets basic scientific principles
Critical study for SIDS endpoint

04-MAR-2003

(282)

Type of experience:

other: kinetics, inhalation

Remark:

Exposure of 34 male workers to combined toluene, styrene and methanol was monitored by personal diffusive sampling of solvent vapours in breathing zone air, analysis of shift-end blood for the 3 solvents and analysis of shift-end urine for hippuric, mandelic and phenylglyoxylic acids and methanol. The exposure of most of the workers was below current occupational exposure limits. Regression analysis showed that a linear correlation exists for each of the 3 solvents between any pairs of the concentrations in air, blood and urine. Namely, toluene, styrene and methanol concentrations in blood obtained at the end of a shift are linearly related to the time-weighted average intensity of exposure to corresponding solvents, and also hippuric, mandelic and phenylglyoxylic acids as well as methanol in shift-end urine. The concentrations of hippuric, mandelic and phenylglyoxylic acids as well as methanol in urine correlated with the respiratory exposure intensity. Comparison of the present results with the exposure-excretion relationship after occupational exposure to the individual solvent showed that no modification in metabolism is induced by the combined exposure when exposure is low, as in the present case.

Reliability:

(2) valid with restrictions

15-DEC-2003

acceptable study, meets basic scientific principles

(283)

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- Remark:** Methanol absorption rate through human skin (forearm, 15-60 min.) has been examined and a value of 0.192 mg/cm²/min) was determined. The excretion of unchanged methanol with urine and exhaled air, after absorption through skin and administration "per os" amounted to 16 % and 22 % of the absorbed dose (1.67 g), respectively. It was estimated, that exposure of one hand to liquid methanol for only 2 min. would lead to the absorption of as much methanol (170 mg), as would be taken up by the lungs from an 8 h exposure to MAC of 50 mg/m³.
- Reliability:** (2) valid with restrictions
acceptable study, meets basic scientific principles
- Flag:** Critical study for SIDS endpoint
06-MAR-2003 (284)
- Remark:** Dermal exposures to methanol were administered in a clinical study designed to compare several biological indicators. Four subjects were exposed in five exposure sessions of varying length. In each session, a sequence of measurements of methanol conc. in blood, breath, and headspace samples of air at exposed and unexposed skin analysed. At exposed skin samples were highly elevated for at least 8 h following exposure, indicating the presence of a methanol reservoir in skin. After exposure, methanol conc. at exposed skin showed a rapid initial decline, then a slower first order decrease. When transfer was restricted, surface conc. at unexposed skin were similar to levels in breath and were strongly correlated to methanol conc. in blood.
- Reliability:** (2) valid with restrictions
basic data given, acceptable restrictions
06-MAR-2003 (285)
- Remark:** The elimination of methanol after doses of 2.5 to 7 .0 ml has been studied in five human subjects. At any time the rate of elimination was found to be proportional to the concentration of methanol in the body. Blood levels of 47 to 76 mg/l were measured 2 to 3 h after oral uptake of 71 - 84 mg methanol/kg bw (6.6 - 7.4 ml per person); methanol disappearance obeyed first-order kinetics with a half-time of about 3 h. Only a very small fraction of ingested methanol (about 2 %) was eliminated via the respiratory and urinary routes. The

rates of absorption of methanol by two human subjects during exposure to vapour concentrations of 400 - 1000 ppm have been investigated. Over short periods the amount of methanol absorbed appears to be approximately proportional to the duration of exposure and to the concentration of vapour in the atmosphere. It is concluded that accumulation in the body would occur at 3000 ppm and the maximum safe concentration for occupational exposure is 300 ppm.

Reliability:

(2) valid with restrictions
acceptable study, meets basic scientific principles
Critical study for SIDS endpoint

Flag:

06-MAR-2003

(286)

Remark:

Methanol is slowly eliminated from the body; hence, repeated exposures result in an increasing concentration in blood and tissue.

Reliability:

(2) valid with restrictions
basic data given, acceptable restrictions

06-MAR-2003

(287)

Remark:

In methanol-exposed male and female workers there was a linear relationship between methanol exposure and formate excretion in the urine after working shift up to an 8-h average of 2000 and 4000 ml/m³, respectively. In unexposed persons, the baseline urinary excretion of methanol and formate were 1.9 ±0.8 and 26.2 ±12.2 mg/l urine.

Based upon a regression equation presented by the authors, the mean added formate concentration in the urine amounted to about 180 mg/l (males) and 100 mg/l (females) after an 8-h average of 2000 ml/m³, and to 17 mg/l (males) and about 10 mg/l (females) after 200 ml/m³ (Yasugi et al. 1992).

The exposure-excretion relationship and possible health effects of exposure to methanol vapor were studied in 33 exposed workers during the second half of 2 working weeks. Urinary methanol concentrations were also determined in 91 nonexposed subjects (Kawai et al., 1991). The geometric mean value for methanol in urine samples from the latter was <2 mg/l. Among the exposed workers, the methanol level in urine samples collected prior to work shift exceeded the 95 % upper limit of normal. Methanol concentrations in urine at the end of shift showed a significant correlation between exposure to

methanol vapour at concentrations of up to 5,500 ppm and the level of methanol measured:
Following exposures to mean 1690 ml/m³, more than 100 mg/l methanol was found in the urine of 8 men, at an average exposure level of 550 ml/m³, 30 - 100 mg/l methanol were detected in 6 men. The highest 8-h exposures from 4000 ml/m³ and higher corresponded to 300 to 500 mg/l (Kawai et al., 1991).

The calculation indicated that a mean level of 42 mg methanol/l urine was excreted in the shift-end urine samples following 8 h exposure to methanol at 200 ppm.

Highly exposed workers (3500 - 5500 ml/m³) more often complained of blurred vision and headache during or after work. Nobody stated to suffer from photophobia. The examination of the eye fundus failed to reveal retinal changes. Among three workers employed in that company between 0.3 and 7.8 years and having been exposed to about 1000 - 1600 and 120 to 3600 mg/m³ (one case), two showed retarded pupil reflex and one mild mydriasis (Kawai et al. 1991). Dimmed vision and nasal irritation were among the most frequent symptoms complained during work.

Reliability:

(2) valid with restrictions
acceptable study, meets basic scientific principles
Critical study for SIDS endpoint

Flag:

06-MAR-2003

(288) (289)

Remark:

Five human subjects were exposed to an atmospheric concentration of 200 ppm of methanol in a test chamber for 7 h per day for 5 consecutive days. Ambient air in the chamber was monitored continuously for methanol, while urine was monitored for methanol and formic acid. Mean urinary methanol concentration were increased from baseline at the end of each exposure session, but returned to baseline in samples collected 16 h following cessation of exposure. The concentration of formic acid in morning urine specimens did not change significantly over the 7 days of the exposure.

Reliability:

(2) valid with restrictions
acceptable study, meets basic scientific principles
Critical study for SIDS endpoint

Flag:

06-MAR-2003

(290)

Remark: The aim of this study was to determine the reference values for methanol in urine of the general population non-occupationally exposed to the xenobiotic under study. Thus, urinary methanol was measured in 84 occupationally non-exposed subjects from the city of Sao Paulo, Brazil, by means of headspace gas chromatography with a flame ionization detector. The results revealed a mean of 2.26 +/- 1.26 mg methanol/l urine and a geometric mean of 2.10 mg/l for the studied population. The reference values varied within the range of 0.50-4.78 mg/l (mean +/- 2 S.D.). Methanol levels in urine did not differ statistically between male and female subjects. Urinary methanol in the total population was less than 4.80 mg/l in 95% of cases.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

06-MAR-2003 (291)

Remark: One hundred and forty-three workers exposed to one or more of toluene, xylene, ethylbenzene, styrene, n-hexane, and methanol at sub-occupational exposure limits were examined for the time-weighted average intensity of exposure by diffusive sampling, and for biological exposure indicators by means of analysis of shift-end blood for the solvent and analysis of shift-end urine for the corresponding metabolite(s). Urinalysis was also performed in 20 nonexposed control men to establish the "background level." Both solvent concentrations in blood and metabolite concentrations in urine correlated significantly with solvent concentrations in air. Comparison of blood analysis and urinalysis as regards sensitivity in identifying low solvent exposure showed that blood analysis is generally superior to urinalysis. It was also noted that estimation of exposure intensity on an individual basis is scarcely possible even with blood analysis. Solvent concentration in whole blood was the same as that in serum in the case of the aromatics, except for styrene. It was higher in blood than in serum in the case of n-hexane, and lower in the cases of styrene and methanol.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

06-MAR-2003 (292)

Type of experience: other: kinetics, model

Remark: Two patients with extremely high blood methanol concentrations (260 and 282 mg/dl) were successfully treated using pharmacokinetic dosing of ethanol, hemodialysis and

supportive measures. Both patients recovered completely without residual ophthalmologic deficits. Early hemodialysis and inhibition of methanol metabolism with effective ethanol concentrations were attributed to the patients' full recovery. Methanol elimination was enhanced by hemodialysis as evidenced by a decrease in half-life from eight to two and a half hours. Methanol dialysance was 98 ml/min. A dosage regimen for ethanol was devised, utilizing dose-dependent pharmacokinetic parameters and the ethanol dialysance (100 to 120 ml/min) from these two patients. An ethanol loading dose of 0.6 g/kg should be administered to an adult with an acute methanol ingestion. This dose will produce a blood ethanol concentration of approximately 100 mg/dl which can be maintained by an ethanol infusion of 66 mg/kg/hour for nondrinkers to 154 mg/kg/hour for chronic ethanol drinkers. Hemodialysis should be initiated if the blood methanol concentration is greater than 50 mg/dl. If hemodialysis is initiated, the ethanol infusion should be increased by 7.2 g/hour.

Reliability:

(2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002

(293)

Type of experience: other: kinetics, nutrition**Remark:**

Blood methanol concentrations were measured in 24 1-year-old infants administered aspartame, a dipeptide methyl ester sweetener. The doses studied included a dose projected to be the 99th percentile of daily ingestion for adults (34 mg/kg body weight), a very high use dose (50 mg/kg body weight) and a dose considered to be in the abuse range (100 mg/kg body weight). Blood methanol values in infants were compared to values observed previously in adults administered equivalent doses of aspartame. Methanol concentrations were below the level of detection (0.35 mg/dl) in the blood of 10 infants administered aspartame at 34 mg/kg body weight, but were significantly elevated (P less than or equal to 0.05) after ingestion of aspartame at 50 and 100 mg/kg body weight. At the latter doses, mean peak blood methanol concentrations and the area under the blood methanol concentration-time curve increased in proportion to dose. Mean (+/- SEM) peak blood methanol concentration was 0.30 +/- 0.10 mg/100 ml at a 50 mg/kg body weight aspartame dose (n = 6) and 1.02 +/- 0.28 mg/ml at the 100 mg/kg body weight dose (n = 8). Blood methanol values in infants were similar to those observed in normal adults.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

Flag: Critical study for SIDS endpoint
26-SEP-2002 (294)

Type of experience: other: kinetics, nutrition

Remark: Blood methanol concentrations were measured in 30 normal adult subjects administered aspartame, a dipeptide methyl ester. The doses studied included the 99th percentile of projected daily ingestion (34 mg/kg body weight) and three doses considered to be in the abuse range (100, 150, and 200 mg/kg body weight). Methanol concentrations were below the level of detection (0.4 mg/dl) in the blood of the 12 normal subjects who ingested aspartame at 34 mg/kg. They were significantly elevated (p less than or equal to 0 .001) after ingestion of each abuse dose, with the mean peak blood methanol concentrations and the areas under the blood methanol concentration-time curve increasing in proportion to dose. Mean (+/- SD) peak blood methanol concentrations were 1.27 +/- 0.48 mg/dl at the 100 mg/kg dose, 2.14 +/- 0.35 mg/dl at the 150 mg/kg dose, and 2.58 +/- 0.78 mg/dl at the 200 mg/kg dose. Blood methanol concentrations returned to predosing levels by 8 h after administration of the 100 mg/kg dose. Methanol was still detected in the blood 8 h after the subjects had ingested aspartame at 150 or 200 mg/kg. Blood formate analyses were carried out in the 6 subjects who ingested aspartame at 200 mg/kg, since recent studies indicate that the toxic effects of methanol are due to formate accumulation. No significant increase in blood formate concentrations over predosing concentrations was noted. No changes were noted in any of the blood chemistry profile parameters measured 24 h after aspartame ingestion, compared to values noted before administration. Similarly, no differences were noted in ophthalmologic examinations carried out before and after aspartame loading.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

Flag: Critical study for SIDS endpoint
26-SEP-2002 (295)

Type of experience: other: review, acute toxicity

Remark: The fatal dose in humans is between 2 and 8 ounces (1 ounce = 28,35g).

Reliability: (2) valid with restrictions
see remark on selection of literature
26-SEP-2002 (296)

Type of experience: other: review, acute toxicity

Remark: Most serious cases of methanol poisoning that have been reported (many fatal; others involved permanent or temporary loss of vision) resulted from the ingestion of methanol in the belief that it was ethanol. Ocular disturbance and blindness in humans resulting from repeated rubbing of the skin with methanol was also reported.

Reliability: (2) valid with restrictions
see remark on selection of literature

26-SEP-2002 (297)

Type of experience: other: review, acute toxicity

Remark: The signs and symptoms most characteristic of methanol poisoning are visual disturbance and metabolic acidosis. Permanent damage (residual scotomata) can result even if complete blindness is avoided.

Reliability: (2) valid with restrictions
see remark on selection of literature

26-SEP-2002 (298)

Type of experience: other: review, acute toxicity

Remark: Methanol poisoning with typical disturbed vision may appear in the occupational setting, by ingestion after drinking of methanol instead of ethanol and continuous inhalation when handling the hot product.

Reliability: (2) valid with restrictions
see remark on selection of literature

26-SEP-2002 (299)

Type of experience: other: review, acute toxicity

Remark: The minimal lethal dose of methanol in humans has not been determined. It has been suggested that about 1 g/kg can cause death if the patient is untreated and has not consumed ethanol. In some clinical cases, the blood methanol content is low in the last phase of the poisoning. In three such cases blood methanol concentration was 0.275, 0.277, and 0.194 g/l, respectively. On the assumption that the body water in diffusion equilibrium with the blood represents about 70 % of the body weight, it has been calculated that 0.19, 0.19, and 0.14 g/kg, respectively, was present in the body. Data on the rate of methanol oxidation in humans probably do not exist. Provided that the rate of methanol oxidation is the same in man as in rhesus monkeys, the amount of methanol oxidized during 18 hours (the average time needed for development of severe acidosis in clinical cases) would be 0.666 g/kg. It seems reasonable still to regard 1 g/kg of methanol as the approximate minimal lethal

Reliability: dose in man.
(2) valid with restrictions
see remark on selection of literature

Flag: Critical study for SIDS endpoint
15-DEC-2003 (300)

Type of experience: other: review, acute toxicity

Remark: Annual report of the American Association of Poison Control Centers National Data Collection System. 3 cases of fatal methanol ingestion were reported. Blood levels were 175, 402, and 39 mg/dl, respectively.

