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CRITERIA DOCUMENTS FROM THE NORDIC EXPERT GROUP 1991

Brita Beije och Per Lundberg (Eds)

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PREFACE

The Nordic Council is an international body for the governments in the five countries, Denmark, Finland, Iceland, Norway and Sweden. One of the committees within the Nordic Council, the Nordic Senior Executive Committee for Occupational Environment Matters, initiated a project with a view to compiling and evaluating scientific information on chemical agents relevant to health and safety at work and the production of criteria documents. The documents are meant to be used by the regulatory authorities in the Nordic countries as a scientific basis for the setting of national occupational exposure limits.

The management of the project is given to a group of scientists: The Nordic Expert Group for Documentation of Occupational Exposure Limits. At present the Expert Group consists of the following members:

Helgi Gudbergsson	Municipal Institute of Public Health, Iceland
Per Lundberg (Chairman)	National Institute of Occupational Health, Sweden
Petter Kristensen	National Institute of Occupational Health, Norway
Vesa Riihimäki	Institute of Occupational Health, Finland
Adolf Schaich Fries	National Institute of Occupational Health, Denmark

The secretariat is located at the National Institute of Occupational Health, S-171 84 Solna, Sweden.

The criteria documents aim at establishing a dose-response/dose-effect relationship and a critical effect, based on published scientific literature. The task is not to give a proposal for a numerical exposure limit value.

The literature is evaluated and a draft is written by a scientist appointed by the Expert Group with the support and guidance of one member of the group. The draft is then sent for a peer review to experts by the secretariat. Ultimately the draft is discussed and revised at the Expert Group Meeting before it is accepted as their document.

Only studies considered to be valid and reliable as well as significant for the discussion have been referred to. Concentrations in air are given in mg/m^3 and in biological media in mol/l or mg/kg. In case they are given otherwise in the original articles they are, if possible, recalculated and the original values are given within brackets.

This volume consists of English translations of the criteria documents which have been published in a Scandinavian language during 1991. The names of the scientists who have written the separate documents are given in the list of contents, where aslo the dates of acceptance by the Expert Group are given.

Solna in December 1991

Brita Beije
Secretary

Per Lundberg
Chairman

CONTENTS

Dimethylethylamine

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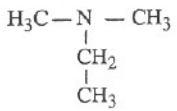
CONTENTS

1. PHYSICAL AND CHEMICAL DATA

Systematic name	N, N-Dimethylethylamine (DMEA)
Synonym	Ethyldimethylamine
CAS number	598-56-1
Formula	$C_4H_{11}N$
Structural formula	
Molecular weight	73.1
Vapour pressure at 20 °C	55.2 kPa (414 mm Hg)
Boiling point	37 °C (101.3 kPa)
Melting point	-140 °C
Flash point	-36 °C (closed cup)
Density	0.675
Odour threshold	$7.6 \cdot 10^{-3}$ ppm (0.023 mg/m^3)
Partition coefficient (K_{ow}) (octanol/water)	5.0
Conversion factors	1 ppm = 3 mg/m^3; 1 mg/m^3 = 0.33 ppm

DMEA is a colourless, volatile and very flammable liquid with an unpleasant fish/ammonia odour. It is a strong base, pKa 10.2. DMEA is soluble in water and most organic solvents (2, 13, 24).

2. OCCURRENCE, USES

2.1. Uses

DMEA is used, as catalyst, in the polymerization of polyurethanes and polyamides (4). The main application of DMEA is in foundries in the production of mould cores. The mould cores are produced of a mixture of sand, phenol resin and isocyanate into which the gaseous DMEA is introduced [Cold-box; Isocure®; Ashland technique; (6)].

2.2. Occupational exposure

About ten studies of occupational exposure to DMEA among workers in gray-iron, steel, and aluminum casting foundries have been reported during the last 15 years (5, 10, 12, 16-18, 20-22, 25; table 1). The full-shift time-weighted averages range from not detectable to 47 mg/m^3. The highest concentration in a workers breathing zone is 125 mg/m^3 [1-h sampling period (22)]. Samples collected in work area show DMEA air concentrations of 155 (9) and 186 (19) mg/m^3, respectively.

N-nitrosodimethylamine and N-nitrosomethylethylamine have been detected in the work area of the core shops when using DMEA in the Ashland technique. However, it has not been shown whether the nitrosamines originate from transformation of DMEA or from impurities in the DMEA (7).

2.3.1. Measurement of dimethylethylamine in the air

Sampling of DMEA in the air may be performed either on solid sorbents, such as silica gel (9), acid-treated silica gel (8) or charcoal (3), or in acidic absorption solution (9, 11). DMEA is desorbed from the solid adsorbents by an organic solvent (e.g. methanol, dichloromethane). Analysis of DMEA in the air samples are performed, without employing derivatization steps, by gas chromatographic methods, employing flame ionisation (8) or nitrogen-phosphorus detectors (3, 11) or by isotacophoresis (9). The detection limit of these methods correspond to a DMEA air concentration of ≤ 0.1 mg/m^3.

2.3.2. Measurement of dimethylethylamine in biological samples

Analysis of DMEA in blood and urine samples may be performed by adding potassium hydroxide to the samples and then extracting the DMEA by an organic solvent (e.g. di-butyl ether). The solvent phase is analysed with a gas chromatographic method, using a nitrogen-phosphorus detector. The detection limit for DMEA in blood and urine samples are 0.04 and 0.1 µmol/l, respectively (11).

3. KINETICS

3.1 Uptake

Four volunteers experimentally exposed (10, 20, 40 and 50 mg/m^3) during 8 h to DMEA showed an uptake of 81 - 94% (23). Orally administered DMEA was recovered to 90% during 24 h after administration. Uptake through the skin has not been studied.

3.2. Distribution

The volume of distribution of DMEA was in average 310 l, which is about 5 times the body water content (23). This must be due to at least one reservoir of DMEA somewhere in the body, in which the DMEA concentration was well above the average.

3.3. Biotransformation

In man, DMEA was biotransformed into dimethylethylamine-N-oxide (DMEAO). In an experimental (23), as well as an occupational (12), study, the metabolite DMEAO represented 90% of the total DMEA and DMEAO excreted in the urine.

The enzyme (monooxygenase) responsible for N-oxygenation of DMEA to DMEAO has not yet been identified. It is, however, generally accepted that the N-oxygenation of tertiary aliphatic amines is attributed to the flavin-containing monooxygenase [FMO; "Ziegler´s enzyme" (27, 28)]. The N-oxygenation requires NADPH, as coenzyme, and oxygen. Primary aliphatic amines increase the activity of FMO. Dealkylation of DMEA into dimethylamine or methylethylamine has not been found (12, 23).

3.4 Elimination

Elimination of DMEA through the lungs is minimal. One hour after the termination of an 8-h experimental exposure period (10, 20, 40 eller 50 mg/m^3), the DMEA concentration in the exhaled air was about 0.3 mg/m^3. The concentration in the exhaled air was independant of the exposure level and was probably originating from DMEA absorbed in the mucous membranes of the respiratory tract and not from an elimination through the lungs (23).

The elimination of DMEA from the body occurrs mainly by urinary excretion of the metabolite DMEAO. However, about 10 % of total DMEA excreted in the urine is the mother compound. The capacity of the body to eliminate DMEA has been studied in four volunteers (23). The total clearance (plasma clearance) was calculated to 125-280 l/h (mean 170 l/h), renal clearance 7-39 l/h (mean 16 l/h) and metabolic (non-renal) clearance 110-260 l/h (mean 160 l/min). The metabolite, DMEAO, which is also eliminated through urinary excretion, showed a renal clearance of 7-18 l/h (mean 12 l/h). The clearance of DMEA was not dependant on the level of exposure. The urinary pH, however, affected the clearance of unmetabolised DMEA. Thus, a decrease in the urinary pH increased the renal clearance and the elimination of DMEA from the body. Furthermore, it resulted in a smaller fraction of DMEAO in the urine. The metabolic clearance of DMEA and the renal clearance of DMEAO were not affected by the urinary pH.