Reliability: (2) valid with restrictions
see remark on selection of literature

Flag: Critical study for SIDS endpoint
26-SEP-2002 (301)

Type of experience: other: review, acute toxicity

Remark: Methanol intoxication causes severe metabolic acidosis and can lead to permanent visual damage or death. Methanol, readily available in common products like antifreeze, is ingested accidentally or deliberately as a substitute for ethanol and in suicide attempts. Because it may become major fuel source in the 21st century and because industrial uses are expanding, deliberate and accidental intoxication is likely to increase. Rapid diagnosis is essential so that appropriate treatment can be instituted quickly. The authors review the pharmacology, clinical and laboratory findings, and pathology and pathophysiology of methanol intoxication. In addition, they discuss the differential diagnosis and treatment of acute intoxication, including the use of 4-methylpyrazole in preventing the conversion of methanol to formate.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (302)

Type of experience: other: review, acute toxicity

Remark: A 41-year-old man ingested orally a large quantity of methanol and was found dead at home. The presence of methanol in body fluids and tissues was determined by head-space gas chromatography. The blood ethanol and acetone were negative. Tissue distribution of methanol showed that the kidney presented the highest content of methanol (5.13 g/kg) followed by liver (4.18 g/kg), vitreous humor (3.96 g/l), heart (3.45 g/kg), urine (3.43 g/l), pericardial fluid

-
- (3.29 g/l), blood (2.84 g/l) and finally stomach content (2.21 g/l).
- Reliability:** (2) valid with restrictions
basic data given, acceptable restrictions
- 26-SEP-2002 (303)
- Type of experience:** other: review, acute toxicity
- Remark:** Methanol ingestion is an uncommon form of poisoning that can cause severe metabolic disturbances, blindness, permanent neurologic dysfunction and death. While methanol itself may be harmless, it is converted in vivo to the highly toxic formic acid. The diagnosis is sometimes elusive and requires a high index of suspicion. Because antidotal treatment is available it is important to recognize methanol poisoning promptly. The presence of metabolic acidosis associated with an increased anion gap and increased osmol gap are important laboratory findings. Specific therapeutic measures include correction of the metabolic acidosis with sodium bicarbonate and administration of enteral or parenteral ethanol to competitively inhibit the metabolic breakdown of methanol to formic acid. Hemodialysis accelerates the elimination of both methanol and formic acid and also assists in correction of the metabolic acidosis. Experimental data suggests that administration of folic acid may be of benefit by hastening the metabolism of formic acid to carbon dioxide. Prompt institution of specific therapy can probably decrease the morbidity and mortality associated with this form of poisoning.
- Reliability:** (2) valid with restrictions
basic data given, acceptable restrictions
- 26-SEP-2002 (304)
- Type of experience:** other: review, acute toxicity
- Remark:** Review of methanol toxicology incl. composition, use/sources, acute toxicity, chronic toxicity, target organ toxicity, absorption-metabolism-excretion, genotoxicity, neurotoxicity, reproductive toxicity, carcinogenicity, epidemiology, environmental fate, environmental toxicity, and regulatory status.
- Reliability:** (2) valid with restrictions
basic data given, acceptable restrictions
- 26-SEP-2002 (145)

Type of experience: other: review, acute toxicity

Remark: There have been about one hundred cases of amblyopia and death from inhalation of wood alcohol recorded in the literature to 1912.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002

(305)

Type of experience: other: acute toxicity

Remark: Eleven patients concomitantly poisoned with methanol are described. Their whole blood methanol conc. ranged from 137.2 mmol/l (4.39 g/l) to 7.4 mmol/l (0.24 g/l). The clinical course in most patients was mild, which was attributed to the concomitant and subsequent ethanol ingestion and rapid transport to dialysing units. One patient suffered permanent visual impairment of one eye, while the others recovered completely. Symptoms of poisoning were most clearly correlated to the degree of metabolic acidosis. All patients were dialysed. Ethanol in conc. even lower than usually recommended may be useful as the only treatment of patients with blood methanol conc. up to 15 mmol/l (0.5 g/l), provided there is no acidosis or visual impairment.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint

27-SEP-2002

(306)

Type of experience: other: acute toxicity

Remark: Case report: a 26-year-old woman ingested 250 to 500 ml methanol during the 38 th week of pregnancy. The initial serum methanol conc. was 230 mg/dl and formate was 33.6 mg/dl. A mild metabolic acidosis was present. Therapy included ethanol infusion, bicarbonate administration and three courses of hemodialysis. Delivery occurred six days after methanol exposure, when methanol was no longer detected in maternal blood. No further complications were noted in the mother and her newborn.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002

(307)

Type of experience: other: acute toxicity

Remark: Case report of a 38-year-old male developing Parkinson disease after methanol intoxication.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

16-APR-2002

(308)

Type of experience: other: acute toxicity

Remark: 64 cases of inhalation toxicity were reported. 6 of them died, 16 were permanently blind, 25 had permanent impairment of vision, and 8 were temporarily blind.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (309)

Type of experience: other: acute toxicity

Remark: Four cases of methanol poisoning were treated with alkali, ethyl alcohol, and the haemodialysis. In 3 cases respirator treatment was also required. Severe acidosis, failing eye-sight, and cerebral damage were present at the start of treatment within 29 to 44 hours of the methanol ingestion. Three patients, one of whom had consumed 90 g, died despite correction of the acidosis and elimination of methanol. Autopsy in these cases showed massive necrosis of the brain and haemorrhages into the putamina. The fourth patient, who had consumed 80 g methanol, survived but died 1 1/2 year later from pneumonia; autopsy showed slit-shaped cysts in the lateral parts of the putamina. All the patients had considerable hypopotassaemia.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint

26-SEP-2002 (310)

Type of experience: other: acute toxicity

Remark: Clinical and pathologic observations in a group of 320 patients with acute methanol poisoning after ingestion of bootleg whiskey containing 35 % methanoal and 15 % ethanol were reported. Amount ingested ranged from 30 ml to 260 ml methanol. There were 37 deaths. Visual symptoms, occurring eighteen to forty-eight hours after ingestion, included blurred vision, loss of central vision and complete blindness. Acidosis was present in every severely poisoned patient. Partial or complete recovery of the initially reduced visual acuity was observed in most patients.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (311)

Type of experience: other: acute toxicity

Remark: In four of six patients with methanol intoxication necrotic changes in the area of putamina were seen. Haemorrhages were also seen in some cases.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (312)

Type of experience: other: acute toxicity

Remark: 14 patients whose vision was affected by methanol intoxication were evaluated by a standard electroretinographic technique. Some increase in mean latencies and peak times appeared and the a- and b-waves amplitude to all parameters of stimuli were significantly below normal range. The changes in the ERG indicate damage to the outer retinal layers including visual cells. The usual ERG observed in methanol poisoning is unlike that found with degeneration of the optic nerve fibre or ganglion cell layer, and is also unlike that found when damage extends to the bipolar layer.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (313)

Type of experience: other: acute toxicity

Remark: A 54-year-old woman developed acute necrotizing pancreatitis following acute methanol poisoning. She died from multiple organ failure 54 hours after the ingestion. In a series of 22 consecutive patients admitted with a diagnosis of acute methanol poisoning, evidence of pancreatic damage was found in 11 patients.

Reliability: (2) valid with restrictions
basic data, acceptable restrictions
26-SEP-2002 (314)

Type of experience: other: acute toxicity

Remark: Occupational methanol poisoning has frequently caused death or blindness. Several cases resulted from work in confined spaces, e.g., varnishing beer vats. Headache and blurred vision were reportedly frequent symptoms. It is believed, that absorption of 8 grams would seriously affect the eyes and that such a dose could result from inhalation of 800 to 1000 ppm for 8 hours. Work room concentration of 500 to 600 ppm were found. It is recommended to keep the levels below 1 ppm.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (315)

Type of experience: other: acute toxicity

Remark: An outbreak of acute methyl alcohol intoxication occurred. Twenty-eight young men attended a drinking party and drank methyl alcohol. The amount consumed by each individual ranged from an equivalent of 60-600 ml of pure methanol. Three had prior ethanol ingestion. All 28 became ill 8-36 hours after drinking and were hospitalized. The most commonly observed clinical syndromes were: acute metabolic acidosis, severe visual impairment and acute pancreatitis. Four died within 72 hours after admission to the hospital. All had severe metabolic acidosis and visual impairment and three pancreatitis. Of 24 who recovered, 16 showed no residual complications, six had bi-lateral visual impairment and had difficulty in speech as well as visual impairment.

A three month follow-up examination showed no change in the findings. Coma, seizures and prolonged acidosis were poor prognostic signs. The estimated amount of consumed methanol and the rapidity of the appearance of signs of toxicity following methanol ingestion did not seem to influence the outcome of poisoning.

Reliability: (2) valid with restrictions

Flag: acceptable study, meets basic scientific principles

26-SEP-2002 Critical study for SIDS endpoint

(316)

Type of experience: other: acute toxicity

Remark: In a suicidal attempt by a 13-year old white girl, methanol (90 - 240 ml of an antifreeze containing 60 % methanol, 39.25 % potassium phosphate) produced classical immediate symptoms and permanent damage to the central nervous system characterized by severe bilateral optic atrophy, rigidity, spasticity, and hypokinesia.

Reliability: (2) valid with restrictions

26-SEP-2002 basic data given, acceptable restrictions

(317)

Type of experience: other: acute toxicity

Remark: In two case reports, the characteristic clinical, computertomograph-scan and neuropathological findings in acute methanol intoxication are described. The significance of acidosis for the survival is stressed. After a latency period of several days, one patient developed the rare condition of haemorrhagic leucocephalopathy with striking computertomograph-scan and morphologic findings.

Reliability: (2) valid with restrictions

26-SEP-2002 basic data given, acceptable restrictions

(318)

Type of experience: other: acute toxicity

Remark: A case is presented of a consultant supervising tank cleaning prior to methanol loading. He wore positive pressure breathing apparatus but no protective clothing. After 2-3 hours working in the confined space of the tank, he worked on deck and continued to wear his methanol-soaked clothing which eventually dried out. Visual symptoms of acute methanol toxicity presented some 8 hours after exposure. The appropriate treatment (with ethanol provided by the ship bond) was carried out in the hospital and the individual recovered completely.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (319)

Type of experience: other: acute toxicity

Remark: Case report: loss of vision after chronic inhalation and skin resorption of methanol.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (320)

Type of experience: other: acute toxicity

Remark: The main toxic effects of methanol is exerted upon the nervous system, particularly the optic nerves and possibly the retinae. The condition can progress to permanent blindness. Once absorbed methanol is only very slowly eliminated. Coma resulting from massive exposures may last as long as 2-4 days. In the body, the products formed by its oxidation are formaldehyde and formic acid, both of which are toxic. Because of the slow elimination, methanol should be regarded as cumulative poison. Though single exposures to fumes may cause no harmful effect, daily exposure may result in the accumulation of sufficient methanol in the body to cause illness. Death from ingestion of less than 30 ml has been reported. Human systemic effects: changes in circulation, cough, dyspnea, headache, lacrimation, nausea or vomiting, optic nerve neuropathy, respiratory effects, visual field changes.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (321)

Type of experience: other: acute toxicity

Remark: Cardinal features in teh mechanism, pathology, symptomatology and therapy of methyl alcohol poisoning are reviewed in the light of a series of 23 cases. The first five of these patients died of respiratory arrest after several hours of coma. They were sporadic cases who had ingested relatively high amounts of both ethyl and methyl alcohol. The sixth case was brought to the hospital in profound shock and died within four hours. The remaining 17 cases were all victims of the same batch of liquor that had killed the six patient, and were treated with alkalis. None died or sustained permanent injury. The two basic mechanisms of poisoning are direct chemical irritation of tissues and systemic acidosis. The former is influenced by the unlimited miscibility of methanol with water and the distribution of water in the tissues of the body. Acidosis is the most important therapeutic consideration. Alkalinization should be prompt and vigorous. It is suggested that ketosis may play a role in acidosis and depression, and that intravenous glucose may be an important adjunct to treatment.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions (322)
26-SEP-2002

Type of experience: other: acute toxicity

Remark: A series of 49 cases of acute methyl alcohol poisoning with recovery is reported (no demonstrable eye lesion after five months). Gastic lavage was not used in any case, because it is of questionable value when poisoning has occurred. Plasma extenders were found valuable in combating peripheral vascular failure. Frequent drainage of the cerebrospinal fluid does not appear essential. The occurrence of hypototassaemia during management was definitely established.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions (323)
26-SEP-2002

Type of experience: other: acute toxicity

Remark: The clincial course of 4 men who drank commercial duplicating fluid containing 40 % to 60 % methyl alcohol is presented. One of the patients died (ingestion of about 12 ounces), and the necropsy findings were severe, acute passive congestion of the lungs, kidneys, spleen, liver and brain, as well as congestion with petechial hemorrhages of

-
- the stomach and segments of the jejunum. Histologic examination was compatible with the cross findings. The other men made a complete recovery.
- Reliability:** (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (324)
- Type of experience:** other: acute toxicity
- Remark:** Report of a common-source epidemic of methanol poisoning which involved 18 people, of whom eight died. Of the 18 known cases, 11 were treated with only one death despite several patients with methanol concentrations of 200 mg or more per 100 ml blood. Among different types of treatment used peritoneal dialysis was shown to be effective, with a clearance rate five to ten times that obtained with forced fluid diuresis.
- Reliability:** (2) valid with restrictions
acceptable study, meets basic scientific principles
04-MAR-2003 (325)
- Type of experience:** other: acute toxicity
- Remark:** Case report of a intoxication in a 55-year-old woman after administration of methanol-soaked patches. Initial symptoms were blurred vision, decrease of vision, and neuritis of the optic nerve. Decrease of vision was permanent.
- Reliability:** (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (326)
- Type of experience:** other: acute toxicity
- Remark:** Report of a child who died after methanol intoxication, presumably due to percutaneous resorption. Methanol soaked pads had been applied to the child's chest for approx. twice 12 hours.
- Reliability:** (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (327)
- Type of experience:** other: acute toxicity
- Remark:** Case report of fuzzy and heavy feeling in the head and tendency to sway in a worker engaged in antimold spraying to mildewproof a living room. The antimold agent was diluted with methanol or ethanol. Since the work generally continues for several days, signs and symptoms had persisted. However, within 10 days of completing the work, these signs any

symptoms completely disappeared. Examination of the urine disclosed that methanol in urine was in the normal range ten days after discontinuation of the work.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (328)

Type of experience: other: acute toxicity

Remark: Case report on a 50-year-old man with methanol conc. of 5 mg/dl after ingestion of alcoholic beverages. The ethanol level was 275 mg/dl.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
05-JUN-2002 (329)

Type of experience: other: acute toxicity

Remark: Methanol poisoning accounting for several deaths were investigated in an Ontario registry. Between 1986 to 1991 43 fatalities were found. Suicide attempts account for 21 cases while 22 were classified as accidental. 14 of these consumed products labelled as methanol or wood alcohol as a substitute for ethanol. 3 cases ingested methanol being improperly stored in containers normally associated with ethanol, and 5 patients were poisoned through consumption of liquor from illicit sources.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (330)

Type of experience: other: acute toxicity

Remark: A patient poisoned with methanol was successfully hemodialyzed with an ethanol-enriched, bicarbonate-based dialysate. Along with a concomitant intravenous infusion of ethanol, the ethanol-enriched dialytic procedure was able to maintain an intradialytic plasma ethanol level of 80 to 102 mg/dl. The patient recovered without any sequelae of methanol intoxication.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (331)

Type of experience: other: acute toxicity

Remark: Observations in 323 patients who had ingested bootleg whiskey containing 35-40 % methanol and 4 % ethanol were reported. 41 deaths occurred. 115 patients showed acidosis and visual disturbances when first seen in the hospital.

Lowest letal dose was 6 ml methanol and highest dose survival was 200 ml methanol.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint
26-SEP-2002 (332)

Type of experience: other: acute toxicity

Remark: 21 cases of percutaneous methanol intoxication in children were reported. 12 of these children died. The children's cloths soaked in methanol were applied overnight and during the day with a rate of one to three applications to each child. Th elapsed time between application and onset of symptoms varied from 1 to 13 h and the time till diagnosis varied from 4 to 48 h. The early manifestations were centralnervous system depression and in some cases hyperpnea and in some cases severe respiratory depression.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint
26-SEP-2002 (333)

Type of experience: other: acute toxicity

Remark: Report of 54 cases of death and blindness from cilombian spirits and other methylated preparations. As to the general effect: headache was mentioned in 19 cases as a conspicuous symptom; gastric pain in 11; nausea and vomiting in 26; dilated pupils in 20. the results as to visual disturbance were 16 total blindness, 3 total blindness of one eye, 15 partial recoveries, 7 recoveries; 10 remaing cases teminted fatally; sight became dim in three hours in one case, six hours in 1 case; twelve hours in 2 cases; twenty hours in 1 case; twenty-four hours in 19 cases; forty-eight hours in 5 cases; three days in 2 cases; six days in 1 case; seven days in 1 case; sight became lost in twelve hours in 2 cases, in twenty-four hours in 10 cases; in thrity hours in 2 cases; in forty-eight hours in 3 cases; in three days in 3 cases; in four days in 3 cases; in five days in 2 cases; in six days in 1 case; in seventeen days in 1 case. Of the remaining 21 facts in this connection are not definite.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint
26-SEP-2002 (334) (335)

Type of experience: other: acute toxicity

Remark: A health hazard evaluation was conducted by the National Institute for Occupational Safety and Health (NIOSH) to determine if vapors from duplicating fluid (99% methyl alcohol) used in direct-process spirit duplicating machines were causing adverse health effects among teacher aides, or had been responsible for the deaths of three former teacher aides. Death certificates and autopsy data were obtained and evaluated. A self-administered symptom questionnaire was distributed to current teacher aides (exposed group) and to a comparison group of teachers. Fifteen-minute breathing zone air samples for methyl alcohol vapor were collected at operator stations using an infrared gas analyzer. No information supported the claim that the three deaths were related to methyl alcohol exposure. Teacher aides reported significantly more blurred vision, headache, dizziness, and nausea than the comparison group. Concentrations of airborne methyl alcohol ranged from 365-3080 ppm; 15 of 21 measurements exceeded the NIOSH-recommended 15-minute exposure limit of 800 ppm. A mean 96% reduction in vapor concentration was accomplished using inexpensive enclosures and existing room exhaust systems.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

Flag: Critical study for SIDS endpoint

04-MAR-2003

(336)

Type of experience: other: acute toxicity

Remark: Significant toxicity can result from intentional methanol inhalation. We report seven cases, involving four patients, of intentional inhalation of CARB-MEDIC carburetor cleaner containing toluene (43.8%), methanol (23.2%), methylene chloride (20.5%), and propane (12.5%). Patients arrived at the emergency department with central nervous system depression, nausea, vomiting, shortness of breath, photophobia, and/or decreased visual acuity. Treatment included correction of acidosis, leucovorin and/or folic acid, ethanol infusions, and supportive care. Hemodialysis was necessary in three cases. Measured blood methanol levels ranged from 50.4 to 128.6 mg/dL. Blood formic acid levels were 120, 193, and 480 micrograms/mL, respectively, in three patients. Ophthalmic examinations revealed hyperemic discs and decreased visual acuity in one patient. One individual was found pulseless with several CARB-MEDIC cans nearby. Attempts at revival were unsuccessful.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002

(337)

Type of experience: other: acute toxicity

Remark: In a large epidemic it was demonstrated that patients acutely intoxicated with methanol can be triaged effectively on the basis of bloodmethanol level, serum bicarbonate concentration, and assessment of specific clinical complications. Additional findings such as elevated MCV and hematocrit may prove useful as markers for nonspecific toxic swelling of cells. Ehtanol should be included in the treatment of all suspected cases, using doses that will maintain a blood ethanol level of 100 mg/dl until methanol levels fall to the range of 20 mg/dl.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint

26-SEP-2002

(338)

Type of experience: other: acute toxicity

Remark: A 46-year-old male presented to the emergency room seven hours after consuming a large container of sterno. He could not see and complained of abdominal and back pain. He was tachypneic, tachycardic, hypertensive and hypothermic. Laboratory results were significant for a severe metabolic acidosis, a serum osmolality of 465 and serum methanol level of 493 mg/dl. Aggressive treatment included ethanol drip, bicarbonate and hemodialysis. He survived and regained his eyesight in spite of this degree of elevation of the serum methanol level.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint

26-SEP-2002

(339)

Type of experience: other: acute toxicity

Remark: A 31-year-old previously healthy man presented with a 7-day history of nausea, intractable vomiting, diffuse abdominal pain, and progressive blurred and tunnel vision. The patient had visual disturbances and an osmolal gap that suggested methanol intoxication.

Reliability: (2) valid with restrictions
basic data given, accpetable restrictions

26-SEP-2002

(340)

Type of experience: other: acute toxicity

Remark: A 27-year-old man admitted to the medical intensive care unit. A coworker found him standing in the bathroom, unresponsive and rigid. The patient received standard emergency department treatment for the unconscious patient with suspected methanol poisoning with negative results. The patient had seizure activities with dilated pupils, clenched jaw, and tonic muscle activity. Deep tendon reflexes were absent symmetrically. Neurological assessment showed a severely depressed level of consciousness. Initial blood levels showed a methanol concentration of 234 mg/dl and an ethanol level of 74 mg/dl.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (341)

Type of experience: other: acute toxicity

Remark: A 44-year-old man was found unconscious beneath an elevated rapid transit right-of-way. On admission to the emergency room, the patient was comatose in metabolic acidosis with high anion and osmolal gaps. The serum methanol was 583 mg/dL. The serum ethanol and ethylene glycol were negative. The patient was treated with ethanol, bicarbonate, and hemodialysis. He expired 40 h after admission. The postmortem methanol concentrations in body fluids were as follows: bile 175 mg/dL, vitreous humor 173 mg/dL, and blood 142 mg/dL. Urine was not available for analysis. Postmortem methanol concentrations in body tissues are given in decreasing order: brain 159 mg/100 g, kidney 130 mg/100 g, lung 127 mg/100 g, spleen 125 mg/100 g, skeletal muscle 112 mg/100 g, pancreas 109 mg/100 g, liver 107 mg/100 g, and heart 93 mg/100 g. The total amount of methanol in the gastric contents was 73 mg.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint
26-SEP-2002 (342)

Type of experience: other: acute toxicity

Remark: Methyl ethyl ketone is a common solvent but data on overdose in humans are scarce. We report a case of co-ingestion of methyl ethyl ketone together with methanol associated with a hyperosmolar coma without anion gap metabolic acidosis. Blood levels of methyl ethyl ketone and its metabolite, 2-butanol, indicated that this solvent did contribute

approximately 20 mosm/L to an observed osmolar gap of 99 mosm/L. At the levels detected, methyl ethyl ketone may have inhibited methanol metabolism, contributing to the low serum formate (1.3 mmol/L) and normal anion gap despite a blood methanol of 67 mmol/L.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (343)

Type of experience: other: acute toxicity

Remark: In 10 adult patients acutely intoxicated with methanol, the base deficit and the total blood CO₂ were highly correlated with blood formate level. No correlation was found between the same parameters and blood methanol level. Formate production is the main cause of acidosis in the early stages of methanol poisoning. The association of a latency period before treatment exceeding 10 hours and a blood formate level above 0.5 g/l (11.1 mmol/l) is predictive of severe methanol poisoning possibly leading to permanent sequelae.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (344)

Type of experience: other: acute toxicity, cns

Remark: Subarachnoid hemorrhage developed in a patient intoxicated with methanol. Computed tomography performed at the time of admission suggested this complication. The hemorrhage was definite and extensive by the 5th day after admission and was accompanied by left caudate and pontine hemorrhage, as well as severe cerebral edema.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (345)

Type of experience: other: acute toxicity, cns

Remark: Bilateral putaminal necrosis is characteristic of methanol poisoning. A 31-year-old male alcoholic had headache, impaired consciousness, neck stiffness, roving eyes with dilated unreactive pupils, papilloedema, abdominal pain, vomiting, and severe metabolic acidosis after a binge. Abnormalities of the cerebrospinal fluid included an initial pressure of 240 mmH₂O, RBC 286/mm³, WBC 8/mm³, and protein 179 mg/dl. Peritoneal dialysis was performed on the 2nd day after drinking. A blood test for methanol was not performed until the 5th day, and its results was negative. However, computed tomography (CT) on the 3rd day showed necrosis and

hemorrhage of bilateral putamina and the cerebral cortex, and post-contrast enhancement of meninges. On the 22nd day, a CT revealed further changes: necrosis of bilateral subcortical white matter, and post-contrast gyral enhancement at the otherwise normal-looking areas of the cerebral cortex.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (346)

Type of experience: other: acute toxicity, cns

Remark: A 37-year-old man with a history of alcohol abuse was brought to the emergency department 1 day after having consumed approximately one half gallon of windshield washer fluid. The patient was found to be unresponsive, with shallow respirations. Toxicological screening revealed a blood methanol level of 331 mg/dl (103 mmol/l) and an ethanol level of 0 mg/dl. MR imaging showed necrosis of the putamen.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (347)

Type of experience: other: acute toxicity, cns

Remark: The ocular manifestation of acute methyl alcohol intoxication were studied in 24 men during an outbreak. The visual acuity, pupillary reactions to light, fundal appearance and visual fields were recorded in all patients within 72 hours of ingestion of methanol and again three months later. Nine patients had no ocular abnormality, 7 had only transient ocular abnormalities, and eight had permanent ocular abnormalities. Complete blindness occurred in two patients, while severe visual deficit resulted in four others. The incidence of permanent ocular abnormalities was found to correlate with the incidence of metabolic acidosis, and with the stated volume of methanol consumed. An inverse correlation was found between stated volume of methanol consumed and onset of blurred vision.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles
Flag: Critical study for SIDS endpoint
26-SEP-2002 (348)

Type of experience: other: acute toxicity, cns

Remark: In two survivors of severe methanol poisoning apart from blindness Parkinsonian-like extra-pyramidal syndrome characterized by poor voice volume, masked face, mild tremor, rigidity, bradykinesia, hyperreflexia, bilateral

Babinski responses, and mild dementia were reported. CT scan in both patients demonstrated symmetrical low density lesion in the fronto-central white matter and putamen bilaterally. EMG showed extensive denervation, mainly involving the legs, but normal motor conduction velocities. Autopsy in one patient who died from suicide 11 months following poisoning revealed cystic resorption of the putamen and the peripheral white matter in addition to widespread neuronal damage throughout cerebrum, cerebellum, brainstem, and spinal cord.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions (349)
26-SEP-2002

Type of experience: other: acute toxicity, cns

Remark: Five optic nerves from three cases of methanol intoxication were examined. The patients died in cardiorespiratory failure thirty hours, two days, and eighteen days, respectively, after presentation with acute bilateral visual loss. The retrolaminary nerve segments showed circumscribed demyelination of loss of myelin staining with relative preservation of axons. Retinal ganglion cells were spared. Electromicroscopy in the case with shortest survival revealed periaxonal intramyelinic spaces behind the lamina cribosa.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions (350)
26-SEP-2002

Type of experience: other: acute toxicity, cns

Remark: The histopathologic effects of methanol on the optic nerve were studied in four patients. Circumscribed myelin damage occurred behind the lamina cribrosa in each nerve. Axons were preserved. Demyelination also occurred in cerebral hemispheric white matter in one patient. This selective myelinoclastic effect of methanol metabolism is probably caused by histotoxic anoxia in watershed areas of the cerebral and distal optic nerve circulations. Juxtalbar demyelination may cause optic disk edema in methanol poisoning by compressive obstruction of orthograde axoplasmic flow. Visual loss may be due to disruption of saltatory conduction. Retrolaminar demyelinating optic neuropathy is an early morphologic correlate of visual loss in methanol intoxication.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (351)

Type of experience: other: acute toxicity, cns

Remark: Case report of a 40-year-old woman with putaminal necrosis after methanol intoxication.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (352)

Type of experience: other: acute toxicity, cns

Remark: Case report of a 62-year-old plumber with putamen necrosis after severe methanol intoxication.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (353)

Type of experience: other: acute toxicity, cns

Remark: Case report of a case of acute methanol intoxication with central nervous system damage producing a Parkinson syndrome with persistent sequel.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (354)

Type of experience: other: acute toxicity, CNS (Chuwers)

Remark: This study assessed whether acute exposure to methanol vapor at the current industrial threshold limit value of 200 ppm for 4 hr has adverse effects on human neurobehavioral performance. Twenty-six healthy subjects (15 men, 11 women; ages 26-51 years) were exposed to methanol or water vapor for 4 hr while seated in a chamber. The subjects served as their own controls in a randomized, double-blind study design. The variables assessed were serum and urine methanol and formate levels; visual performance (color discrimination and contrast sensitivity); and neurophysiological (auditory evoked potentials) and neurobehavioral performances. Exposure to methanol increased serum concentrations and urinary excretions of methanol, but did not affect formate levels. Overall visual, neurophysiological, and neurobehavioral test outcomes were not significantly affected, unless certain between-subject variables are considered. Slight effects on P-300 amplitude and Symbol Digit testing were noted.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

Flag: Critical study for SIDS endpoint
29-APR-2004 (355)

Type of experience: other: acute toxicity, cns

Remark: Report of with areas of decreased attenuation symmetrically localised, especially in the putamen in the computerised tomography in a 62-year-old plumber after intake of methanol-containing home-made liquor.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (356)

Type of experience: other: acute toxicity, CNS (Muttray)

Remark: Twelve healthy subjects were exposed for 4 h to 200 ppm and to 20 ppm (control) in an exposure chamber in a cross-over design. The EEG was recorded before (reference) and at the end of each exposure with, the subject's eyes closed and opened and during a choice reaction test (color word stress test). Spectral power was calculated by fast Fourier transformation. Subjective symptoms and effects of blinding with 20 ppm methanol were assessed by questionnaires. The study was a single-blind one. During subjects' exposure to 200 ppm, their scores for prenarcoctic and irritating symptoms were not different from controls. In the closed-eye condition of subjects, the spectral power of the theta-band and of some electrodes of the delta-band was significantly less at the end of exposure to 200 ppm, than that of controls. In the open-eye condition and during the color word stress test no significant changes were found. The changes in the theta-band suggest a slight excitatory effect of 200 ppm methanol. The effect was weak, as scores of acute symptoms did not change.

Reliability: (2) valid with restrictions
acceptable study meets basic scientific principles

Flag: Critical study for SIDS endpoint
29-APR-2004 (357)

Type of experience: other: review, therapy

Remark: Review on the roles of alkaline salts and ethyl alcohol in the treatment of methanol poisoning.

Reliability: (2) valid with restrictions
see remark on selection of literature
26-SEP-2002 (358)

Type of experience: other: review, therapy

Remark: Treatment of methanol poisoning is well established and consists specifically of the administration of alkali and ethanol, and hemodialysis. Since methanol poisoning often occur as accidents with a lot of patients involved, it is not always possible to follow sophisticated protocols for treatment. If the patients are awake with no severe hyperventilation or acidosis, ethanol administration is most important because it is widely available and can be given orally. Under such circumstances every person suspected of having consumed some of the suspect liquid should be given ethanol orally or intravenously aiming at a blood concentration of about 22 mmol/l. In severely poisoned patients in coma with pronounced acidosis and hyperventilation it is vital to correct the acidosis as soon as possible. Before any laboratory analyses are available, at least 200 to 300 mmol of bicarbonate should be infused intravenously, followed by ethanol infusion. If methods of determination of methanol or formate are not available, the magnitude of the osmolal and anion gaps along with the clinical judgement should provide the basis for treatment.

Reliability: (2) valid with restrictions
see remark on selection of literature

26-SEP-2002

(359)

Type of experience: other: review, therapy

Remark: Twelve cases of methanol poisoning are reviewed. The clinical presentation and biochemical features are described and the results of treatment with alkali, ethanol and dialysis reported. The outcome of methanol poisoning appears to be related more to the interval between the time of ingestion and the start of therapy and to the degree of acidosis than to the initial serum methanol level. Therefore, early and aggressive treatment with bicarbonate and ethanol and subsequent institution of hemodialysis are strongly recommended whenever methanol can be detected in the blood, especially when metabolic acidosis of the anion-gap type is present, when mental or visual disturbances are present, or when more than 30 ml of absolute methanol has been consumed.

Reliability: (2) valid with restrictions
see remark on selection of literature

26-SEP-2002

(360)

Type of experience: other: review, therapy

Remark: A unique opportunity presented itself for reviewing and comparing the results of two different methods of dialytic therapy for the treatment of methanol poisoning. The group that was treated with hemodialysis initially had a faster fall in serum methanol level, regained consciousness faster, had a shorter hospital stay, and most importantly, had no residual effects when compared with the group treated with peritoneal dialysis. In this group, one patient died and another was permanently blinded.

Reliability: (2) valid with restrictions
see remark on selection of literature

26-SEP-2002

(361)

Type of experience: other: therapy

Remark: A case report of severe methanol poisoning is reported which required 21 hours of hemodialysis to bring their serum methanol levels down to a nontoxic level.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002

(362)

Type of experience: other: therapy

Remark: Nine patients with methyl alcohol poisoning who were treated with hemodialysis were described. The time from ingestion to dialysis varied from 4 to 100 hours. Predialysis blood methanol levels ranged from 3 to 570 mg/dl. All patients were acidotic and had an increased anion gap. Two patients died, seven recovered, but three had permanent visual impairment. There was little correlation between the blood methanol level or anion gap and visual outcome. The interval from ingestion to treatment appears to be more important than the initial biochemical status. Prompt hemodialysis is recommended, if the blood methanol level is above 50 mg/dl, when an amount of methanol exceeding the minimal lethal dose (30 ml) is known to have been ingested, when there is evidence of acidosis or when an abnormality has developed in vision, funduscopic examination or mental state. Concurrent therapy with alkali and ethanol is vital.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint

26-SEP-2002

(363)

Type of experience: other: therapy

Remark: The diagnostic value of determination of the anion and osmolal gaps was studied in 6 patients poisoned with methanol and in 5 poisoned with ethylene glycol. Increased osmolal gap was present on admission in all patients, whereas increased anion gap was present in all except one. In the methanol-poisoned patients the mean blood values were: pH 7.27, anion gap 24 mmol/l, osmolal gap 81 mosmol/kg H₂O, methanol 67 mmol/l, ethanol 11 mmol/l and in the ethylene glycol-poisoned patients: pH 6.93, anion gap 38 mmol/l, osmolal gap 35 mosmol/kg H₂O and ethylene glycol 24 mmol/l. In the absence of alcoholic acidosis or diabetic coma the finding of a simultaneous increase in both the anion and osmolal gaps indicates methanol or ethylene glycol poisoning. Thus determinations of the anion and osmolal gaps are mandatory whenever facing metabolic acidosis of unknown etiology.

Reliability: (2) valid with restrictions
acceptable study meets basic scientific principles

26-SEP-2002

(364)

Type of experience: other: therapy

Remark: 4-Methylpyrazole (4-MP), an inhibitor of alcohol dehydrogenase, may be useful for the treatment of methanol and ethylene glycol intoxications. A placebo-controlled, double blind, multiple dose, sequential, ascending-dose study has been performed to determine the tolerance of 4-MP in healthy volunteers. Oral loading doses of 4-MP were followed by supplemental doses every 12 h through 5 days, producing plasma levels in the therapeutic range. A slight, transient elevation in one or both serum transaminase values was observed in 6 of the 15 subjects treated with 4-MP. This effect was not dose related nor apparently mediated through a hypersensitivity reaction. Serum triglyceride levels were increased in 30% of 4-MP treated subjects, but also in 25% of the placebo subjects. 4-MP treatment did not produce any other significant changes in objective clinical parameters nor in subjective side effects. The results suggest that a mild, transient increase in liver function tests might be observed in some subjects treated with multiple doses of 4-MP. Nevertheless, the slower elimination rate and lesser degree of toxicity of 4-MP would make it preferable to ethanol in therapy of these poisonings.