After termination of the exposure the elimination curves of DMEA and DMEAO displayed a two-phase pattern. The half-lives for DMEA were 1.5 and 7 h, and for DMEAO 2.5 and 8 h, respectively (12, 23).

Elimination through faeces has not been studied.

3.5. Biological exposure indicators

The half-lives for DMEA and its metabolite DMEAO in the body were 1.3 and 3 h, respectively, as determined by the slope of the semi-logaritmic plasma concentration-time curve (23).

There was a good correlation between the DMEA air concentration and the concentration of DMEA+DMEAO in plasma at the end of the exposure. There was also a close correlation between the DMEA air concentration and the urinary excretion of DMEA+DMEAO. Experimental exposure (light-moderate physical work) to 10 mg DMEA/m^3 during 8 h displayed a concentration of DMEA+DMEAO in plasma of 4.9 µmol/l and a 2-h post-shift excretion of DMEA+DMEAO in urine of 75 mmol/mol creatinine (23). The corresponding concentrations after occupational exposure (moderate-heavy work) to 10 mg DMEA/m^3 were 5.7 µmol/l and 135 mmol/mol creatinine, respectively (12).

4. GENERAL TOXICOLOGY

DMEA is a very corrosive amine thus, having a direct toxic effect (1). The lipid solubility of DMEA (octanol/water = 5) is less than that of other amines used in the industrial setting. Thus, DMEA has a lesser ability to cross biological membranes than these other amines. (22).

5. EFFECTS ON ORGANS

5.1. Effects on skin and mucous membranes

No studies of the effects on the skin of DMEA in man or animals have not been reported.

Volunteers exposed experimentally to DMEA, 8 h to 40 - 50 mg/m^3, or 15 min to 80 and 160 mg/m^3, respectively, showed eye irritation (22). Eight hours expoure to DMEA of 10 - 20 mg/m^3 did not cause irritation of the eye (table 2).

Studies of occupationally exposed workers record eye irritation after 8-h exposure to DMEA of 1 to 47 mg/m^3, including peak exposure of 125 mg/m^3 [table 1; (16, 17, 20-22)].

5.2. Effects on respiratory organs

Core workers (n=30) handling the Ashland technique with DMEA as catalyst ran an

increased risk of bronchial obstruction than other workers (n=29) of the same foundry (14). The DMEA air concentration was not reported.

In two studies of occupational exposure to DMEA [0-47 mg/m^3; n=9 (16) and 4-26 mg/m^3; n=26 (10), respectively] pulmonary function tests were performed by forced vital capacity (FVC), forced expiratory volume in one second ($FEV_{1,0}$), and maximal expiratory flow after 0.5 second (MMEF) in the core workers. The pre-shift and post-shift pulmonary function tests showed no changes.

5.3. Effects on the liver

No information is available.

5.4. Effects on the kidneys

No information is available.

5.5. Effects on the gastrointestinal tract

No relevant studies have been reported. Subjective symptoms recorded concomitantly with studies of the DMEA air concentration in core shops are shown in table 1.

5.6. Effects on the heart and circulatory system

Core workers displayed more often high blood pressure than other workers from the same foundry (18).

5.7. Effects on blood and blood forming organs

No information is available.

5.8. Effects on central and peripheral nervous system

Core workers exposed to DMEA recorded more often subjective facial pains (trigeminus-neuralgia; tic douloureux) than other workers from the same foundry (18).

Studies, *in vitro*, of the effects on guinea pig ileum showed that DMEA caused contractions of the ileum. The effects were probably a direct stimulation of the nicotinic receptors in the ganglions (15).

5.9. Effects on other organs

Ophthalmological examinations of four volunteers after experimental exposure to DMEA disclosed epithelial corneal edema in parts of the cornea not covered by the eyelid. A discrete increase of the thickness of the cornea was also recorded (22). These effects caused visual disturbances (hazy vision and halo effect). A slight conjunctival injection was seen in the affected subjects. The symptoms lasted for some hours after the end of exposure and then faded gradually (22). The subjective symptoms, hazy vision and halo effect, have also been reported after ocuppational exposure to DMEA [table 1; (18, 20-22)].

Mydriasis (dilated pupils, not responsive to light) has been reported in the manufacturing of pharmaceuticals. The exposure is unclear, as the affected workers were not only exposed to DMEA, but also to triethylamine and chloro-substituted homologues of both amines (26).

6. IMMUNOTOXICITY AND ALLERGY

No information is available.

7. MUTAGENICITY AND GENOTOXICITY

No information is available.

8. CARCINOGENICITY

No information is available.

9. REPRODUCTION AND TERATOGENICITY

No information is available.

10. EXPOSURE, EFFECT AND RESPONSE RELATIONSHIPS

10.1. Effects of short-term exposure

The DMEA air concentrations reported in occupational settings are shown in table 1 together with symptoms reported during the sample collection (20-22) or with symptoms frequently occurring during the last year (16-18). In table 2 are shown the effects on the eyes of volunteers after experimental exposure to DMEA (22).

Tabell 1. Air concentration of DMEA and recorded symptoms.

Reference	DMEA (mg/m^3)			Symptom
	Breathing zone		Area samples	
	Full shift	Part of shift		
16	nd-47	nd-105 (4 h)	-	Eye, nose, and throat irritation. Headache. Discomfort in the chest.
17	2.2-35	nd-46 (1 h)	1-9	Eye irritation. Headache, dizziness and stomach pain.
18	0.3-35	-	-	Haze. Halo effect. Core workers had more often high blood pressure and neuralgia than other foundry workers.
22	0.5-28	0.1-125 (1 h)	-	Visual disturbances, eye irritation and corneal edema.
10	4-26	-	9	No recorded symptoms.
21	1.6-24	nd-29 (15 min)	2-4	Visual disturbances and eye irritation. Watery eyes. Nausea, headache and other systemic effects.
20	1.2-2.3	-	1.8-2.1	Headache. Eye and nose irritation. Visual disturbances. Cough. Nausea and dizziness.

nd = not detectable

Tabell 2. Effects on the eyes after experimental exposure to DMEA of four volunteers (22).

Exposure		Eye irritation (n)	Visual disturbances (n)	Latency[1] (h)	Corneal edema (n)	Duration[2] of visual disturbances (h)
Air concentration (mg/m^3)	Time					
10	8 h	0	0	-	0	-
20	8 h	0[3]	0[3]	-	0[3]	-
40	8 h	3	1	6	1	1
50	8 h	3	4	3-7	3	1-3
80	15 min	3	0	-	0	-
160	15 min	3	0	-	0	-

1 = time from start of exposure to confirmation of visual disturbances
2 = time from end of exposure until the visual disturbances ceased
3 = Two subjects only were exposed to 20 mg/m^3

10.2. Effects of long-term exposure

No information is available.

11. RESEARCH NEEDS

It is important to evaluate whether DMEA has a systemic effect in man or not, e.g. on the nervous system, respiratory organs, and the heart. Studies of the toxic effects of DMEA on the skin, the uptake of DMEA through to the skin, and the synergistic effects of DMEA with other compounds are also needed. The mutagenic, genotoxic, carcinogenic, and teratogenic effects of DMEA have not yet been studied.

12. DISCUSSION AND EVALUATION

Experimental exposure to 40-50 mg DMEA/m^3 caused corneal edema, eye irritation, and visual disturbances. The effects reported after occupational exposure to, as low as, 1 mg DMEA/m^3 may be caused by peak exposure; the corebox mashines may leak 20 % gaseous DMEA.

Exposure to 10 mg/m^3 caused a slight increase of the corneal thickness but no visual disturbances. Mydriasis has been reported after exposure to DMEA, but are probably caused by concomitant exposure to the much more toxic chloro-substituted homologues of DMEA.

There is no sign of persistent effects on the eyes. Visual disturbances, however, are unpleasant and may increase the risk of accidents at work and in the traffic after work.

DMEA may have an increasing effect on the blood pressure and may also affect the trigeminus.

The critical effects of DMEA are on the eyes.

13. SUMMARY

B. Åkesson: Dimethylethylamine. Nordic Expert Group for Documentation of Occupational Exposure Limits. Arbete och Hälsa 1991.

A critical survey and evaluation of the relevant literature, to be used as a basis for establishing an occupational exposure limit for dimethylethylamine, is presented.

Dimethylethylamine causes irritation on the mucous membranes of the eye and has a toxic effect on the cornea involving corneal edema with an increase of the thickness of the cornea. Subjective symtoms are visual disturbances (foggy vision) and a halo effect. Experimental exposure to dimethylethylamine levels of 40 - 50 mg/m^3 caused eye irritation, corneal edema and visual disturbances. An increase of the corneal thickness without subjective symptoms was observed already at 10 mg/m^3. Effects reported after much lower levels of occupational exposure to dimethylethylamine are most probably due to short, high exposure peaks. Thus, the critical effect of occupational exposure to DMEA is the effect on the eyes.

In English. 28 references.

Key words: Dimethylethylamine, exposure, visual disturbances, corneal edema, corneal thickness, eye irritation, metabolism, dimethylethylamine-N-oxide, occupational exposure limit, hypertonia, nervous system.

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Appendix 1.

Occupational threshold limit values for dimethylethylamine in some countries.

Country	mg/m^3	ppm	Year	Ref.
Denmark	75	25	1988	(1)
Finland	-	-	1987	(2)
France	15	5	1988	(3)
	75[1)]	25[1)]		
Germany	75[2)]	25[2)]	1990	(4)
Great Britain	-	-	1988	(5)
Iceland	75	25	1978	(6)
Netherlands	75	25	1989	(7)
Norway	60	20	1989	(8)
Sweden	-	-	1990	(9)
USA (ACGIH)	-	-	1990-91	(10)
(NIOSH)	-	-	1990-91	(11)

1) 15 minutes peak exposure
2) MAK i.e. 10 minutes peak exposure
- = no threshold value

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Isophorone

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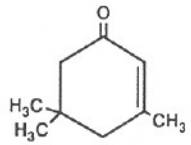
Contents

1. Physical and Chemical Data

Chemical name:	3,5,5-Trimethyl-2-cyclohexene-1-on
CAS No:	78-59-1
Synonyms:	Isophorone, Trimethylhexenone, Isoacetofenone, α-Isophorone
Formula:	$C_9H_{14}O$
Structure:	



Molecular weight:	138,2
Melting point:	-8,1° C
Boiling point:	215° C
Vapor pressure (20° C):	0,03 kPa
Density (20° C):	0,92 g/ml
Conversion factors:	1 mg/m^3 = 0.174 ppm 1 ppm = 5.744 mg/m^3

Isophorone at room temperature is a colourless liquid with a peppermint-like odour. The reported odour threshold is 0.2 ppm (1.15 mg/m^3) (1).

Isophorone is soluble in water and mixes with most organic solvents.

2. Occurrences and Uses

2.1. Uses and air concentrations in the working environment

Isophorone is an excellent solvent for many types of fat and oil. It is used as a solvent for lacquer, ink and thinner. In ink and thinner, used in a screen printing plant, the concentration of isophorone was between 10 and 75 % (19).

When determining air concentrations of solvents in a screen printing plant, isophorone levels were between 3.5 ± 1.8 and 14.5 ± 6.2 ppm (20.1 ± 10.3 and 83.3 ± 35.6 mg/m^3 respectively) at different manufacturing processes. In the breathing zone, concentrations of 8.3 ± 5.6 to 17.8 ± 5.5 ppm (47.7 ± 32.2 and 102.2 ± 31.6 mg/m^3, respectively) were recorded. The sampling time was 50-90 min and reported values are time-weighted averages (19).

2.2. Methods for the analysis of air concentrations

Isophorone in air is collected on a charcoal tube, using a 12-liter sample. Sampling at an air flow of 0.2 l/min is recommended. After desorption with carbon disulfide the sample is analyzed by gas chromatography. The method is considered suitable for air concentrations in the range 2 to 400 mg/m^3 (15, 26).

Lately other solid adsorbents than charcoal have been used. When comparing different adsorbents, XAD polymers are recommended prior to charcoal mainly because isophorone decomposes on charcoal adsorbent during storage (11).

Another method involves adsorption on porous polymers (Tenax-GC), thermal desorption and analysis by gas chromatography. The method has a "safe" sampling volume of 3700 liter (28 000 l/g of adsorbent) and a suitable desorption temperature is 170° C (3).

3. Kinetics

3.1. Uptake, biotransformation and elimination

There are no data on uptake, biotransformation or elimination of isophorone in man.

A single dose of isophorone (1 g/kg bw) given by oral gavage to rabbits was partly excreted in exhaled air. In urine, collected during 48 h, unmetabolized isophorone was found. Furthermore, 5,5-dimethyl cyclohexene-3-one and 5,5-dimethyl cyclohexene-1-carboxy acid, both formed by methyl oxidation of isophorone, were analyzed. Isophorol, formed by reduction of the keto-moiety and excreted as a glucuronic conjugate, as well as dihydroisophorone and cis-and trans-trimethyl-3,5,5-cyclohexanol were also found in the urine. The latter is probably formed via dihydroisophorone by dismutation (8, 24).

3.2. Biological exposure indicators

There are no methods available.

4. General Toxicology

The following toxicity data are reported from animal studies (16).

LD_{50} rat	oral	2330 mg/kg bw
LD_{50} rat	skin	1500 mg/kg bw
LC_{20} rat	inhalation, 4 h	1840 ppm (10569 mg/m^3)

In a study (22) 10 rats and 10 guinea pigs were exposed for 6 weeks (8h/day; 5 days/week) to 0, 25, 50, 100, 200 och 500 ppm isophorone (0, 144, 287, 574, 1149 and 2872 mg/m^3). At the highest dose level 9 out of 20 animals died. At 200 ppm 3 out of 18 died and at 100 ppm 2 out of 16 animals died. At all three dose levels, growth rate was reduced.

The LD_{50} value of isophorone in combination with other organic chemicals has been determined. Totally 26 chemicals were tested in combination vith isophorone. In each test-pair a 50% (v/v) mixture was intubated in rats. The results indicate an additive effect (23).

5. Effects on Organs

5.1. Effects on skin and mucous membranes

In a study (20) twelve subjects were exposed to isophorone for 15 min. At 10 ppm (57,4 mg/m^3) 40% of the subjects objected to the odour but the majority indicated that they could accept exposure for 8 h. At 25 ppm (144 mg/m^3) 70% objected to the odour , and irritation of the eyes, nose and throat was seen in a majority of the subjects.

In Patty´s Industrial Hygiene (10) there is a notation, without reference, that occupational exposure to 40 ppm isophorone (230 mg/m^3) or more is irritative to eyes, nose and throat.