Reliability: (2) valid with restrictions

26-SEP-2002 basic data given, acceptable restrictions (365)

Type of experience: other: therapy

Remark: 1. Osmolal and anion gaps are helpful in the diagnosis and evaluation of intoxications with methanol and ethylene glycol. Reported reference values for osmolal gap and anion gap are $-1 (+/- 6)$ mosm kg^{-1} H_2O and $16 (+/- 2)$ mmol l^{-1} , respectively. However, we have repeatedly found unexplained increased gaps in patients admitted to our department, and the relevance of the established reference values has been questioned. 2. Osmolal and anion gaps were determined in an unselected population of patients consecutively admitted to an emergency medical department. In the case of unexplained gaps, the blood samples were analysed with respect to the presence of alcohols and organic acids. 3. We included all accessible patients admitted during 14 days. Appropriate blood samples were obtained in 177 patients (88 male, 89 female), with a mean age of 65 years (range 17-94). 4. The mean and (standard deviation) for osmolal and anion gaps in our material were 5.2 mosm kg^{-1} H_2O (7.0) and 12.9 mmol/l (4.2). Neither methanol nor ethylene-glycol was detected in serum from any patients. Small amounts of ethanol were found in 5 patients, and high lactate levels explained in part the most extensively increased anion gaps. However, the calculated analytical standard deviation accounted entirely for the variation in the material.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (366)

Type of experience: other: therapy

Remark: A patient intentionally ingested an unknown amount of methanol and was admitted to the hospital 6 h later. On admission, the methanol concentration in blood was estimated as approximately of 134 mmol/L, based on the calculation of the osmolal gap. Intravenous ethanol administration and hemodialysis were promptly started. During hemodialysis, several blood samples were collected for determination of methanol and ethanol concentrations. Initially, we used gas chromatography with split-mode injection of pretreated serum samples; however, methanol concentrations turned out to be significantly lower than expected, based on calculated osmolal gap values. Because no explanation for the excess serum osmolal gap was apparent, we reanalyzed samples, using head-space gas chromatography. The methanol concentrations

measured were significantly higher and osmolal gap values were no longer excessive.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (367)

Type of experience: other: therapy

Remark: 4-Methylpyrazole (4-MP), an inhibitor of alcohol dehydrogenase, may be useful for the treatment of methanol and ethylene glycol intoxications. A placebo-controlled, double blind, multiple dose, sequential, ascending-dose study has been performed to determine the tolerance of 4-MP in healthy volunteers. Oral loading doses of 4-MP were followed by supplemental doses every 12 h through 5 days, producing plasma levels in the therapeutic range. A slight, transient elevation in one or both serum transaminase values was observed in 6 of the 15 subjects treated with 4-MP. This effect was not dose related nor apparently mediated through a hypersensitivity reaction. Serum triglyceride levels were increased in 30% of 4-MP treated subjects, but also in 25% of the placebo subjects. 4-MP treatment did not produce any other significant changes in objective clinical parameters nor in subjective side effects. The results suggest that a mild, transient increase in liver function tests might be observed in some subjects treated with multiple doses of 4-MP. Nevertheless, the slower elimination rate and lesser degree of toxicity of 4-MP would make it preferable to ethanol in therapy of these poisonings.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (365)

Type of experience: other: irritation

Remark: In an experimental study, 12 volunteers were exposed in an inhalation chamber to 20 and 200 ppm methanol. At 200 ppm (20 ppm) 5 (3) volunteers reported slight irritation of the nose and 6 (3) of the throat.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions
15-APR-2002 (368)

Type of experience: other: irritation

Remark: From 1989 - 1993 two workers with local skin- and eye irritation, as well as malaise, after accidental local and inhalative exposure to methanol were send to the local clinic for further treatment.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

Flag: Critical study for SIDS endpoint
04-MAR-2003 (369)

Type of experience: other: irritation

Remark: Local eye contact with liquid methanol resulted in severe chemosis and lesions of the cornea, which however receded in a few days when properly treated.

Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles

Flag: Critical study for SIDS endpoint
26-SEP-2002 (370)

Type of experience: other: sensitisation, skin

Remark: In four patients with eczema positive patch-test reactions to methanol (10 %) were described.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (371)

Type of experience: other: review, chronic toxicity

Remark: Cases of chronic poisoning from repeated exposure to methanol vapor were manifested by conjunctivitis, headache, giddiness, insomnia, gastric disturbance, and bilateral blindness. In one fatal case of occupational methanol intoxication by inhalation a female worker was exposed about 12 hours. A postevent study of the process revealed methanol vapor concentrations ranging from 4,000 to 13,000 ppm.

Reliability: (2) valid with restrictions
see remark on selection of literature

26-SEP-2002 (372)

Type of experience: other: chronic toxicity

Remark: A case of chronic methanol poisoning, with marked diminution of vision, resulted apparently from the exposure at 1200 to 8000 ppm for 4 years. Also reports of headaches among workers exposed at 300 ppm during operation of duplicating machines were recorded.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (373)

Type of experience: other: chronic toxicity

Remark: A case of chronic poisoning from methanol vapor with marked diminution of vision and enlargement of the liver was reported. The employee was engaged in centrifuging material which contained 35 to 40 % methanol. Although he had been employed in this occupation for four years, his symptoms did not develop until blackout conditions became necessary with a resultant decrease in ventilation. Air analysis at the work place revealed levels from 1.6 to 10.9 mg/l (appr. 1,200 to 8,300 ppm).

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (374)

Type of experience: other: chronic toxicity

Remark: A study of the wood heel industry in Massachusetts found average methanol vapour concentrations ranging from 160 to 780 ppm, with no definitive evidence of injury to the exposed workers.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (375)

Type of experience: other: chronic toxicity

Remark: A report indicated severe recurrent headaches in workers exposed to methanol in concentrations between 200 to 375 ppm.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (376)

Type of experience: other: chronic toxicity

Remark: From 1978 - 1982 only one new case of methanol-induced occupational disease was compensated in Germany.

Reliability: (2) valid with restrictions
basic data given, acceptable restrictions

26-SEP-2002 (377)

Type of experience: other: chronic toxicity

Remark: Nineteen workers exposed to the fumes of acetone and methanol in a collar fusing plant were studied. Physical examinations revealed no abnormalities. Blood studies revealed no disturbances in hematopoiesis. Absorption of acetone was demonstrated by finding this substance in the urine of every member of the group. The concentration of solvents in the workroom atmosphere (methanol: 22 and 25 ppm, acetone: 45 and 40 ppm) was apparently not high enough

Reliability: to cause pathological changes.
(2) valid with restrictions
basic data given, acceptable restrictions
26-SEP-2002 (378)

Type of experience: other: carcinogenicity

Remark: No studies have been reported in the literature on the carcinogenicity of methanol in humans.

Reliability: (2) valid with restrictions
see remark on selection of literature
26-SEP-2002 (84)

Type of experience: other: review, reproductive and developmental effects

Remark: The Panel reviewed four epidemiological studies that examined folate supplementation and cleft palate (Peer et al, 1964, Tolarova and Harris, 1995, Shaw et al, 1995, and Cziezel and Dudas, 1992). The Panel concluded that in the aggregate the studies suggest a reduced risk of oral clefts following supplementation with vitamins containing folic acid. The epidemiological findings weakly support the hypothetical mechanism of action that methanol reduction of folate activity in the rodent causes oral cleft defects. However, that extrapolation is difficult due to the various differences, including different folate levels between rodents and humans.

No human data on the reproductive toxicity of methanol have been located.

Reliability: (2) valid with restrictions
see remark on selection of literature
04-MAR-2003 (187)

Type of experience: other: review, reproductive and developmental effects

Remark: No studies have been reported in the peer-reviewed literature on the reproductive and developmental effects of methanol in humans.

Reliability: (2) valid with restrictions
see remark on selection of literature
26-SEP-2002 (84)

Type of experience: other: review, reproductive and developmental effects

Remark: Study reviewed a variety of occupations and associated exposures to complex mixtures in women who gave birth to infants with and without cleft lip or palate. No association was found between methanol exposure and oral clefts, but several limitations of the study were raised: small number of subjects exposed to methanol, lack of individual exposure data, and confounding by other chemical exposures.

Because of these limitations the Panel judged the study results to be uncertain and concluded there are insufficient data upon which to evaluate the developmental toxicity of methanol in humans.

Reliability: (2) valid with restrictions
acceptable study, meets bsci scientific principles

Flag: Critical study for SIDS endpoint
26-SEP-2002 (379) (187)

Type of experience: other: kinetics, nutrition

Remark: Aspartam was administered to humans at a single dose of 500 mg per individual in 100 ml tap water.

Four adult volunteers fasted for 8 h and avoided alcohol, fruits, fruit drinks or vegetable for 24 h.

Blood methanol was measured at 0, 30, 45, 60, 90, 120, and 180 min following ingestion.

The dose of aspartame was representative of the daily average sugar consumption and corresponded to about 50 mg methanol = 0.7 - 0.8 mg/kg.

Result: Baseline blood methanol: 1.4 - 2.6 mg/L

Mean incremental increase (maximum after 45 min): <= 1 mg/L

Aspartame consumption by adults at a dose equivalent to the daily intake of sugar results in methanol blood levels similar to endogenous levels, in particular when divided in smaller fractions over the day.

Flag: Critical study for SIDS endpoint
29-APR-2004 (380) (187)

Type of experience: other: kinetics, nutrition (Leon)

Remark: This longer-term study demonstrated that ingesting aspartame equivalent to a methanol dose of 7.5 mg/(kg bw*d) per day resulted in blood methanol levels around 10 mg/LL in adults.

Flag: Critical study for SIDS endpoint
29-APR-2004 (381) (187)

5.11 Additional Remarks

Type: other: review

Remark: A. The toxic effects of methanol in humans and their implications are reviewed. The highest estimated exposure to methanol when used as fuel (EPA 1988) is up to 650 mg/m3.

Acute toxicity in humans affects, as previously reported, the central nervous system and primarily the optic nerve.

The background level of methanol in blood was measured as 0.73 mg/l (0.32 - 2.61 mg/l) [Sedivec et al. 1981: 31 persons) and the background level of formate, the major metabolite, was measured between 3 and 19 mg/l [0.07 - 0.4 mM].

Based on these values a background body burden of 0.5 mg/kg body weight was estimated and at worst case exposure scenarios additional body burdens between 0.03 and 1.1 mg/kg body weight were calculated (Tab. 6, p. 34).

The clearance of methanol from the body was calculated as Vmax 48 mg/kg/h in primates and 30 mg/kg/h in rats.

Metabolism in humans contributed to 96.9 % of the clearance, whereas renal and pulmonary excretion contributed only to 0.6 and 2.5 % respectively. Over 90 % of the dose was exhaled as CO2. The half-time was approx. 2.5 - 3 hours.

The contribution of formate to methanol toxicity is not clear according to the author.

According to the data reviewed no toxic effects of methanol are expected when the substance is used as fuel even at worst case exposure scenarios.

B. The minimal lethal dose to humans is considered to range from 0.3 to 1 g/(kg*d). A blood level of 500 mg/l in acutely poisoned patients generally is regarded as requiring hemodialysis. This concentration can be achieved by ingestion of 0.4 ml methanol/kg, which is only 28 ml for an adult person (70 kg), but just 4 to 6 ml for small children up to one year old.

Acute methanol intoxication (after single dose) evolves in a well-defined pattern: mild depression of the CNS, an asymptomatic latent period for several hours to >=2 days (most common 12 - 14 hours).

The toxicity syndrome that emerges consists of uncompensated metabolic acidosis with superimposed toxicity to the visual

system. Clinical symptoms include headache, dizziness, nausea, and vomiting, abdominal pain, and difficult, periodic breathing (Kussmaul breathing) which may progress to coma and death from respiratory failure.

In parallel, visual disturbance to total blindness may occur.

Ophthalmologic examination would reveal hyperemia of the optic disc, followed by the appearance of oedema projecting into the surrounding of the retina from the optic disc, while the papilla itself is not oedematous.

The severity of retinal oedema is predictive of restoration of vision. Pallor of the optic disc is an end-stage sign of irreversible effects of the visual system and may appear one to two months after an acute methanol dosage (or possibly chronic exposure).

Post-mortem signs of damage were observed in the basal ganglia in the brain, specifically the putamen (which area participates in the control of motor activities). Survivors of severe methanol intoxication may continue suffering from residual lesions of the putamen.

Test substance: Methanol, no further data.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

10-FEB-2003 (186)

Type: other: reviews

Flag: Critical study for SIDS endpoint

18-FEB-2003 (382) (84) (186) (187)

Type: other: Ocular toxicity / folate / rat

Remark: 1. Changes in the electroretinogram (ERG) and the potassium-induced Müller cell depolarization (KIMD) of folate-reduced rats have been observed after oral methanol administration of 1.5 - 2.5 g/kg whereby formate in blood increased, concentrations ranging from approx. 6 to 15 mM at 48 h (Lee et al., 1994). Moreover, methanol induces a decrease in retinal ATP, which suggests interference with oxidative phosphorylation. Formate is known as inhibitor of mitochondrial cytochrome oxidase in Müller cells (supporting glial cells).

No effects were noted at 1 g methanol/kg per os despite folate deprivation. No adverse effects are produced by doses of up to 3.5 g/kg in rats maintained on normal diet (Garner

et al. 1995b).

2. However, creating a blood formate profile by infusion of formate similar to that observed after administration of a retinotoxic methanol dose failed to alter the ERG. Retinal levels of formate after infusion were only 16 % of those measured after methanol administration (Garner et al., 1995. p. 105).

3. Intraretinal metabolism of methanol to formate is necessary for the initiation of methanol-induced retinal toxicity. Retinal and vitreous humor formate levels are approx. 15.3 umol/g and 19.2 mM, resp., after a single oral methanol dose of 3.0 g/kg bw to folate-reduced rats. The enzymes responsible for methanol metabolism, catalase and aldehyde dehydrogenase, are both present in Müller cells.

4. The acute effect of methanol on the ERG involves a severe decrease in the b-wave amplitude at approx. 48 h following a single dose and has been observed in humans, in primates and in folate-reduced rats (Garner et al., 1995, p. 101). The b-wave is generated by the Müller cell, the primary glial cell, in response to an increase in extracellular potassium released by the bipolar cells. Exactly parallel, a dose-related evocation loss of the potassium-induced Müller-cell depolarisation is noted.

5. The electrophysiological as well as histopathological effects seen after methanol treatment correlate closely to those induced by alpha-amino adipic acid, a specific Müller-cell toxin.

Conclusion:

The inhibition of cytochrome oxidase in Müller-cell mitochondria by intracellularly generated formate may explain the mechanism of methanol-induced retinal toxicity.

Reliability:

(2) valid with restrictions
Test procedures in accordance with accepted standard methods, sufficiently documented

Flag:

Critical study for SIDS endpoint

18-FEB-2003

(383) (384) (385)

Type:

other: Absorption / excretion / inhalation / human

Method:

Methanol absorption after inhalative exposure and excretion in urine was measured in groups of 4 - 5 human volunteers to be able to extrapolate exposure in the working environment from excreted urine levels.
Exposure concentrations were approx. 100 to 300 mg/m3.

Retention of methanol via lung was determined from the concentration difference between inspired and expired air.

Methanol in urine (8-h shift) served as a quantitative measure for retained methanol.
Blood levels were not measured.

Result:

The mean normal urine level was 0.73 mg/l (range from 0.32 - 2.61 mg/l), data selected from a control group of 31 individuals.

A mean of 57.7 % of the inhaled dose was retained, range 53.4 - 61.3 %, irrespective of the exposure level of selected magnitude.

Urine excretion represented nearly 1 % of the retained dose at a normal diuresis.

Average urine concentrations reached a peak after 8 h (Fig. 3) and were fairly proportional to the exposure levels (approx. 3.3, 7.0, and 9.5 mg/l). After 18 - 24 h from the start of exposure (about >= 12 h after termination), urine methanol has approached baseline level again.

The excretion half-life was about 1.5 to 2 h.

In cross-over drinking experiments it could be shown that the urinary methanol excretion correlated strictly with diuresis, i.e. irrespective of the urine volume produced at the same exposure level, the urine concentrations were identical and were dependent only on the exposure level.

This suggests that methanol distributes only passively into the urine in relation to the blood level.

This also implies that the total quantity excreted into the urine cannot be the criterion for the exposure level, but only its concentration.

The mean equation of regression (Fig. 5) between retained methanol quantity (body burden) [X in mg] and the whole-shift urine concentration [Y in mg/l] could be formulated as

$$Y = 0.7470 + 0.00763X$$

Test substance:

Methanol, no further data.

Reliability:

(2) valid with restrictions

2e: Study well documented, meets generally accepted scientific principles, acceptable for assessment.

Flag:

Critical study for SIDS endpoint

Type: other: Absorption / distribution / rat, mouse

Remark: In mice exposed to methanol in air at concentrations of 2500 to 10000 ppm (3.3 - 13 mg/l) absorbed the substance rapidly. Blood methanol concentrations were approximately 1000 mg/l at 1 hour and 3000 - 4000 mg/l at 7 hours exposure.

The fractional absorption was higher in mice (approx. 85 %) than in rats (56 - 87 %) (p. 251).

Comparison of rat and mouse:
The same environmental methanol vapour concentration leads to very different blood methanol level and is in mouse higher although the maximum elimination rate is about twice as high in mice as in rat (Pollack and Brouwer 1996):

At the teratogenic exposure concentration in mice of 5000 ml/m³, the following blood levels would be achieved

mouse: 2313 +-338 mg/l(exposed as group);
rat: 1047 +-298 mg/l.

In humans: 224 mg/l.

Test substance: Methanol, no further data.

Conclusion: The same environmental methanol vapour concentration leads to very different blood methanol level. Therefore, risk assessment for methanol should be based on blood concentrations following inhalation exposure, not on exposure concentrations itself.

The differences between the teratogenic potential of methanol in rats versus mice, with mice being approximately twofold more sensitive than rats may be due to the differences in blood concentrations.

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

18-FEB-2003

(386) (387)

Type: other: Toxicokinetics / formate / HEI study monkeys

Method: Comparative study designed to clarify methanol and formate kinetics in blood at low and high exposure concentrations to methanol in rats and monkeys with extrapolation to humans (Horton et al. 1992):

Animals: Male F344 rats (190 - 230 g) and young adult male Rhesus monkeys (Macaca mulatta), 4 rats and 3 monkeys per test group.

Inhalation exposure: single for 6 h, but in 3 monkeys also repeated inhalation (2000 ppm, 6 h/d, 5x/wk, 14 d) (not documented: see Horton et al., Discussion, p.33)

Test concentrations of methanol: nominal 50, 200, 1200, and 2000 ppm (analytical values were close to nominal).

Analytcs chamber: continuously by IR gas analyzer for methanol.

Analytcs blood: for methanol GC, for formate enzymatically coupled to a fluorescence measurement of a reactant (NADH2).

Sampling up to 12 h post-exposure.

Blood gas analysis for pH, bicarbonate, and pCO2 was performed.

Result:

Kinetic key data:

Generally, the elimination rate from blood decreased with increasing concentrations, in rats only at the highest dose significant. At the lowest concentration, 200 ppm, the elimination was only slightly lower in monkeys than in rats (approx. -30 %), but was distinctly slower at the higher burdens. No significant increases in blood values above background were evident at 50 ppm methanol.

	T1/2[h]	
	Rat	Monkey
200 ppm	0.8 +-0.2	1.1 +-0.2
1200 ppm	1.0 +-0.1	3.2 +-1.2
2000 ppm	2.1 +-0.1	2.9 +-0.6

Blood levels after 6-h exposure [ug/ml]:

	Rat	Monkey
----- Methanol:		
200 ppm	3.1 +-0.4	3.9 +-1.0
1200 ppm	26.6 +-2.0	37.6 +-8.5
2000 ppm	79.7 +-6.1	64.4 +-10.7

Formate: The peak blood levels in methanol-exposed rats and monkeys ranged between 5.4 and 13.2 ug/ml (approx. 0.25 - 0.6 mM) without showing statistically significant differences to control animals [note: Data are not documented in Horton et al., 1992: However, Medinsky and Dorman (1995) present data in Fig. 1 attributed to the studies by Horton et al. (1992).]

Blood acidosis: no signs of acidosis which is in line with the formate blood levels in normal range.

Repeated inhalation in monkeys (data not shown: see Horton et al., 1992, Discussion, p. 33), carried out as confirmatory test to examine accumulation toxicology: In blood samples collected after 1 and 2 weeks of exposure, no accumulation of methanol or formate.

Prediction of in-vivo time-course data by the PBPK model: Below 1500 mg/m3 (1200 ppm), all three species (rat, monkey, human) would exhibit similar post-exposure blood levels of methanol which would be proportional to atmospheric concentrations. Above 1200 ppm, increase in blood would become non-linear for rat and monkey, but remain linear for human.

Test substance: Methanol purity >98%
Conclusion: Comparison of monkey blood-methanol elimination rate constant with those derived for humans (HEI, 1987) and rats shows an interspecies similarity in the overall elimination of methanol after low doses (Horton et al., 1992, Discussion).

The dose of methanol required to saturate the folate-dependent metabolism of formate, calculated from an equation derived by the Health Effects Institute (HEI, 1987), is about 250 mg/kg. If one assumes a 6-kg monkey breathes 118.8 l/h and 100% absorption of methanol occurs, a dose equivalent to 308 mg/kg is absorbed during a 6-h exposure to 2000 ppm methanol(2600 mg/m3).

While this amount of methanol could overwhelm the formate metabolism pathway if given as a bolus, the saturation of metabolism with this amount divided over 6 h is unlikely (Horton et al., 1992, Discussion).

Reliability: (2) valid with restrictions
2e: Study well documented, meets generally accepted scientific principles, acceptable for assessment.
Flag: Critical study for SIDS endpoint

18-FEB-2003

(388) (389) (243)

Type: other: Absorption / inhalation / human

Method: Six human volunteers (from 29 - 55 years) were subjected to a controlled diet-regimen (without obvious methanol-delivering nutrition and additives) throughout the study and exposed to 200 ppm (260 mg/m3) methanol for 6 hours. Five individuals were each tested at rest or at light

exercise.

Remark:

Blood methanol concentrations increased from 1.8 ug/ml (mean endogenous level) to 7.0 ug/ml at rest and to 8.1 ug/ml under light exercise (increase in mean pulmonary ventilation at a factor of about 2.7 from average 10.5 to 26.6 L/min and increase in respiratory rate at a factor of about 1.7 from 11.2 to 18.6 breathes/min).

Blood formate levels did not increase.

Blood levels at rest (from Tab. III and V):

Test person	Methanol[ug/ml]		Formate [ug/ml]	
	pre-exposure	post-exposure	pre-exposure	post-exposure
1	2.53	5.44	8.11	8.12
2	3.57	7.55	10.57	13.06
3	1.32	7.93	10.35	7.24
4	0.57	8.08	9.08	8.98
5	1.12	5.83	7.31	6.12
Mean	1.82	6.97	9.08	8.70
+SD	+1.21	+1.24	+1.26	+2.38

Blood levels at exercise (from Tab. IV and VI):

Test person	Methanol[ug/ml]		Formate [ug/ml]	
	pre-exposure	post-exposure	pre-exposure	post-exposure
1	1.99	9.82	5.36	8.90
2	2.00	7.40	9.28	11.14
3	3.15	7.47	9.35	9.54
4	0.54	6.40	9.07	9.91
5	1.95	9.57	10.83	8.10
Mean	1.93	8.13	8.78	9.52
+SD	+0.93	+1.49	+1.82	+1.02

The results show that no formate mediated toxicity is expected when workers are exposed to 200 ppm methanol, the current Occupational Safety and Health Administration 8-hr time-weighted average permissible exposure limit. Methanol, no further data.

Test substance:**Conclusion:**

At 200 ppm methanol or below, methanol blood levels may increase 3.5 to 4-fold above the endogenous methanol concentration in human blood, while the formate level

remains unchanged, irrespective of physical activity whether at rest or light exercise.

This was confirmed e.g. by D'Alessandro et al. (1994) in similar experiments on human volunteers (comp. also Medinsky and Dorman, 1995).

Reliability:

(2) valid with restrictions

2e: Study well documented, meets generally accepted scientific principles, acceptable for assessment.

Flag:

Critical study for SIDS endpoint

18-FEB-2003

(390) (191) (243)

Type:

other: Risk Assessment Model / inhalation

Method:

A "multicompartment biologically based dynamic" inhalation model based on kinetic methanol inhalation data from rats (Horton et al., 1992), monkeys (Dorman et al., 1994) and humans (Sedivec et al., 1981; Osterloh et al., 1996; Batterman et al., 1998) was developed to describe the time evolution of methanol and its metabolites in the whole body and in accessible biological matrices.

Result:

Predictions from simulations (PBPK modelling) of continuous inhalation of 200 ppm methanol in humans for 5 days (Bouchard et al. 2001, p. 177/178):

Based on the following assumptions:

- a negligible background burden of methanol,
- an absorption fraction of 0.577 (Sedivec et al. 1981),
- a pulmonary ventilation rate of 10.8 L/min (Sedivec et al. 1981; Batterman et al. 1998),
- an apparent distribution volume for methanol of 0.7 l/kg (corresponding to human body fluid),
- an apparent distribution volume for formate of 4.6 l/kg (estimations by Bouchard et al. 2001, Tab. 2, p. 173 and p. 180),
- a daily urine volume of 1.5 l,

near steady state will be reached in 20 h. After 5 d, methanol in blood and urine is estimated at 5.5 mg/l (171 umol/l) and 8.1 mg/l (252 umol/l); formate in blood and urine is 1.16 mg/l (3.5 mol/l) and 1.5 mg/l (31.7 umol/l = 0.97 mg/g creatinine or 2390 umol/mol creatinine).

This shows that exposure concentrations of <500 ppm are not sufficient to raise formate levels significantly, while methanol increases.

The model, adapted to kinetic data in humans exposed acutely to methanol, predicts that 8-h inhalation exposures ranging from 500 to 2000 ppm, without physical activities, are needed to increase concentrations of blood formate and

urinary formic acid above reported background values (4.9-10.3 and 6.3-13 mg/l, resp.).

Therefore, acc. to authors, blood and urinary methanol levels are the most sensitive biomarkers of absorbed methanol.

Pulmonary retention: Using the experimental human data of Osterloh et al. (1996), Sedivec et al. (1981) and Batterman et al. (1998), the best fit in the model for the average absorption fraction was higher than that given by Sedivec et al. (1981), namely about 80 % and corresponded to the retention of 79 % given by Batterman et al. 1998 (Bouchard et al., 2001, p. 177).

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

18-FEB-2003 (275) (391) (241) (389) (281) (277)

Type: other: Absorption / distribution / inhalation / rat

Method: Whole-body exposure system:

1. Adult female Long-Evans rats (n = 12) were exposed to 4500 ppm methanol for 3 consecutive days once for 6 h. Blood methanol levels were measured 1 and 6 h during and 1-h post exposure as well as background levels before each exposure.

2. Adult female Long-Evans rats (n = 12) were exposed to 4500 ppm methanol during pregnancy and lactation along with the progeny (gd 6 through post-natal day[PND] 21).

(see also other entry under 5.8.2)

Result:

exposure time	1 h	6 h	1 h post exp.
	[in approx. averages mg methanol/ml]		
day 1	0.25	0.90	0.95
day 2	0.40	0.60	0.60
day 3	0.30	0.50	0.60

After the first day, the levels were higher which can be explained with adaptation to the new environment (higher activity). The pre-exposure levels (1 h before first exposure and 17 h after each previous exposure interval) were non-detectable.

During pregnancy and lactation, blood levels in dams were in the range of 0.50 +/-0.07 to 0.55 +/-0.09 mg/ml. But in

offsprings, the level was about 2x that of dams, at 1.26 +-0.23 mg/ml, and gradually decreased to that of the dams after 52 PND.

Test substance: Methanol, HPLC grade
Reliability: (1) valid without restriction
1c: Test procedures in accordance with accepted standard methods, well documented
Flag: Critical study for SIDS endpoint
18-FEB-2003 (232)

Type: other: Absorption / distribution / oral / rat

Result: After oral administration of 100 and 2500 mg methanol/kg to female SD rats, gastrointestinal absorption was 100 % within minutes (abs. half-life 1.5 min and 7.6 min, respectively) (p. 16).

The maximum elimination rate is about twice as high in mice as in rat: 117. +-3 and 60.7 +-1.4 mg/(kg*h) (Pollack and Brouwer 1996, Tab. 7, p. 21; Ward et al. 1995).

After inhalation, the mean fractional respiratory absorption of methanol in rats and mice was found to about 85 %, respectively, at exposure concentrations from 1000 to 5000 ml/m³, and tended to be lower (60 - 70 %) at 10000 - 20000 ml/m³ in rats, but not in mice (Pollack and Brouwer 1996, Tab. 12, p. 27; Tab. 15, p. 33).

Blood levels in rats were about 800 (calc.) - 1200 mg/l (measured) at 5000 ml/m³ (8 h) (Fig. 37, p. 34).

Blood levels in mice were about 3000 mg/l at 5000 ml/m³(8 h) (Fig. 37, p. 34).

Reliability: (2) valid with restrictions
2e: Study well documented, meets generally accepted scientific principles, acceptable for assessment.
Flag: Critical study for SIDS endpoint
27-NOV-2003 (387) (392)

Type: other: Toxicokinetics / formate / folate deficiency / HEI study monkeys

Method: Pharmacokinetic study to evaluate the contribution of methanol-derived formate to the endogenous formate pool in nonprimates under folate-sufficient and folate-deficient condition (Medinsky et al., 1995, 1997):

Four 12 year old female cynomolgus monkeys (*Macaca fascicularis*) were used: lung-only exposure under

isoflurane-anaesthesia. Exposure time = 2 h.
14C-radiolabelled methanol was used as atmospheric tracer material.

Exposure concentrations/situation: nominal 10, 45, 200, and 900 ppm (normal folate) and 900 ppm (folate-deficient) in the every same monkeys two months later after folate-deficient diet. Measured concentrations corresponded well to nominal ones.
(Achieved folate deficiency corresponded to stage-III and greater.)
Air analytics of methanol by GC.

Blood analysis of methanol and formate by HPLC and liquid scintillation spectroscopy: The methanol-dependent fraction of formate was estimated by using 14C-radiolabelled methanol.

Remark: -----
It is unclear why the endogenous animal-specific total formate blood levels were not determined in the same test animals. This restriction is not considered to invalidate the overall result and conclusion.

Result: -----
Methanol levels in blood:
Average 2-h concentrations were 0.65 +-0.3, 3.0 +-0.8, 21 +-16, 106 +-84, and 211 +-71 uM following 10, 45, 200, 900 and 900 ppm(deficient).
AUCs were linearly related to the inhaled methanol dose, the T1/2 were largely unaffected by the methanol concentrations and ranged from mean t1/2 of 0.56, 0.88, 0.62, and 0.95 h, respectively, with 1.31 h under folate deficiency showing a positive increasing trend, although not significant (Dorman et al., 1994; Medinsky et al., 1997).

Formate levels in blood:
Average 2-h concentrations were 0.07 +-0.02, 0.25 +-0.09, 2.3 +-2.9, 2.8 +-1.7, and 9.5 +-4.7 uM following 10, 45, 200, 900 and 900 ppm(deficient).

The endogenous formate blood level was not measured, but assumed to be 0.1 to 0.2 mM (taken from other sources). The range of the author's group is given as 0.29 to 0.56 mM (see Discussion).

Test substance: -----
Radiolabelled methanol from Sigma Chemical Corporation, >98% pure, methanol HPLC pure.

Conclusion: The results suggest that low-dose short-term exposure to methanol would not result in toxic elevated methanol or formate levels in blood even under folate deficiency, because, even under folate deficiency, the methanol-related formate levels remained at a factor of 10 to 100 below endogenous blood concentrations and are several orders of magnitude lower than levels of formate known to be toxic (>7

mM) [see also: Medinsky and Dorman, 1995, p. 709].

Reliability:

(2) valid with restrictions
2e: Study well documented, meets generally accepted
scientific principles, acceptable for assessment.

Flag:

Critical study for SIDS endpoint

18-FEB-2003

(241) (393) (243)

Type:

other: Metabolism / formate / monkey ip

Method:

8 weeks old male Fischer 344 rats and male monkeys (Macaca fascicularis) received ¹⁴C-labeled methanol per i.p. injection in doses of 25; 125; 600 and 3000 mg/kg. 5 animals per dose group were tested (monkeys were repeatedly used). Blood was collected after 6; 12; 24; 30 and 48 hours.

The following parameters were analysed:

- formic acid
 - organic acid
 - folic acid
 - pH
 - biochemistry (glucose, blood urea nitrogen, Na, K, Cl).
-

Result:**Rats:**

In 25 to 600 mg/kg dosage groups no abnormality was seen and there were hardly any individual difference in blood radioactivity.

In the 3000 mg/kg group, however, animals grew weak immediately after administration, with one death during 48 hours. Moreover, somewhat serious toxic symptoms, such as difficulty in blood collection and diarrhea, were seen. The disappearance of radioactivity in blood delayed markedly when compared with the other groups. The recovery was noted in only on animals which had shown a decrease of the blood level within 48 hours.