A decrease in respiratory rate in mice has been used as an index of sensory irritation. A recording was made 10 min before exposure and during 5 min exposure. At least four different concentrations of the test substance were used and the RD_{50} value was then calculated. The RD_{50} value represents the concentration associated with a 50 % decrease in the respiratory rate. For isophorone, the RD_{50} value was calculated to be 27.8 ppm (159.7 mg/m^3) (6).

The application of 0.5 ml isophorone is reported to have a mild irritative effect on rabbit skin (25).

5.2. Effects on respiratory organs

There are no data on humans.

From animal studies there are reports on congested lungs and pneumonia in rats and guinea pigs exposed to 500 ppm isophorone (2872 mg/m^3) for 6 weeks, 8 h/d, 5 d/w (22).

5.3. Effects on liver

There are no data on humans.

In rats and guinea pigs exposed for 6 weeks, 8 h/d, 5 d/w to 500 ppm isophorone(2 872 mg/m^3), congestion and cloudy swelling were reported in the liver cells (22).

5.4. Effects on kidneys

There is no information regarding man.

When rats and guinea pigs were exposed to 50 ppm isophorone (287 mg/m^3) or more for 6 weeks, 8 h/d, 5 d/w, pathological changes of the kidneys were seen. Dilatation of Bowman's capsule and cloudy swelling of tubular epithelium were typical injuries. At 500 ppm (2872 mg/m^3) kidneys were congested and necrosis

of the epithelium was also reported. At this dose, albumin was excreted in the urine (22).

In a later study, rats were given 5 i.p. injections of isophorone per week for 2 weeks. Each dose represented 10 % of the LD_{50} value. A 24-hour urine collection was carried out every 2 days, and albumin, $ß_2$-microglobulin and ß-N-acetylglucosamidase were determined. The values were not different when comparing exposed animals to controls (2). (According to an unpublished studie reported in Patty's Industrial Hygiene (10) the i.p. LD_{50} in rats is 400-800 mg/kg bw.) See also chapter 8.

5.5. Effects on digestive tract

No information is available.

5.6. Effects on heart and blood vessels

There is no information regarding man.

Twentyfour hours' continuous exposure to 300 ppm isophorone (1723 mg/m^3) caused accelerated heart rate in guinea pigs (21).

5.7. Effects on blood and blood-forming organs

There are no data on humans.

In rats and guinea pigs exposed to 500 ppm isophorone (2827 mg/m^3) for 6 weeks, 8 h/d, 5 d/w, unspecific changes in blood cells were observed. These changes were not seen at 200 ppm (1149 mg/m^3) or lower (22).

5.8. Effects on central nervous system

In an unpublished report cited by NIOSH (14) complaints of tiredness and sickness are reported from workers exposed to 5-8 ppm isophorone (28.7-46.0 mg/m^3). After improvement of the ventilation system, the air concentration was lowered to 1-4 ppm (5.7-23.0 mg/m^3) and the complaints ceased.

High concentrations of isophorone (saturated air) caused death in rats probably due to paralysis of the respiratory center (21).

Effects on the central nervous system has been studied with a method originally developed for detecting the efficacy of antidepressant drugs (7). The effect was determined by measuring the duration of immobility in mice subjected to a 'behavioural dispair', swimming test. The concentration of isophorone that produced a 50% decrease in immobility was calculated to be 110 ppm (632 mg/m^3). The significance of this experiment in evaluating the degree of toxicity is not known.

5.9. Effects on peripheral nervous system

There are no data on effects of isophorone in man or experimental animals.

5.10. Effects on eyes

Undiluted isophorone (0.1 ml) in the rabbit eye caused conjunctivitis and opacity of the cornea (25). According to a previous report (5), isophorone caused corneal injury, grade 4 (on a 10-grade scale where 10 represents the most severe effect).

Opacity of the cornea or corneal necrosis was found in guinea pigs, but not rats, exposed to 840 ppm isophorone (4823 mg/m^3) for 4 hours or more (21).

6. Immunotoxicity and Allergy

There are no data on immunotoxicity or allergy caused by isophorone.

7. Mutagenicity and Genotoxicity

Isophorone has been tested in the Ames assay with strains TA 100, TA 1535, TA1537 and TA 98 of Salmonella typhimurium in the presence and absence of liver S9 from Arochlor induced hamster or rat. The mutation frequency was not increased in the dosing interval 33 - 1000 µg/plate (13).

With the mouse lymphoma L5178Y/TK$^{+/-}$ assay contradictory results have been published. One study (18) using the dosing interval of 0.13-1.3 µg/ml, showed only negative results, both in the presence and absence of S9 mix. In another study, which was done within the National Toxicology Program in the US (NTP) (12, 17), the number of mutants was significantly increased in the absence of S9 mix. Isophorone was not tested in the presence of S9 mix. The discrepancy between the two laboratories is difficult to explain in view of the fact that the studies were performed under similar conditions, and in the same dosing interval (up to the letal dose 1600 µg/ml).

Isophorone did not induce DNA repair (Unscheduled DNA Synthesis) in primary rat hepatocytes exposed to 0.005 - 0.20 µl/ml incubation. The toxic dose in this assay was 0.40 µl/ml (18).

Gulati et al (9) studied the effect of isophorone on the induction of chromosomal aberrations (ABS) and sister chromatid exchange (SCE) i Chinese hamster ovary (CHO) cells. No increase in ABS was detected at doses up to 1600 µg/ml, whereas a significant induction in SCE was observed in the absence of S9, and at isophorone doses between 500-1000 µg/ml. It should be noted, however, that isophorone is cytotoxic at these dose levels.

O'Donoghue et al (18) has studied the genotoxic effect of isophorone in vivo. No micronuclei were induced in the bone-marrow of i.p. exposed mice (0.54 ml/kg body weight, 497 mg/kg).

The results from the studies with isophorone (Ames, mouse lymphoma, UDS in primary hepatocytes, ABS and SCE in CHO cells, MN in bone marrow) are basically negative, with a few equivocal results. Thus, it seems unlikely that isophorone possesses a genotoxic hazard to the human.

8. Carcinogenicity

A cancer bioassay with rat and mouse has been performed within the National Toxicology Program (4, 17). Isophorone dissolved in corn oil was given by oral gavage 5 days/week. In a pilot study groups of 5 rats and 5 mice of each sex were given 0, 125, 500, 1000 or 2000 mg/kg body weight for 2 weeks. At the highest dose 4/5 female rats, 1/5 male rats and all 5 male mice died during the experiment. No substance related effects were observed.

In a second study, 10 rats and 10 mice of both sexes were given 0, 62.5, 125, 250, 500 or 1000 mg/kg body weight for 16 weeks (oral gavage, 5 days/week). No compound-related effects were observed in the rats, wheras 3/10 female and 1/10 male mice died during the course of the study. These deaths were judged to be compound-related. The body weight of the male mice exposed to the highest dose levels were approximately 10 % lower than the corresponding controls.

In the two year study, 50 rats and 50 mice of each sex received 0, 250 (low dose) or 500 (high dose) mg/kg isophorone in corn oil by gavage, 5 days per week for 103 weeks. The survival of high dose male rats was lower than that of the vehicle control and the low dose animals. There were no chemically related clinical signs of toxicity in either rats or mice during the two year study. However, certain organs and organ systems in both species showed histopathological changes in response to the isophorone exposure.

The pathology study revealed in male rats; kidney tubular hyperplasia in 4/50 in the high dose group, 1/50 in the low dose group and 0/50 in the control group; tubular adenoma in 2/50, 0/50 and 0/50 respectively; tubular adenocarcinoma in 1/50, 3/50 and 0/50 respectively; epithelial hyperplasia in kidney in 5/50, 5/50 and 0/50 respectively. Furthermore, in the high dose group 5 cases of preputial carcinoma were observed, as well as an increased incidence of fat metamorphoses in the cells of the adrenal cortex (26/50 in high dose, 21/50 in low dose and 7/50 in controls).