In animals of 25 to 600 mg/kg dosage groups, the major excretion route was exhalation: 70-80 % of administered dose was excreted as CO₂ and methanol. The excretion of methanol increased with increased dosage.

The excretion into urine and feces was very low: 6.1-8.3 % and 2.1-2.7 % respectively. The excretion was almost completed within 48 hours.

At 3000 mg/kg dosage group 2 animals died within 24 hours, and one additional animal within 48 hours after administration. The excretion was very slow: about 22 % was excreted into exhaled breath as CO₂ or methanol and about 8 % in urine. There were hardly any feces, and therefore the fecal excretion of radioactivity remained very few.

Monkeys:

The major route of excretion was exhalation at 25 to 600 mg/kg, 50 % of the administered dose being excreted

as CO₂. Even after 48 hours, total excretion remained low: about 60 % (probably due to the test procedure: loss of CO₂ during anesthetization and blood collection). Methanol excretion was 0.82-3.8 %, urinary excretion 5.9-7.5 % and excretion via feces 0.35-0.53 %.

At 3000 mg/kg the excretion were 25 % as CO₂, 9 % as methanol, 20 % via urine, the excretion was delayed in this group.

Reliability:

(2) valid with restrictions
Test procedures in accordance with accepted standard methods, sufficiently documented

Flag:

Critical study for SIDS endpoint

18-FEB-2003

(161)

Method:

a) Application of 0.19 - 0.21 ml methanol to the skin of 6 human volunteers by means of a closed application glass chamber (11.2 cm²), absorption was measured from 15 to 60 min p.a., 22 determinations in total. Residual available methanol was washed off with water and determined spectrophotometrically (Dutkiewicz et al. 1980).

b) Exposure of one hand to methanol (Dutkiewicz et al. 1980).

Result:

a) Following occluded exposure in 6 volunteers, average skin absorption rate of methanol is reported to be at 0.192 mg/cm²/min (Dutkiewicz et al. 1980).

b) Exposure of one hand to liquid methanol for 2 min would result in a body burden of 170 mg, similar to that resulting from exposure to an approximate air concentration of 50 mg/m³ (38 ml/m³) for 8 h (Dutkiewicz et al. 1980) (see also: IPSC/WHO, 1997).

c) In 12 humans one hand of whom was exposed to liquid methanol for 2 to 16 min, the average uptake through the skin was 8.1 +-3.7 mg/(cm²*h) = 0.135 +-0.062 mg/(cm²*min) (Batterman and Franzblau 1997).

Full exposure of one hand for 16 min resulted in a blood level equivalent to that reached after inhalation of 400 ml/m³ for one 8-h working shift with a maximal blood level of some 11 mg/l (corrected for background value) (Franzblau and Batterman 1995; Batterman and Franzblau 1997).

Test substance:

Methanol, no further data.

Conclusion:

The percutaneous absorption may be considerable on direct contact of skin to methanol and constitute a substantial part of the body burden in relation to inhalation.

Both independent sources arrived at similar quantitative kinetic results for skin permeation in humans and appear to be reliable.

Reliability:

(2) valid with restrictions
Special study design, sufficiently documented, meets generally accepted scientific principles, acceptable for assessment

Flag:

Critical study for SIDS endpoint

27-NOV-2003

(276) (382) (394) (395)

Method:

In normal (CB6-F1) and FDH-deficient mice (NEUT2), acute methanol toxicity and formate elimination after formate i.p. application were compared. The homozygous NEUT2 mice lack cytosolic 10-formyltetrahydrofolate dehydrogenase (FDH). ¹⁴C¹⁴CO₂ from exhaled air was collected after treatment of mice with a low (5 mg/kg) or a high dose (100 mg/kg) of ¹⁴C-formate. For discrimination between catalase-dependent and other biotransformation routes, 3-aminotriazole (3-AT: 1 g/kg i.p.) was employed as selective inhibitor of catalase.

Result:

Acute LD50 were virtually similar in either strain at about 6000 mg/kg methanol.

At 5 mg/kg formate, the difference in CO₂ formation in the NEUT2 strain was about one half of the normal mice as expected. However, the inhibitor 3-AT failed to reduce CO₂ generation in either case.

At 100 mg/kg formate, CO₂ exhalation was similar in both strains (which is in line with the congruent acute toxicity in either strain), but also inhibited by 3-AT to the same extent.

There was no significant difference in formate-related CO₂ release in either strain after 3-AT inhibition, although the FDH-deficient strain should -in theory- be unable to oxidise formate under inhibitory condition.

Conclusion:

At low formate burden (here 5 mg/kg), the function of the catalase is poor or not operating, but is active at high doses (here 100 mg/kg). The folate-dependent FDH system appears to function under normal metabolic conditions.

The residual metabolic capacity of the FDH-deficient mouse strain to oxidise formate is apparently a folate-independent alternate oxidation system not yet characterised.

Therefore, there appear to be at least three metabolic pathways in mice for the oxidation of formate arising from methanol or other sources:

- 1. the common cytosolic folate-dependent 10-formyltetrahydrofolate dehydrogenase (FDH) pathway,
- 2. the catalase-dependent route, and
- 3. a yet unknown route, likely folate-independent.

Reliability:

 (2) valid with restrictions
 Test procedures in accordance with accepted standard methods, sufficiently documented

Flag:

Critical study for SIDS endpoint

27-NOV-2003

(396)

Method:

Sodium formate metabolism was studied in monkeys (*Macaca fascicularis*) after i.v. infusion using ¹⁴C-radiolabelled formate /doses 1, 2.5, 5 and 10 mmol/kg). Tests were performed under folate-sufficient and folate-deficient conditions.

Result:

At the high dose levels of formate, the rate of formate metabolism to CO₂ was less than that observed in rat (Fig. 3). At lower doses the rate of oxidation to CO₂ was about 25 % of that in rat.

The catalase-peroxidase pathway was shown to play no role in the oxidation of formate in monkeys (using catalase inhibitor) (Fig 4).

The half-life of formate elimination was about 38 min at 2.5 mmol/kg formate, in the absence or presence of a catalase inhibitor.

Folate depletion reduced formate metabolism significantly while folate supplementation accelerated it (Fig. 6 - 9). There was a striking increase in blood formate following methanol application under dietary folate deficiency (approx. 5 vs. 2 mEq/l blood after 0.5 g methanol/kg).

 Elimination half-lives for formate from blood ranged from some 30 - 50 min in the monkey and about 12 - 23 min in rats, depending on the doses (Clay et al. 1975, p. 57).

Reliability:

(2) valid with restrictions
 2e: Study well documented, meets generally accepted scientific principles, acceptable for assessment.

Flag:

Critical study for SIDS endpoint

27-NOV-2003

(162) (397)

Method: The folate-dependent formate oxidation and the activities of several folate-dependent enzymes were measured in vitro with liver homogenate and liver cytosolic fraction of rat (male SD), mouse (Swiss-Webster), monkey (female Cynomolgus, *Macaca fascicularis*) and humans (from victims of traumatic head injuries, without evident liver diseases).

Result: Human and monkey liver tissue showed much lower 10-formyltetrahydrofolate dehydrogenase activity, the enzyme catalyzing the final step of formate oxidation to CO₂, about 25 % and 37 %, respectively, than in rat liver. Furthermore, total folate and H₄folate in human liver were only 40 and 50 %, respectively, of that observed in rat liver.

In monkey liver, H₄folate was comparable to that in humans, but total folate was comparable to that in rat.

Mouse liver contained much higher H₄- and total folate levels than rat and monkey.

The

The in-vitro rates of formate oxidations were approx. 30 to 40 mg/(kg bw*h) in monkey, about 75 - 80 mg/(kg*h) in rats, and approx. 300 mg/(kg*h) in mice [humans not shown].

Test substance: Methanol, no further data.

Conclusion: The authors explain the higher toxicity of methanol in humans and monkeys compared to rodents by the low H₄folate levels as well as the reduced enzymic capacity of formate oxidation.

Reliability: (2) valid with restrictions
2e: Study well documented, meets generally accepted scientific principles, acceptable for assessment.

Flag: Critical study for SIDS endpoint

27-NOV-2003 (398)

Type: other: Ocular toxicity / folate / human / rat

Method: Comparative biochemical study to quantify folate and 10-formyl tetrahydrofolate dehydrogenase in human and rat retina and thus explain the higher ocular toxicity of methanol towards human being.

Result: The cell-specific localisation of the enzyme, 10-FDH, was found to be similar in rat and human retina, preferentially located in the Müller-cell type, the principal glia of the retina (by immunohistochemistry).
The amount of 10-FDH found in cytosolic as well as in the

mitochondrial fraction, was about 3x higher in humans than in the SD rats (Western blot analysis).

However, the retinal folate levels were lower in humans (about 14 % of that in rats), compared with the high folate liver pools, the retina contains very much less folate.

Conclusion: Formate oxidation was found to be about 50 % lower in human than in rat retina (Eells et al, 1995). This is in line with this finding that lower folate levels in human retina may limit conversion of formate into CO₂ and result in higher ocular toxicity in humans.

Reliability: (2) valid with restrictions
Test procedures in accordance with accepted standard methods, sufficiently documented

Flag: Critical study for SIDS endpoint

18-FEB-2003 (399) (400)

Type: other: Risk Assessment / Reproductive parameters

Remark: A range of toxicological investigations, especially teratogenicity studies, was evaluated to perform a risk assessment for consumer and occupational exposure scenarios. The author came to the conclusion that exposure to the current TWA (time weighted permissible exposure limit) does not pose any risk.

Test substance: Methanol, no further data.

Reliability: (2) valid with restrictions

18-FEB-2003 (240)

Remark: The authors evaluated published data of formate production in humans and animals after methanol exposure. They came to the conclusion that even high methanol exposure up to 2.6 mg/l did not produce toxic blood formate levels and therefore exposure to methanol likely to be encountered during normal use of methanol-based fuels (up to 0.06 mg/l) will no pose an unacceptable risk.

Test substance: Methanol, no further data.

18-FEB-2003 (401)

Remark: Review on important studies for essential endpoint, intended to perform a risk assessment for the general human population: Reference concentrations are derived.

The study by NEDO (1987) on monkeys is employed as central basis for this estimation.

Result: -----
A 1-h reference concentration (RfC) was derived to be at about 100 mg/m³, taking into account sensitive people. A very conservative chronic RfC was obtained and proposed at 0.38 mg/m³, based on the assumption that 10 ppm has to be used as NOAEL and 100 ppm as the LOAEL for "neurotoxic effects". This appears to be not in compliance with the authors' observations and is not suggested in the report.

Reliability: -----
(2) valid with restrictions

18-FEB-2003

(161) (177)

Remark: Conclusions on the role of formate:
"From the studies of formate in rodents in vivo and in vitro... it appears that formate is toxic to the developing embryo at concentrations far in excess of those achieved after teratogenic methanol exposure in the mouse, but within the range of levels achieved in humans after acute high-dose methanol poisoning. Thus, although methanol appears to be the proximate murine teratogen, formate is still of concern in terms of potential developmental toxicity in humans." (p. 386)

Conclusions on the role of folic acid:
"These results (note: including studies on folate-deficient diet) suggest that dietary folate intake may be an important consideration in assessing the risk of methanol-induced developmental toxicity." (p. 386)

18-FEB-2003

(255)

Type: other: Absorption / inhalation / rat

Method: A flow-through inhalation chamber system (coupled with the barometric method of evaluation of the tidal volume) is introduced which allows the on-line monitoring of the essential ventilation parameters, the tidal respiration volume and respiration frequency of the test animal, under unrestrained conditions as well as in- and efflux vapor concentrations. This enables to obtain more accurate and thus representative data about extraction of the inhalant from air flow. Concomitant blood sampling via a fixed cannula further allows to analyse blood levels at any time interval.

Female SD rats were used, exposed to 1000, 5000, 10000, 15000, and 20000 ppm methanol for 8 h.

Result: Based on this technique, the mean ventilation volume for 8 h was 280 - 230 L/kg b.w. (mean 250 L/kg), a slight downward trend discernible with increasing exposure concentration. The respiration frequency was found to be significantly lower than commonly published (about -20 to -30%), while the tidal volumes were similar. At 5000 ppm, an extraction (retention) factor (absorption) of about 0.9, at 15000 ppm of about 0.6 resulted from the plots of cumulative mass vs. exposure time. No data given at the lower exposures.

The highest blood concentrations were at about 3500 mg/L (No exposure-blood correlation is shown.).

Conclusion: Based on these results, the respiratory uptake of methanol by rats can be assumed to be approx. 100 % at exposure concentrations of <5000 ppm.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

18-FEB-2003

(402)

Type: other: Ocular toxicity / rat model

Method: Nonprimate model in rats in which the folate-dependent formate oxidation (on the level of methionine synthetase) has been selectively inhibited by treatment with N2O in inhaled air.

Animals: Male Long-Evans rats (250 - 300 g)

Methanol administered i.p. at an initial dose of 4 g/kg followed by a supplemental dose of 1 or 2 g/kg 12 h later.

Thus, blood formate levels were from 7 - 15 mM or 4 - 6 mM for 30 - 40 h.

The "non-intoxicated" controls received 4 and 2 g/kg methanol without N2O, the non-treated controls either the vehicle or N2O in air.

Retinal function was assessed by flicker electroretinography (ERG). Histopathology of retina and optic nerve was carried out by light microscopic visualisation.

Result: Methanol intoxicated rats developed formic acidemia, metabolic acidosis and visual toxicity within 36 hours.

Histopathological effect on retinal structure:

In the high-dose group (7 - 15 mM blood formate vs. methanol-treated control with 0.5 to 2 mM formate), prominent vacuolation in the photoreceptors near the junction of inner and outer segments, with accumulation of densely stained material in the inner segments near the outer limiting membrane. Mitochondrial swelling and disruption was noted in the retinal pigment epithelium, photoreceptor inner segments and optic nerve (Eells et al., 2000; Seme et al., 2001). Ultrastructural studies by electronmicroscopy revealed that the retinal morphology (as represented by the mitochondrial-rich, inner segment of the photoreceptor) was similar to the control after recovery of 72 h, but subtle photoreceptor changes were still present as a spacing between the cell nuclei of the outer nuclear layer which suggests residual histological alterations from formate-induced, previous edema (Seme et al., 2001).

In the low-dose group (4 - 6 mM formate in blood), no histopathological changes were apparent at the light-microscopic level (Wallace et al., 1997).

However, visual dysfunction was already visible in functional diagnostic, occurring as reductions in the flash evoked cortical potential (FEP) and in electroretinogram (ERG) at formate concentrations lower than those associated with morphological changes and provide functional evidence of direct retinal toxicity in methanol poisoning (Wallace et al, 1997). Rod- and cone-mediated ERG responses were attenuated in a formate- and time-dependent manner (Seme et al., 1999, 2001).

Biochemical effects:

Retinal ATP, ADP, and GSH were significantly depleted following methanol-treatment under inhibition of formate oxidation after 72 and 144 h with GSH levels about 1/2 of controls, and after recovery still decreased, while energy metabolites showed no difference from the control values (Seme et al., 2001).

Test substance: Methanol, HPLC grade from Sigma

Conclusion: Functional tests provide functional evidence of direct retinal toxicity in methanol poisoning at stages not yet pronounced in histopathological changes.

Hypothetical mechanism: Formic acid binds to cytochrome aa3 and inhibits cytochrome oxidase activity with inhibition constant values between 5 - 30 mM, which is in the range of concentrations found in the retina and vitreous humor of methanol-intoxicated rats. This may explain the effect on mitochondria and resulting visual dysfunction (Eells et al., 2000).

The rat model appears to be a useful tool to also elucidate the sequelae of methanol intoxication in humans who are more sensitive than rodents.

Reliability:

(2) valid with restrictions
2e: Study well documented, meets generally accepted scientific principles, acceptable for assessment.

18-FEB-2003

(403) (404) (405) (406)

Type:

adsorption

Remark:

In shortly described experiments, monkeys (normal or folate deficient) exposed to low levels of methanol (10 - 900 ppm = 0.013 - 1.17 mg/l) showed a methanol-derived increment of formate in blood which were 10 - 1000 fold lower than those levels normally present in the body.

It is concluded that exposure to methanol at the threshold limit value of 200 ppm (0.26 mg/l) is unlikely to pose an unacceptable risk in healthy or even moderately folate-deficient individuals.

Test substance:

Methanol, no further data.

Reliability:

(2) valid with restrictions

29-JAN-2003

(407)

Type:

Biochemical or cellular interactions

Remark:

The cytotoxicity of methanol on C6 rat glioma cells was measured by various methods: trypan blue exclusion, protein synthesis, neutral red assay and induction of heat shock protein synthesis. Methanol showed a very low toxicity with first toxic effects in all test systems at concentrations between 1 and 10 Mol (ca. 32 - 320 g/l).

Test substance:

Methanol from Sigma Chemicals, Germany, no further data.

14-MAR-1997

(408)

Type:

Biochemical or cellular interactions

Remark:

The cytotoxicity of methanol was tested in chicken embryo forebrain neuron cultures using the MTT and the neutral red assay to predict acute toxicity.
LC50 in the MTT assay was 482.2 uMol and in the neutral red assay 340.6 uMol. The toxicity of methanol was low in comparison with the other 49 test substances.

Test substance:

Methanol from BDH, analar analytical grade, no further data.

14-MAR-1997

(409)

Type: Biochemical or cellular interactions

Remark: In neuronal cell cultures (pheochromocytoma PC12) alcohols in combination with neuronal growth factor leads to greatly enhanced neurite extension and cell differentiation. The effect increases with increasing chain length, methanol being the less potent substance. The role of this effect in alcohol toxicity on brain is not understood.

Test substance: Methanol, no further data.

14-MAR-1997 (410)

Type: Biochemical or cellular interactions

Remark: Rat hepatocytes were exposed to methanol for 20 hours at concentrations of up to 39.55 ug/ml. The treatment had no effect on LDH-leakage, MTT-reduction, cellular protein content and neutral red uptake.

Test substance: Methanol, no further data.

20-MAR-1997 (411)

Type: Distribution

Remark: After oral administration of a single dose of methanol to dogs (1.97 g/kg) about 10 % was excreted unchanged in the urine. The methanol concentration in the organs was nearly half as high as that found in the urine. About 20 % of the administered dose was excreted in urine as formic acid. Formic acid concentrations in tissues was about half the serum concentration.
After oral administration of 2.38 g/kg methanol to rabbits 10 % were excreted unchanged in urine. No further details are reported.

Test substance: Methanol, no further data.

15-AUG-2002 (84) (412)

Type: Distribution

Remark: The examination of the toxicokinetics of intravenously administered methanol to female rats as a single dose of 50 or 100 or 2500 mg/kg resulted in a markedly non linear elimination of methanol from the systemic circulation suggestive of a significant capacity limited rate of elimination. The data from the 2500 mg/kg group was described by a kinetic model incorporating parallel first-order and saturable elimination processes, a portion of this apparent linear elimination pathway was due to renalexcretion of the unchanged alcohol.

Test substance: Methanol, no further data.

24-MAR-1997

(84) (413)

Type: Metabolism**Remark:** The metabolism of methanol (and ethanol) by catalase and alcohol-dehydrogenase was studied in alcohol-dehydrogenase positive and negative deer mice. Metabolism by catalase was the predominant pathway for both alcohols but both enzymes contributed to the total metabolism (Bradford et al., 1993).

Methanol metabolism in alcohol-dehydrogenase deficient deer mice was inhibited by 4-methylpyrazole, which inhibited fatty acyl CoA-synthetase, thus reducing the availability of H₂O₂ for catalase dependent alcohol metabolism (Bradford et al., 1992).

Test substance: Methanol, no further data.

19-MAR-1997

(414) (415)

Type: Toxicokinetics**Remark:** In monkeys, four daily skin application of 0.5 ml (daily dose 1.56 g/kg) could be lethal. Rabbits proved to be distinctly more insensitive to methanol either dermally applied or also after inhalation exposure. Individual variation was nonetheless large in either species.**Test substance:** Methanol, no further data.

05-MAR-1997

(158)

Type: Toxicokinetics**Method:** Comparative toxicity and metabolic study
1. in normal mice a) under folate-sufficient, b) under folate-deprived conditions, and
2. in a C57BL mouse strain suffering catalase deficiency (catalase considered to be the prime enzyme oxidising formate) as well as a strain with normal catalase activity.

Methanol and formate levels in blood as well as urinary formate excretion were compared with acute toxicity (LD₅₀) under respective conditions after oral administration of methanol (2 - 8 g/kg bw).

Remark: Those mice with catalase deficiency showed a somewhat lower LD₅₀(oral and i.p.) than those with normal catalase level, irrespective of folate status. Folate- and catalase deficient mice excreted considerably higher amounts of formate into the urine (30.8-40.8 %), with significantly higher blood formate levels than in those normal, but formate-deficient mice (15 to 17 umol/ml vs. 0.8 - 1.4 umol/ml (24 h after 6 and 5 g/kg methanol oral).

In no case, folate deficiency alone could not explain the poor decrease in LD50 which was unexpected in relation to the pronounced increase in the formate level with 24 h.

A low supplementation with methionine (0.2%) had no influence on the LD50 under catalase- and folate-deficient conditions, while at 1.8 % methionine, there was an apparent LD50-decrease from 7.1 g/kg to 6.4 g/kg.

Test substance: Methanol, no further data.
Conclusion: The authors concluded that the formate concentrations in plasma do not correlate well with methanol-induced lethalties in catalase-deficient, but folate-sufficient mice or folate-deficient mice. In addition, urinary excretion, not oxidation, is the primary means to get rid of high levels of formate.

The results in mice with catalase or folate deficiency -acc. to authors - appear to correspond to conditions in methanol-poisoned monkeys: high formate level at low pH in blood.

15-AUG-2002 (136)

Type: Toxicokinetics

Remark: The pharmacokinetics of methanol was compared in Fisher rats and Rhesus monkeys. After i.v. application to rats 96.6 % were metabolized, 2.6 % exhaled and only 0.8 % excreted in urine. The kinetic parameters measured or cited were: AUC at 2000 ppm 623.6 (rats) and 479.8 (monkeys) ug x h/ml, t1/2(half-life) 2.1 hours in rats and 2.9 hours in monkeys, Vmax15.41 mg/h x kg, Km 33.92 mg/l.

Test substance: Radiolabelled Methanol from Sigma Chemicals Corporation, no further data.

18-FEB-2003 (389)

Type: other: Absorption / distribution / inhalation / monkey

Method: Comprehensive study design in analogy to Segment-I study limited to exposure of female monkeys (*Macaca fascicularis*) and qualitative/clinical signs of toxicity. It comprised

- methanol pharmacokinetics
- maternal toxicity (see other entry: 5.8.2)
- reproductive toxicity (see other entry: 5.8.2)
- developmental neurotoxicity (see other entry: 5.8.2).

Groups of 11-12 adult female monkeys were exposed to methanol vapor in the concentrations of 0; 200; 600 or 1800 ppm, daily for 2.5 hour before breeding, during breeding

and during pregnancy.

Blood kinetics for methanol, blood concentration of formate as well as folate were determined 2x/week 30 min after the 2.5-h exposure in female monkeys during the prebreeding period, after 87 d of exposure and 2x during pregnancy (Kinetic studies 1 to 4). The number of animals was 9 to 10 per group.

Result:

Methanol: No significant differences in the blood concentrations between the 4 phases (measured 0.5 h post-exposure, n = 11 or 12 (non-pregnant); n = 9 or 10 (pregnancy) (Fig 7, Tab. 11, Fig. 14, Part I):

Baseline range approx. 2 ug/ml [approx. 0.06 mM]
200 ppm: range approx. 5 ug/ml [approx. 0.15 mM]
600 ppm: range approx. 10 ug/ml [approx. 0.30 mM]
1800 ppm: range approx. 35 -40 ug/ml [1mM-range]

At 1800 ppm, after 5 h elimination, the residual methanol level was near baseline (max. 2-fold higher).

The mean estimated elimination half-lives (for 600 and 1800 ppm) ranged between about 60 to 90 min.

Formate: Irrespective of concentration levels or exposure intervals, there was no evidence of a significant increase above the background range of about 0.15 - 0.30 mM (approx. 7 to 14 ug/ml) (Fig. 8, Fig. 15, Tab. 12, Part I).

Folate in serum: There was no significant shift in the folate levels during pregnancy, independent of exposure (Tab. 13, Part I).

Reliability:

(2) valid with restrictions
Test procedures in accordance with accepted standard methods, well documented

18-FEB-2003

(416)

Type:

other: Ocular toxicity / animal model

Remark:

1. In a preliminary study, F-344 rats fed control and folate-deficient diets and exposed to 1050 mg/m³ methanol (20 h/d, 7 d/wk, 13 weeks) showed degeneration of the retina and optic nerve under both conditions, but not yet after 4 weeks of exposure. However, Long-Evans rats fed either diet failed to develop any ocular lesion. The authors suggest that spontaneous degeneration was specific for F-344 rats and are therefore not suitable for ocular toxicity studies. (Lee et al. 1991).

2. To create an animal model for human methanol toxicity folate-deficient rats (male Long-Evans) were exposed to methanol (single oral administration of 2 - 3.5 g/kg) and formate accumulation in blood was measured.

Methanol concentrations in blood did not greatly differ between normal and folate deficient rats, while formate accumulated in folate-deficient but not in normal rats. Inhalation of 1200 or 2000 ppm for 6 hours led also to increased blood formate levels only in folate deficient rats.

If in fact formate accumulation is responsible for methanol toxicity the animal model may be suitable to test these effects. (Lee et al. 1994)

Test substance: Methanol, no further data.

15-DEC-2003

(417) (418)

Type: other: Ocular toxicity / formate /monkey

Method: Test design to elucidate the mechanism of methanol-induced toxicity syndrome and ocular toxicity: here influence of formate (McMartin et al., 1978).

Four male rhesus monkeys (Macaca mulatta) received buffered Naformate i.v. to a loading of 1.25 mmol/kg bw., with a mean infusion rate of 3.1 mEq/kg/h. This procedure produced no acidosis.

(see also other investigations of this group under 5.4)

Result: After 10 h, all animals accumulated formate in blood between 10 to 30 mEq/L. Blood pHs were maintained between 7.4 and 7.6. Pupillary reflexes were rapidly altered, and in most animals no response to light was observed between 24 and 48 h. Ophthalmology revealed marked optic disc edema (mainly in the prelaminar region, central portion of the proximal part of the optic nerve without significantly reaching to the distal part.

The retina including the ganglion-cell layer were completely normal. (McMartin et al., 1978)

Conclusion: These results with formate were completely consistent with those findings after methanol-intoxication (see other entries of this group under 5.4).

Formic acid and not formaldehyde (McMartin et al., 1979) has to be considered the causative agent for ocular damage within the methanol-intoxication syndrome, irrespective of acidosis.

The mechanism of formate toxicity may be seen in the

inhibition of oxidative phosphorylation by formate based on the on findings that this substance is an efficient inhibitor of cytochrome oxidase.

Reliability: (2) valid with restrictions

18-FEB-2003 (419) (183)

Type: other: Review

Remark: The reviewer describes the effects of methanol intoxication in humans. Methanol affects primarily the central nervous system involving the visual system up to optic atrophy. The minimal lethal dose in humans is reported to be 80 g. Methanol causes a variety of histopathological changes in the central nervous system and especially in the optic nerve.

Test substance: Methanol, no further data.

17-FEB-2003 (420)

Type: other: Review

Remark: The toxicity, kinetics and metabolism of methanol is reviewed. According to the reviewer methanol is readily resorbed by all exposure routes and metabolized in the liver firstly to formaldehyde and subsequently to CO₂. It is of moderate acute toxicity with the central nervous system and especially the optic nerve as primary target organs. Methanol was not carcinogenic in rats and mice in inhalation carcinogenicity studies but in reproductive toxicity studies it was teratogenic and embryotoxic in rats and mice.

Test substance: Methanol, no further data.

17-FEB-2003 (421)

Type: other: Review

Remark: Potential Health Effects of Gasoline and Its Constituents: A Review of Current Literature (1990 - 1997) on Toxicological Data

17-FEB-2003 (422)

Type: other: Risk Assessment Model / inhalation

Remark: Quantitative Models of Risk Assessment for Developmental Neurotoxicants, based on inhalation data in SD rats from Nelson et al., 1995:
The use of continuous foetal weight data was as sensitive as foetal brain malformation data in estimating excess risk to

methanol inhalation exposure in developing rat. Excess risk (assumed to be the additional risk that foetal weight is below the first percentile of control animals) was estimated by using a logistic model: 0.1 at 16000 ppm, 0.01 at 5400 ppm, and 0.001 at 980 ppm.

17-SEP-2002

(423) (225) (226)

Type: other: Toxicokinetics / ethanol influence

Method: Comparative study with ADH inhibitors: Male CD-1 mouse i.p injected 1 g/kg methanol followed by the inhibitor (i.p.). Elimination of methanol from blood was determined (t/2, AUC, mean elimination rate).

Result: Ethanol (4 g/kg) doubled the elimination half life as compared with control (approx. 9 vs. approx. 5 h); chloral hydrate (0.4 g/kg) had no significant impact on methanol elimination. But in combination (0.2 g/kg) with ethanol (4 g/kg), methanol elimination was retarded at a factor of >3 as compared with the control, which corresponded to about a twofold decrease of the elimination rate as compared with ethanol alone.

17-SEP-2002

(424)

Remark: Acute methanol poisoning in men and monkeys: latency, acidosis, ocular toxicity, not observed in most other species tested. Formic acid as likely causative agent is discussed: formate accumulation mainly due to limitation/low level in hepatic tetrahydrofolate along with a low 10formyl-THF dehydrogenase.

Test substance: Methanol, no further data.

18-FEB-2003

(270)

Method: In anesthetised dogs, the acute cardiovascular effect of methanol was investigated after i.v. infusion (20-% solution).

Remark: Methanol distributed rapidly in the body fluid.

Methanol concentrations of 1.3-2.0 mg/ ml blood reduced cardiac stroke volume, cardiac output per minute, systemic blood pressure and blood flow through the Arteria femoralis and Ateria carotis. On the other hand, peripheral resistance increased. Death caused by cardiac arrest came about at a methanol concentration in blood of 4 mg/ml.

Test substance: Methanol, no further data.

18-FEB-2003

(425)

Remark: In anesthetised dogs, methanol retention was about 85 % of inhaled methanol at exposure concentrations from 360 to 960 mg/m³. Absorption was dependent on ventilation rate.

Test substance: Methanol, "pure", no further data.

18-FEB-2003

(426)

Remark: In vitro study on enzyme preparations and in vivo: The influence of glutathione and other SH-compounds on the metabolic inactivation of the alcohol and aldehyde dehydrogenase by aldehyde and methanol was examined by poisoning male adult mice by an i.p. dose of 8.6 g/kg methanol. After 10 min, 1, 2 or 3 hours, the mice were treated either with 4 mg/kg 2,4-dimercaptopropanol, 120 mg cysteine or 27 mg mercaptoethanol. Mean survival time of methanol-treated control animals was no longer than about 10 hours. But following preventive treatment, mice survived considerably longer: the earlier the protective agent was applied, the longer was the survival up to 12-fold compared with the control with a few treated animals alive for 2 months (end of experiment).

Test substance: Methanol, from Fisher Scientific Comp., no further data.

Conclusion: These findings suggest that part of methanol intoxication in mice may be caused by formaldehyde-exerted inactivation of alcohol and aldehyde dehydrogenase, as SH-reagents are also effective in the mitigation of poisoning.

18-FEB-2003

(427)

Method: In this study, enzyme activities responsible for the oxidation of methanol to formate were investigated in liver homogenate and the eye of Long-Evans rats.

Remark: There was no evidence of an alcohol dehydrogenase-dependent metabolism of methanol in the liver and in the eye.

In liver, conversion of methanol was catalysed by an acid-labile catalase and acid-stable alcohol-oxidising enzyme. In the eye, there was only acid-labile catalase activity.

Km value(catalase, eye) = 366 mM,
Km value(catalase, liver) = 1.06 mM,
Vmax value Werte(catalase, eye) = 19.8 umol/h/g,
Vmax value Werte(catalase, liver) = 18.3 umol/h/g

Km value(alc-oxid. enzyme, liver) = 147 mM,

Vmax value(alc-oxid. enzyme, liver) = 30.5 umol/h/g tissue.

Metab. capacity(tissue weight xvmax)[eye] = 2.7 umol/h

Metab. capacity(tissue weight xvmax)[liver catal.] = 255

umol/h

Metab. capacity (tissue weight x vmax)[liver alc.oxid.] =

425 umol/h.

Test substance:

Methanol, no further data.

Conclusion:

The results demonstrate that acid-labile catalase is the sole active system in rat eye to metabolise methanol, while in liver both, catalase and an acid-stable alcohol-oxidising enzyme system significantly contribute to the biooxidation of methanol to formaldehyde.

18-FEB-2003

(428)

Remark:

The toxicity of methanol in relation to formate metabolism was investigated in isolated hepatocytes (Sprague-Dawley rats):

In isolated hepatocytes, formate arising from methanol is further oxidised by folate-dependent pathways (10-formyl-THF synthetase and 10-formyl-THF dehydrogenase) rather than by the catalase-peroxidase reactions.

In these experiments, oxidation rate of methanol to CO2 was significantly lower than found in vivo. After addition of methionine, formation of CO2 from methanol increased, which was ascribed to a restoration and stimulation of the formate oxidation and is based on the fact that in in-vitro liver preparations, the methionine content is at a factor of about 10 lower than in vivo.