No kindney neoplasms were diagnosed in female rats. However, an increased incidens of nephropathy was observed at both dose levels. No other neoplastic or non-neoplastic damages were observed. In male mice, hepatocellular neoplasm (adenoma and carcinoma) was observed in 29/50 in the high dose group, 18/50 in the low dose group and 18/40 in the control group. Subcutaneous fibrosarcoma was observed in 14/50, 8/50 and 6/48 animals, respectively, and lymphoma or leukemia was observed in 5/50, 19/50 and 8/48 animals respectively. The only damage, neoplastic or non-neoplastic, that was observed in female mice was hepatic neoplasia. The incidence was 8/50 in the high dose group, 6/50 in the low dose group and 4/50 in the control group.

The primary effects of long term exposure to isophorone are the proliferative damages in male rats. Carcinoma of the preputial gland (male rats) and hepatocellular neoplasms (male mice) also seem to be compound-related. The authors (4) assume that isophorone may have a promotor effect in male rats and mice, or alternatively some biotransformation product is the cause. According to NTP (17) there are some evidence for carcinogenicity in male rats, equivocal evidence in male mice and no evidence in female rats or mice.

9. Reproduction Toxicology

There are no reports on reproduction toxicology effects of isophorone.

10. Correlation between Exposure, Effect and Response

There are only a few reports dealing with effects in the human due to isophorone exposure. The data are shown in Table I. In animal studies there are only a few in which the animals have been exposed to isophorone by inhalation. The results are summarized in Table II.

Table I. Human effects due to isophorone exposure

Dose	Exposure	Effect/response	Ref
229.8 mg/m^3	work related	irritation of eye, nose, throat	10
143.6 mg/m^3	15 min	irritation of eye, nose, throat	20
57.4 mg/m^3	15 min	40% reacted to the smell, can be accepted during an 8 hours working day	20
28.7-46.0 mg/m^3	work related	tiredness and nausea	14
5.7-23.0 mg/m^3	work related	no complaints about tiredness or nausea	14

Table II. Effects on experimental animals after isophorone inhalation

Dose	Exposure	Species	Effect/response	Ref
10569 mg/m^3	4 h	rat	LC_{20}	16
4823 mg/m^3	4 h	guinea pig	opacity of cornea + necrosis	21
1723 mg/m^3	24 h	guinea pig	increased heart rate	21
2872 mg/m^3	6 weeks 8h/d, 5d/w	rat guinea pig	inflammation of lung, kidney, epithelial necrosis, unspecified changes in blood, 9/20 animals died	22
1148.8 mg/m^3	6 weeks 8 h/d, 5 d/w	rat guinea pig	cloudy swelling in kidney tubular epithelia, reduced body growth, no unspecified changes in blood, 3/18 animals died	22
631.5 mg/m^3	?	mouse	50% reduction in behavioural dispair swimming test	7
287.2 mg/m^3	6 weeks 8 h/d 5 d/w	rat guinea pig	cloudy swelling in kidney tubular epithelia reduced body growth, 2/16 died	22
159.7 mg/m^3	approx 5 min	mouse	RD_{50} (50% reduction of breathing rate)	6
143.6 mg/m^3	6 weeks 8 h/d, 5 d/w	rat guinea pig	no observed effects	22

11. Research Needs

In order to obtain a better picture of effects caused by isophorone, more research is needed, especially toxicokinetic studies, including analysis and quantitation of uptake, biotransformation and excretion. Furthermore, suitable methods for biological monitoring are also needed.

Epidemiological studies are totally missing, and thus such studies are needed, as well as studies regarding the teratogenic effects of isophorone.

12. Discussion and Evaluation

The available information regarding occupational exposure is second hand information and thus difficult to evaluate. From the experimental studies, one can draw the conclusion that 15 minutes exposure to concentrations as low as 50 mg/m^3 affects the olfactory organ and is regarded as irritating.

It is not possible to evaluate the long-term effects, as the inhalation studies performed on experimental animals have only lasted for six weeks. No clinical or pathological changes were observed when rats and guinea pigs were exposed for 6 weeks to 143.6 mg/m^3 isophorone in inhaled air. Short-term exposure to about 160 mg/m^3 caused respiratory tract irritation in mice, which was measured as a reduction of the breathing rate.

Based on the available information the critical effect of isophorone exposure seems to be mucous membrane irritation.

13. Summary

Brita Beije & Per Lundberg. Isophorone 95. The Nordic Expert Group for Documentation of Occupational Exposure Limits. Arbete och Hälsa 1991.

The document summarizes available data concerning health effects due to isophorone exposure.

Isophorone is used in industry as a solvent for fat/grease, oil, lacker, ink and dilutents.

There are few data regarding the effects in exposed humans. However, irritation of eyes, nose and throat has been reported after exposure to 25 ppm (144 mg/m^3) isophorone. According to another report, workers have complained about fatigue and nausea already at 5-8 ppm.

In animal studies, using exposure via inhalation of high concentrations of isophorone, effects have been reported on the lungs (accumulation of blood and inflammation), liver (accumulation of blood and cloudy swelling) and kidneys (accumulation of blood and necrotic epithelium) of rats and guinea pigs, heart frequency in guinea pigs, growth in mice and guinea pigs, blood cells from rats and guinea pigs, and eyes (opacity of cornea) in guinea pigs.

There are no reports regarding immunotoxicity or allergy. The genotoxic effect of isophorone has been studied in several short term tests in vitro with bacteria or mammalian cells. The results are mainly negative. No micronuclei were induced in the bonemarrow of mice exposed to isophorone by i. p. injection. In NTP's cancerbioassay there was some evidence of carcinogenicity in male rats, and equivocal evidence of carcinogenicity in male mice. There was no evidence of carcinogenicity in female rats and mice.

In Swedish, 26 references.

Key words: isophorone, exposure, inhalation, mucuos membranes, respiratory organs, liver, kidney, blood cells, oral gavage, cancerbioassay, occupational exposure limits.

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Appendix I. Maximum air concentrations of isophorone stated in occupational health regulations or recommendations in some countries

Country	mg/m^3	ppm	Year	ref
Denmark	25	5	1988	1
Finland 8h	28	5	1987	2
15 min	56	10		
Iceland	25	5	1978	3
Netherlands A)	25	5	1989	4
Norway	25	5	1989	5
Sweden A)	30	5	1990	6
USA (ACGIH)A)	28	5	1990-91	7
(NIOSH)B)	23	4	1989	8
GermanyC)	28	5	1990	9

A) TLV = threshold limit value (ceiling limit)
B) TWA = time weighted average
C) MAK = maximum concentration in workplace

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Microorganisms

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Contents

List of abbreviations used in this text and short descriptions of some terms discussed in this report:

Terms related to microorganisms

Gram–positive	Staining method reflecting cell wall structure of bacteria. The cell walls of Gram–positive bacteria are mainly composed of peptidoglycan, but Gram negative have a weak peptidoglycan layer and an outer layer rich in LPS.
Peptidoglycans:	Component of the cell wall of all bacteria, notably Gram-positive. Usually built up by repeating units of N-acetylglucoseamin-N-acetylmuramic acid, cross-linked by short peptide chains. Has adjuvant and inflammatory activity.
Glucans	Component of the cell wall of mold-spores. Composed of linear poly-b-D-glucose with 1-3 links (cross linked at 1-6 positions). Has adjuvant and inflammatory activity.
Endotoxin	Toxic component of cellwalls of Gram-negative cells containing LPS
LPS	*Lipo-polysaccharides* —component of the walls of Gram-negative bacteria with most of the toxic effects of endotoxin.
Lipid A	Active part of LPS
LAL	Limulus amebocyte lysate—assay to quantify endotoxin activitys
fmlp	*formyl-methionyl-leucyl-peptides*—bacterial peptides with unique N-terminal sequence. Has inflammatory activity.
Lectin	Glycopeptides (usually of plant origin) which bind to sugar residues of foreign molecules.

Terms related to human responses to microorganisms

Allergic alveolitis	Restrictive lung disease caused by immune reactions to inhaled microorganisms and other antigens.
Adjuvans	Increases immune response to antigens
ODTS	*Organic Dust Toxic Syndrome* —A non-hypersensitive febrile condition caused by inhalation of organic dusts.
Complement	Proteins interacting in recognition and defence against foreign particles, using a non-immune recognition mechanism.
Opsonization	Foreign particles are covered with complement fragments or with antibodies, thus facilitating phagocytosis.
Cytokines	Hormone-like agents released by immune-competent cells, inflammatory cells and other cells to control inflammatory, immune and wound healing responses.
LTC-4	Leucotriene C4—Agent with bronchoconstrictor and vascular effects released by activated cells.
IL-1	Interleukin-1—see TNF for explanation.
TNF	Tumor necrosis factor—IL–1 and TNF are cytokines causing fever and other reactions to inflammation.
FEV_1	Forced expiratory flow in 1 second.

1. Introduction

Microorganisms are indispensable for life. They colonize human skin and intestines and are required for proper function of the digestive tract. They are present almost everywhere and we inhale large numbers present in ambient air. Microorganisms can grow in extreme environments. Some thrive at temperatures of 80-90°C at pH < 2 in hot sulphur springs of volcanic origin, others multiply at near freezing temperatures. Higher organisms have developed defence systems which allow coexistence, but are always at risk of succumbing to microorganisms which possess means to overcome the defences, causing infections. Injury can occur also in the absence of infection, as a result of activation of such defence mechanisms.

2. Scope of the report

The present report discusses injury caused by inhaled microorganisms in the absence of infection. Thus occupational exposures causing infection or spreading of pathogenic microorganisms from patients, postmortem material, diagnostic laboratories or animals are not commented upon. The topic of contamination of the environment with genetically manipulated microorganisms also lies outside the scope of this document.

3. Occupations with high exposures to microorganisms

Farmers are exposed to high concentrations of many types of microorganisms. Farmers producing swine or chicken and dairy products have different exposures and health problems (26, 74, 136). Farmers and workers engaged in the production, storage and transport of grain and in fermentation of barley in breweries are at risk for respiratory diseases caused by inhaled microorganisms (9, 11, 50). Workers exposed to certain types of wood products, such as chips of wood or cork, which may become mouldy, as well as workers exposed to partly fermented material such as in mushroom production and in composting are exposed to mold spores (4, 53, 59, 140). In cotton and flax processing exposure to microorganisms or fragments of microorganisms may cause health problems (54, 107).

Microorganisms can also be inhaled in the form of aerosols from contaminated liquids in sewage plants (72, 120), from cutting liquids or from humidifiers in for example printing offices (13, 48).

Dusts with high spore-counts have been reported from museums (60). It has been suggested that exposure to microorganisms or fragments of microorganisms may play a role in the complaints reported from "sick buildings", but so far very little evidence for this has been produced.

4. Types of microorganisms, cell wall structures

Microorganisms can be subdivided in eucaryotes, such as fungi (yeasts and molds), and procaryotes such as bacteria. Eucaryotes have a nuclear membrane enclosing the genetic material (cell nucleus), while the genetic material of procaryotes are scattered in the cytoplasm. Fungi are multicellular organisms arranged in a "mesh" (syncytium) which can be very large. They can produce conidia (spores) which easily become airborne and spread to distant sites. Actinomycetes are bacteria, but are similar to fungi in the sense that they exist in syncytia and can produce large numbers of spores. Spores are highly resistant to many environmental influences. Thus the vacuum employed in electron microscopy will cause most bacteria and yeasts to collapse, but spores of fungi and actinomycetes remain intact.

Bacteria are classified according to their response to the Gram-staining method. The cell walls of bacteria which are Gram–positive have a three–dimensional layer of peptidoglycan, which is built up by disaccharides cross-liked with short peptide chains (see section 8.3 and figures 1 and 3). Gram negative bacteria form a monolayer of peptidoglycan. The Gram–negative bacteria in addition contain three polymers outside the peptidoglycan layer; lipoprotein, outer membrane and lipopolysaccharide (see section 8.2 and figures 1 and 2). Actinomycetes are Gram–positive bacteria and are distantly related to mycobacteria causing tuberculosis in man. Some bacteria are enclosed by a capsule of carbohydrate origin.

The cell wall of fungi is composed of an exoskeleton made up by sugars arranged in a mesh structure with crosslinks. In addition soluble components, often rich in mannoprotein mainly composed of carbohydrage (a highly branched polymannose), which can be extracted from the cell wall (16). The structural element is often built up of linear chains of glucose, in β links with many crosslinks. Many fungi have chains of poly-β-D-glucose with 1→3 links (cross linked at 1→6 positions) which are called glucans, other may have 1→4 links (cellulose) and others are composed of poly-β– (1→4)-N-acetylglucosamine (chitin). Fungi have no peptidoglycan or LPS in their cell walls.

Many fungi release odorouse components which may cause sanitary problems. The metabolite 2-isopropyl-3-methoxy-pyrazine from the actinomycete streptomyces (39) has a smelling threshold of only 0.002 ppb (122).

5. Toxins from microorganisms.

Many microorganisms have constituents or can excrete substances which are toxic to other organisms (endo- and exotoxins). Some exotoxins are used for medical purposes, such as antibiotics and cyclosporine, the latter used to suppress immune responses to organ transplants. Some toxins could be considered as resources to

suppress or destroy competitors. Other toxins function to increase the capacity of microorganisms to overcome the defence systems of higher organisms.

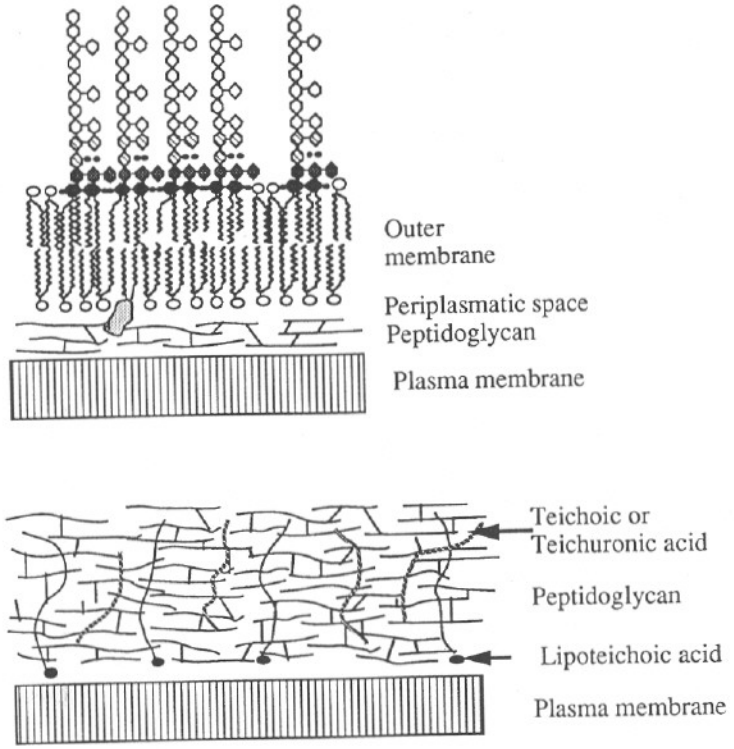

Figure 1. Cell wall structure of Gram–negative (top) and Gram-positive bacteria (bottom). Redrawn from Sonesson (126), based on Hammond et al (44) and Rietschel et al (102).