In hepatocytes from folate-deficient rats, oxidation to formate was poor or absent even after addition of 5-formyltetrahydrofolate or folate, but could be restored by addition of methionine.

The functional role of methionine remains to be elucidated.

Test substance:

Methanol, radiolabelled from New England Nuclear, no further data.

18-FEB-2003

(429)

-
- Method:** Methanol was administered by gavage to normal and folate-deficient rhesus monkeys (*Macaca fascicularis*) at a dose of 3000 mg/kg and 2000 mg/kg bw, respectively. Formaldehyde and formate were analysed in blood, urine, and in the cerebral fluid, the orbit fluid of the eye, liver, kidney, brain, and optic nerve.
-
- Result:** There was no evidence of any formaldehyde increase at a time period when signs of acidosis and methanol intoxication occurred, while significant increases in formate were found in blood and other body fluids. For verification, one untreated monkey was infused ¹⁴C-radiolabelled formaldehyde (1mmol/kg bw, 0.2 M solution) over 3-4 min. The half-life time was 1.5 min.
-
- Test substance:** Methanol, radiolabelled from New England Nuclear, no further data.
- Conclusion:** Formaldehyde cannot be considered as the main factor in the development of methanol-induced toxicity.
- Reliability:** (2) valid with restrictions
- 18-FEB-2003 (419)
- Remark:** Human alcohol dehydrogenase exhibited a 10-fold higher Km value for methanol associated with a 10-fold lower activity than for ethanol. This striking difference suggests that there are different kinetic mechanisms of dehydrogenation of either alcohol.
- Test substance:** Methanol, purified, from Merck, Germany, no further data.
- 18-FEB-2003 (430)
- Remark:** Formic acid arising from methanol is eliminated at different rates by different species: most rapidly in rats with T/2 of approx. 12 min, then in rabbits with T/2 of about 32 min., dog with T/2 of 77 min., and human with T/2 of about 55 min.
- Higher folate levels in blood appear to correlate to lower formate levels. Pretreatment of dogs with amethopterin (folic-acid antagonist) induced a pronounced increase in formate of plasma and urine. On contrast, prophylactic administration of folic acid completely prevented formic-acid accumulation in blood.
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- Test substance:** Methanol, no further data.
- 15-FEB-2003 (431)

Test substance: Methanol, no further data.

15-FEB-2003 (268) (222) (422) (432) (433) (434) (435) (436) (437) (84) (438)
(439) (440) (187) (309) (300) (441) (442) (443) (444)

Remark: In alcohol-free persons, the endogenous methanol blood level ranges from 0.5 - 1 mg/l (1.5 mg/l not exceeded), the urine level from 0.32 - 2.61 mg/l, and the endogenous breath level between 0.21 - 0.79 ug/l. Long-term ingestion of significant amounts of ethanol (drinks, wine etc.), the background methanol levels may shift to higher values due to cumulation of endogenously formed methanol, for example through competitively inhibition of methanol by ethanol.

Test substance: Methanol, no further data.

15-FEB-2003 (445)

Remark: Penetration test in male human voluteers: Methanol, applied to the skin for 6 h on a soaked cotton patch under occlusive conditions was absorbed, through the human skin at significant amounts, and excretion into the urine increased after 2h with the maximum after 6 h.

Test substance: Methanol, no further data.

18-FEB-2003 (446)

Remark: Laquer thinner containing methanol, which is used for inhalation abuse, affected male fertility with a significant decrease in testicular and prostatic weights and plasma testosterone levels. Methanol exposure alone did not produce such effects.

Test substance: Methanol, no further data.

18-FEB-2003 (447)

Remark: The metabolism of formate was compared in monkeys and rats. Formate oxidation was 50 % lower in monkeys than in rats. The activity of a range of folate-dependent enzymes in liver tissue as well as the hepatic H4folate levels were compared. H4folate levels were 40 % lower in monkeys than in rats. Enzyme activities were partly higher and partly lower in monkeys than in rats, and their specific contribution to methanol toxicity could not be clarified.

Test substance: Methanol, no further data.

18-FEB-2003 (448)

Remark: A range of publications with respect to the role of formate toxicity in methanol toxicity in humans are reviewed. The difference of methanol toxicity in primates and rodents is suspected to derive from formate accumulation due to lower formate metabolism. In fact humans and monkeys possess low hepatic H4folate levels and low rates of formate oxidation. Formate itself produces blindness in monkeys, the major toxic effect of methanol.

Test substance: Methanol, no further data.

18-FEB-2003

(270)

Remark: The role of liver catalase activity and H2O2 formation on methanol oxidation in the liver is discussed. According to the author the methanol oxidation is mainly dependent on the rate of H2O2 generation, despite the fact that catalase plays a role in methanol metabolism in liver tissue.

Test substance: Methanol, no further data.

17-FEB-2003

(449)

Remark: The pigtail monkey was tested for effects of toxic methanol exposure to establish an animal model for human methanol toxicity. Blood acidosis was measured as major toxic effect of methanol and the responsible acid was identified. In pigtail monkeys but not in rhesus monkeys significant blood acidosis was produced by a single i.p. dose of 4 g/kg bodyweight methanol. Blood concentration of formate was correlated with acidosis, no other organic acid was found at increased levels in acidotic monkeys.

Test substance: Methanol, reagent grade, no further data.

Reliability: (2) valid with restrictions

18-FEB-2003

(162)

Remark: Male rats were exposed to methanol at 200 ppm (0.26 mg/l) for up to 6 weeks (8 hours/day, 5 days/week) did not show an effect on serum testosterone levels. Exposure to 800 ppm (1.04 mg/l) for up to 13 weeks had no effect on testes to body weight ratio and had no effect on testicular morphology in normal and folate deficient rats. A greater incidence of testicular degeneration was seen only in 18 month old folate-deficient rats exposed to 800 ppm methanol for 13 weeks (20 hours/day, 7 days/week, 12 - 13 rats/group). The results are equivocal.

Test substance: Methanol, no further data.

18-FEB-2003

(450)

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- Remark:** Male rats were exposed to methanol at 200 ppm (0.26 mg/l) for 6 hour/day for up to 1 week. Compared to controls methanol treatment for 1 week led to decreased plasma levels of testosterone, luteinizing hormone and corticosterone. The changes were not statistically significant.
- Test substance:** Methanol, no further data.
- 18-FEB-2003 (190)
- Remark:** 81 - 88 % of inhaled methanol was retained in the respiratory tract in dogs. The inhaled fraction was related to the concentration in air with a 73 % retention at 390 mg/m³ and a 90 % retention at 720 mg/m³.
- Test substance:** Methanol, no further data.
- Reliability:** (2) valid with restrictions
- 18-FEB-2003 (426)
- Remark:** Methanol is metabolised in rodents and primates in different ways and different speed. In rodents methanol is primarily metabolised to formaldehyde by the catalase-peroxidase system. The V_{max} value was determined at 30 mg/kg/hour. In humans and monkeys the alcohol dehydrogenase oxidizes methanol to formaldehyde more rapidly. The V_{max} value in primates is 48 mg/kg/hour. Formaldehyde is finally oxidized to CO₂ by folate dependent enzymes. Primates have less folate than rodents and therefore the detoxication of methanol may be limited by the last steps of metabolism in primates.
- Test substance:** Methanol, no further data.
- 18-FEB-2003 (451)
- Remark:** 1. A high dose of methanol (single 3 g/kg, oral) to male Wistar rats gave some evidence of increased LPO with a transient time-dependent increase in MDA in liver and serum (about +20 % and 40 %, respectively, reversed after 7 d) and with transient decrease in components of the antioxidant system: GSH in liver after 12 h and 2 d, recovered at day 5 (in between no data), various enzymes also showed a transient decrease (Cu,Zn-Superoxide dismutase, GSH-Peroxidase, GSSG-Reductase).
- Blood methanol peak was ca. 2300 ug/ml after 6 h and rapidly declined (Skrzydowska et al. 1997, 1998).

2. After oral doses (1.5, 3, 6 g/kg bw) to male Wistar

rats, the activity of proteases (elöastase and cathepsin G) were increased from 12 h to 5 d in relation to the methanol dose. Concomitantly, the activity of their inhibitors was reduced. Similar disorders in the proteolytic-antiproteolytic balance in favour of protease action can also be observed in human acute intoxications with methanol. The further implications are addressed: blood platelet action, protein degradation, release of anaphylotoxins and other peptides (e.g. kinins) including vasoactive mediators, activation of other proteases etc. (Skrzydowska et al. 1999).

3. The effect of methanol intoxication (6 g/kg bw) on protein modification in the liver of rats was investigated. An increase in free radicals were determined 6 and 12 hours after intoxication.

Malondialdehyde and carbonyl groups in protein were increased; the levels of amino groups and sulphhydryl groups and the amount of tryptophan in proteins were decreased, whereas the amount of bi-tyrosine was increased. According to the authors changes in the protein structure resulted both from free radical action and formaldehyde generation during methanol intoxication (Skrzydowska et al., 2000).

15-FEB-2003

(452) (453) (454) (455) (456) (457)

Remark:

The PBPK approach was used to develop a model of methanol disposition during gestation in rats and mice. To validate this model, concentrations of methanol in the dams and the conceptus were determined after methanol exposure of rats on GD 14 and 20 and of mice on gd 18.

In pregnant animals, conceptual/maternal AUC and C_{max}-ratios decreased with increasing dose at both gd 14 and 20 and at gd 18 in the mouse. Additionally, the conceptual/maternal diffusion-constant ratio consistently decreased with increasing dose in pregnant rats and mice.

These results are consistent with earlier observations that methanol limits its own delivery to the conceptus.

It was further shown that the maximal rate of methanol elimination (v_{max}) in vivo decreased at term in both species with 65 to 80 % of that in non-pregnant rodents. This was also found in vitro in rodent liver homogenates. This appears to suggest that not only altered disposition but also a change in metabolic capacities makes the difference in gestation. Fetal rodent liver was capable of metabolizing methanol in vitro, but only at a rate of <5 % of respective

adult livers. (Ward and Pollack, 1996; Pollack and Brouwer, 1996)
Reliability: (2) valid with restrictions

15-FEB-2003 (387) (458) (459)

Method: In-vitro study on human hepatic enzyme fraction to examine various inhibitors of ADH with methanol as substrate

Result: In methanol intoxication, cimetidine may potentially be a better antidote than 4-methylpyrazole because of its slightly higher inhibitory activity in particular at higher methanol concentrations.

18-FEB-2003 (460)

Remark: According to Caprino and Togna, 1998:

"Intoxication [of methanol] has delayed onset characterized by acidosis, mental confusion, ocular toxicity with visual disturbance [recently suggested as due to intraretinal metabolism of methanol, rather than to elevated blood formate levels (Garner et al., 1995a,b)], reversible or permanent blindness, and, in severe cases, death. These effects represent a classic example of lethal synthesis in which toxic metabolites can cause fatality after characteristic latency period."

.....

"...no interactive effects between methanol and gasoline were found in a short-term inhalation study in rats (Poon et al., 1995), and a study on perinatal exposure to low concentrations of methanol resulted... in no significant abnormalities in the brain of treated rats (Stern et al., 1996)."

"There is little evidence from available information of human health effects from low-level exposure which demonstrates that methanol vapors from motor vehicle fuel can cause acute adverse effects to health. Lee et al. (1992) demonstrated that after 6-hr exposure to current methanol threshold limit value (TLV) of 200 ppm, the formate does not accumulate in blood in human subjects at rest and during exercise. This was confirmed by other authors (D'Alessandro et al., 1994; Osterloh et al., 1996)."

15-FEB-2003 (422) (383) (384) (191) (281) (461) (232)

Remark: Compilation of human case reports on acute methanol intoxications with a collection of the blood levels found in sublethal and lethal events at various time intervals after ingestion.

A provisional nomogram has been derived, beginning at a 3-h concentration of 20 mM and ending at a 130-h concentration of 0.5 mM. Concentrations at any time-point above this straight line result either in life-threatening poisoning or a lethal outcome.

15-FEB-2003

(462)

Result: After oral application of methanol (3 g/kg bw), the thiobarbituric acid-reactive substances (TAS) indicating lipid peroxidation were significantly increased in brain tissue from 6 h to 7 d after treatment, also activities of liver GSH peroxidase and GSH reductase were reduced after 6 h already.

N-Acetylcysteine exerted a protective antioxidant effect in the brain and liver of methanol-treated rats: TAS remained on the background-level after prior i.p. treatment with N-acetylcysteine (150 mg/kg). It resulted in a lower degree of parenchymal damage (Kasacka and Skrzydlewska, 2001).

Furthermore, concomitantly, methanol-induced GSH-depletion, which was significant after 24 h and steadily increased until 7 d, could be mitigated, and enzyme activities involved in the removal of reactive oxygen species were also similar to the untreated control while significantly increased without protective counteraction.

A Trolox-Derivative, an analog of alpha-tocopherol, is introduced as a beneficial antioxidant which prevents or reduces methanol-induced LPO in Wistar rats (Skrzydlewska and Farbiszewski, 1997).

18-FEB-2003

(463) (464) (465) (466)

15-FEB-2003

(241) (242) (389) (191) (243) (255)

Method: Comprehensive study programme on three species (rat, mouse, monkey) including metabolic, pharmacokinetic and short-, long-term and reproduction studies and carcinogenicity studies:
Here two-generation reproduction study in rats (see other entry, 5.8.2).

In 9-week old F1 pups, blood methanol was measured, but not formate (p. 191). Exposure: continuous for 19 to 20 h/d.

Remark: Blood levels of methanol measured in the F1 offsprings (age 9 weeks) (NEDO, 1987, p. 191):

controls (baseline): approx. 2 - 3 ug/ml
10 ppm methanol: approx. 3 - 3.5 ug/ml
100 ppm " : approx. 1 - 4.2 ug/ml
1000 ppm " : approx. 53 (males)-100 (females) ug/ml.

There no data on formate.

Reliability: (2) valid with restrictions
Test procedures in accordance with accepted standard methods, sufficiently documented

Flag: Critical study for SIDS endpoint

18-FEB-2003 (161)

Remark: Under in-vitro conditions, in the presence of liver microsomes or liver nuclei fraction, hydroxymethyl radicals were formed from methanol. This bioactivation was observed only under anaerobic conditions which also resulted in the production of formaldehyde. Formaldehyde was the primary intermediate during aerobic incubation. But both formaldehyde-generating processes appear to be different, because the anaerobic one was strongly inhibited by diphenylene iodonium, while the aerobic one was not.

18-FEB-2003 (467)

Result: Male rats were exposed to methanol vapour at concentrations of 200, 5000 or 10000 ppm (0.26, 6.5 or 13 mg/l) for 6 hours and killed immediately after exposure or at 24 hours. Rats were divided in acclimated (2 weeks prior to handling) and non-acclimated groups. At 6 h, sham-exposed rats already showed an increase in the level of serum LH. Effects on serum hormone levels (luteinizing hormone, follicle stimulating hormone, testosterone, prolactine) were inconsistent with partly no effects and partly contradictory results. According to the author, methanol treatment led only to increased prolactin levels in serum under both

acclimated and non-acclimated conditions.

Test substance: Methanol, no further data.

18-FEB-2003 (148)

Result: The oral administration of ¹⁴C-methanol to rats is reported to have resulted in covalent binding to hemoglobin. A linear relationship from 10 to 100 umol/kg was found.

Reliability: (4) not assignable
Only abstract, no detailed data.

15-FEB-2003 (215)

Remark: In clinical cases, the methanol level is low in the last phase of poisoning.

In Rhesus monkeys given a single methanol dose of 1 g/kg, 37 mg(kg*h) was oxidized (Makar et al, 1968).

Provided that the rate is the same in man, the amount of methanol oxidized during 18 h (the average time needed for development of severe acidosis in clinical cases) would be 0.7 g/kg.

Acc. to Roe (1982), it seems reasonable to regard 1 g/kg of methanol as the approximate minimal lethal dose in man.

Reliability: (4) not assignable
Secondary source

Flag: Critical study for SIDS endpoint

18-FEB-2003 (468) (300)

Remark: In Rhesus monkeys given a single methanol dose of 1 g/kg, 37 mg(kg*h) was oxidized (Makar et al, 1968).

Reliability: (4) not assignable

18-FEB-2003 (468) (300)

6.1 Analytical Methods

-

6.2 Detection and Identification

-

7.1 Function

-

7.2 Effects on Organisms to be Controlled

-

7.3 Organisms to be Protected

-

7.4 User

-

7.5 Resistance

-

8.1 Methods Handling and Storing

-

8.2 Fire Guidance

-

8.3 Emergency Measures

-

8.4 Possib. of Rendering Subst. Harmless

-

8.5 Waste Management

-

8.6 Side-effects Detection

-

8.7 Substance Registered as Dangerous for Ground Water

-

8.8 Reactivity Towards Container Material

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10.1 End Point Summary

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10.2 Hazard Summary

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10.3 Risk Assessment

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