Some toxic effects are results of activation of defence systems developed in higher organisms to identify common structural elements in microorganisms and elicit an inflammatory defence reaction. Examples of such structures are lipopolysaccharides (LPS or "endotoxin") in Gram–negative bacteria, β- (1→3)-D-glucan in many fungi and peptidoglycan in bacteria.

5.1 Mycotoxins:

Several fungi produce mycotoxins (56), which may cause injury to for example cattle feeding on mouldy material. Some have been implicated in human disease, following ingestion or inhalation. The amount of toxins which may be inhaled is, however, very small compared to the amounts that can be ingested. Thus even after a full working day in heavily contaminated air, at most a few micrograms of microorganisms are inhaled.

Fungal mycelium is the dominant part of ingested material and spores predominate in inhaled fungal material. Spores appear to have higher concentrations of some mycotoxins (such as Aurasperone C from *Aspergillus niger*) and lower of other mycotoxins (such as aflatoxin from a mutant of *A parasiticus*) (93) compared to mycelium. Aflatoxin, a strong cancerogen in some laboratory animals has been implicated as a possible occupational hazard through inhalation of dust from contaminated corn or peanuts (12, 30, 46), however, recently the role of aflatoxin as a human carcinogen has recently been questioned (132). Mycotoxins which can cause tremor, such as verruculogen and fumitremorgen C, from some *Aspergillus* and *Penicillium* spp. have been implied as a possible cause of tremor in association with acute febrile symptoms (65). However, although some strains isolated from saw mills are able to produce tremorgens, it has not been shown that inhaled doses are sufficient to cause symptoms. Ochratoxin A, which is nephrotoxic, hepatotoxic and a carcinogen has been demonstrated in stored grain, and it could be a human hazard, but probably by ingestion, rather than by inhalation (1).

The tricothecene T-2 toxin from the field fungus *Fusarium*, Patulin and Gliatoxin, from *Aspergillus* and *Penicillium* interfere with human inflammatory cell functions in vitro, reducing the efficiency of the clearance of microorganisms (130).

5.2 LPS/ Endotoxin, peptidoglycans, β- (1→3)-D-glucan and fmlp.

By far the most studied toxin from microorganisms is LPS (lipopolysaccharide) from Gram–negative bacteria. Lipopolysaccharides are composed of a hydrophilic polysaccharide extending out from the cell wall and a lipid part (lipid A) embedded in the outer lipid layer of the cell membrane. Lipid A is responsible for most, but not all of the toxic effects of LPS (87). Other toxic effects (such as complement activation) and immune-modulating effects can be elicited from lipid-free extracts of Gram–negative cells and from unrelated substances present in conventional LPS preparations (87). Such crude preparations are called endotoxin, which thus denotes a less well defined and more variable entity with possibly greater biological effects than Lipid A alone.

Peptidoglycans, β-(1→3)-D-Glucan and fmlp–like peptides (described in sections 7.2 and 8.3) are components of many types of microorganisms with potent biological effects. These effects are due to activation of receptors on inflammatory

cells which probably have been evolved with the purpose of identifying invading microorganisms and activating proper defence measures.

6. Defence mechanisms.

Phagocytosis: Plants are equipped with enzymes which can degrade the cell wall of microorganisms and lectins, which bind to carbohydrates in microorganisms and cause a local reaction limiting invasive growth. Some lectins (such as phytohemagglutinins) are also potent mitogens for animal cells and are used to cause proliferation of for example human immune cells *in vitro*. In animals the immune system is often employed in the defence. Invertebrates do not have a fully developed immune system with antibodies and lymphocytes, but use for their defence phagocytic cells similar to human macrophages, which engulf and digest microorganisms (123). In the human lungs there are about 5×10^9 macrophages (22). Most of them are found on the surface of the lung alveoli ("lung sacs"), where the gas exchange takes place. Macrophages are equipped with receptors which recognize common cell wall components in microorganisms. These include LPS, peptidoglycan fragments and β- (1→3)-D-glucan. In addition there are several receptors recognizing simple sugar residues such as the mannose-fucose receptor (131).

The particles are first attached to the cell membrane and subsequently the membrane invaginates, forming a vacuole (sac). The next event is fusion of the vacuole with other vacuoles containing enzymes such as lysozyme, which digest the microorganisms within the vacuoles. Following heavy exposure each alveolar macrophage may ingest typically 5-10 spores and up to 20 spores (37).
The complement system is also important in the non-immune recognition of microorganisms (105). This is composed of several proteins which interact in a complicated pattern with foreign particulate surfaces, coating the foreign particle and releasing chemotactic factors attracting macrophages and other inflammatory cells to the site. The alveolar macrophages have receptors for complement fragments attached to the foreign particle surface helping to attach and ingest the foreign particle. Complement can sometimes "puncture" the cell membrane of foreign cells causing cell death.
Immune recognition: In higher animals the immune system participates in the defence against inhaled microorganisms. Antibodies directed against several components of the cell walls are produced by B-lymphocytes. The antibodies identify and coat the surface of the microorganisms. Such antibody coated particles are ingested by alveolar macrophages which have receptors binding to the non-occupied portion of the antibody (fc-receptors). Coating of foreign particles with complement or antibodies is called opsonisation. There is also evidence that components of the cell walls of microorganisms enhance cellular immune responses (88, 133, 142) and induce natural killer cells (118).

Adjuvans function of microorganisms. In many vaccination protocols and in experimental models where antigen is inhaled or injected in order to cause the production of antibodies, Freund´s complete adjuvans is usually injected subcutaneously in order to promote the reaction. The active component is an emulsion of mycobacteria in oil, which strongly enhances the immune–response. Many peptidoglycan fragments can replace whole mycobacteria and the minimal fragment is a muramyl dipeptide N-acetylmuramyl-L-alanyl-D-isoglutamine (2). Also LPS (82) and β- (1→3)-D-glucan (88, 133) have adjuvant activity.
Spore resistance to killing and secondary defence line: Many fungal spores have more or less well developed defence systems against phagocytosis (62, 70, 103, 104). Spores of the *Aspergillus fumigatus* type are attached to macrophages in the early phase of phagocytosis, but the subsequent ingestion phase is blocked by a factor produced by the spores. Many spores diminish or inhibit the respiratory burst of phagocytic cells (69). Three to four hours after attachment, spore laden macrophages seem to leave the alveolar space and enter the interstitial tissue. The spores subsequently start to swell and form mycelial buds as a preparation for growth. In this phase the microorganisms are vulnerable to oxidants and neutrophil cationic proteins from polymorphonuclear phagocytes, which are recruited from the blood and accumulate where budding microorganisms are found (69). Human infection with the fungus *Aspergillus* and animal models of the disease are strongly promoted by absence or defective function of neutrophil granulocytes (70). Viable spores can be found in the lungs for weeks after experimental inhalation of spores in animals (66) and can be cultured from lung sections in human subjects two days after exposure to large amounts of mold dust (34).

7. Injury following inhalation of microorganisms.

7.1 General mechanisms.

Phagocytosis and activation of inflammatory cells: The receptors for LPS, peptidoglycan and β- (1→3)-D-glucan are not only utilized for the attachment and ingestion of microorganisms. They also activate the macrophages and cause a series of events resulting in local inflammation and systemic effects due to the release of hormone-like substances, cytokines (58) and other inflammatory mediators. The different receptors have different effects, although some are common. In addition fragments produced by activation of complement and products of activated macrophages recruite other inflammatory cells from the blood. Antibodies may form complexes with soluble components from the microorganisms and these complexes may activate complement.
Inflammation: Activated inflammatory cells may cause damage by the production of oxygen radicals aimed at destroying the invading cell, and from release of enzymes, toxic proteins and other substances stored in the inflammatory cells or produced

and released upon activation. The inflammatory reaction has important functions in the defence against microorganisms and in wound healing, but can be excessive and cause undue damage in some situations.

Fibrosis: A prolonged and intense inflammation due to inhaled microorganisms may lead to fibrosis and result in irreversible damage. This process is associated with the presence of several inflammatory cell types. It may require an amplification loop between T-lymphocytes and macrophages causing a state of high activation, possibly involving the release of interferon-gamma from activated T-cells (82). Factors which may influence the reaction is the dose and duration of the adjuvant activity and the presence of antigen.

7.2 Specific effects

Inhalation of spores: In humans, repeated inhalation of high doses of mold dust causes alveolar and airways inflammation. Lymphocytosis and increased numbers of mastcells are conspicuous findings (7). The initial response following heavy exposure may, however, be dominated by neutrophils in bronchoalveolar lavage fluid (67).

In experimental animals, intratracheal instillation of spores from *Aspergillus fumigatus* may result in mortality and signs of invasive growth of the fungus, neutrophil accumulation and necrosis in lung tissue, where fungal mycelium is found. Some strains of the fungus appear to be more toxic than others. Viable spores were found up to 3 weeks after exposure. Three weeks after a single exposure, chronic granulomatous changes with macrophages and giant cells are found (66). Guinea pigs daily exposed to dust from mouldy hay for some weeks initially show increased numbers of macrophages and neutrophils, and after three weeks a pronounced increase in lymphocytes is seen (36).

Inhalation of endotoxin: Alveolar macrophages are activated, become adherent and release neutrophil chemotactic factor (139) and platelet activating factor (109). This is followed by a large influx of granulocytes, first to the interstitium (maximum 4-12 hours), later into the airspaces (139). Upon repeated exposure the neutrophilia is reduced to normal levels (36). Release of platelet activating factor diminishes upon repeated exposures, and is fully recovered only after a free interval of 72 hours. Elastase levels in lavage fluid from rats is increased, and bronchial reactivity increases, coinciding in time with interstitial granulocyte infiltration, rather than with appearance of granulocytes in the alveoli (96). Increased bronchial reactivity has also been demonstrated in sheep following intravenous infusion of endotoxin (51).

LPS inhalation also causes increased levels of prostaglandin E2 and tromboxan B2, but not prostaglandin F2 in peripheral blood. There is a pronounced sequestration of granulocytes and platelets in pulmonary capillaries, and cellular infiltration of the interstitium, evidence of capillary leakage into the interstitium, (but not into the alveoli) and endothelial cell changes with increased numbers of pinocytotic vesicles (15).

Interaction with the endotoxin receptor results in activation of phagocytic cells and production of for example oxygen radicals, platelet activating factor, leucotrienes, interleukin-1, tumor necrosis factor and other cytokines (82). Interleukin-1 and tumor necrosis factor probably causes general symptoms such as fever. LPS also potentiates effects of other cell activators. LPS activates complement and following intravenous injection (10) or inhalation of an aerosol of LPS (15) the presence of complement is required for some, but not all, effects of LPS. Thus sequestration of neutrophils and platelets in pulmonary capillaries require complement, but not the interstitial accumulation of inflammatory cells. The effects of parenteral injection of endotoxin, seem to critically depend on the presence of neutrophils (10).

Inhalation of glucan, peptidoglycan and fmlp like peptides: Very little is known concerning the effects of inhalation of these agents. They have well known in-vitro effects on inflammatory cells and glucan and peptidoglycan have strong immunomodulating effects following parenteral administration. Zymosan, an extract from baker's yeast cell walls, has long been recognized as an important particulate activator of phagocytic cells. It is composed of β- (1→3)-D-glucan and a mannoprotein. The effects are due to the glucan component. β- (1→3)-D-glucan is an efficient activator of complement, but the effects on inflammatory cells can be observed even in the absence of opsonins. These effects are probably induced by interaction with a glucan receptor and include production of leucotrienes and oxygen radicals, and tumor necrosis factor. Acylated glucan fragments are very potent activators of alveolar macrophages and may produce tumour necrosis factor and interleukin-1 at nanogram concentrations, and are thus almost as potent as LPS (23, 40, 73, 118, 123, 124, 131). β- (1→3)-D-glucan is a potent immunostimulant. Even oral administration of β- (1→3)-D-glucan has profound immunomodulating effects (133).

Peptidoglycan: Peptidoglycan fragments also act as strong adjuvants potentiating immune reactions (2). If peptidoglycan fragments are protected from degradation in vivo, the adminstration of such material may result in chronic inflammation and arthritis (121).

N-formyl-methionyl-leucyl-phenylalanine (fmlp) like peptides. Bacteria produce unique, amino-terminal peptides when they initiate protein synthesis. These peptides begin with the unique sequence, N-formyl-methionyl-, before continuing on with the rest of the chain. This special peptide sequence is identified by receptors on the mammalian phagocytes and the peptides are potent chemoattractants, leading macrophages to the bacteria (14). Fmlp-like peptides also activate the respiratory burst needed to destroy the bacteria (6). The highly potent peptide fmlp is an important tool in the study of responses of phagocytic cells.

7.3 Human diseases/symptoms

There are several diseases/symptoms which have been attributed to inhalation of dust containing microorganisms. These include

1) Allergic alveolitis
2) Febrile reactions (Organic dust toxic syndrome, ODTS)
3) Chronic inflammatory changes in airways with chest oppression, cough and "monday exacerbation of symptoms"
4) "Mucous membrane irritation".
5) Allergic asthma and rhinitis

Allergic alveolitis is caused by either inhalation of fungal or actinomycete spores or by dust containing bird droppings. The latter disease occurs mostly in the home environment (bird fanciers disease), and seem to have a low prevalence among persons handling hens (83).

Allergic alveolitis caused by inhalation of mold dust is usually associated with high and repeated exposures (98) and is most common in occupations where exposure to mold dust may be excessive, such as in farming, when handling mouldy wood chips (59) or in trimming departments of sawmills with defective drying kilns (5). The disease has been reported from a wide variety of exposure situations (119) and many different types of microorganisms have been implicated, including gram negative bacteria (29). If rigid criteria are employed, the yearly incidence of the disease is rather low (2-4 per 10000 in the scandinavian farming community (75, 134). A large proportion of farmers have evidence of immune stimulation by inhaled microorganisms, but have a normal lung function and a good prognosis (21, 76). Allergic alveolitis is a serious restrictive lungdisease, with a prolonged course, and often causes a residual defect in lung function (20, 84).
Febrile reactions are characterized by chills/fever, sometimes muscle and joint ache and a feeling of malaise, resembling the general symptoms of influenza (34, 77, 99). Fever reactions are 30 to 50 times more common than clinically recognized cases of allergic alveolitis in the farming environment (75) and usually occur following excessive exposures to fungal and actinomycete spores (34, 76, 77). Toxic symptoms also occur in environments with little evidence of fungal spore exposure and a low prevalence of allergic alveolitis. Thus they occur in cardrooms of cotton producing factories (106), in printing shops with humidifiers which have not been cleaned (33, 48, 112) and in swine confinement buildings (26). Toxic reactions do not seem to require prior sensitization.

Farmers who have experienced a toxic reaction show a neutrophil dominance in bronchoalveolar lavage fluid (67) and evidence of a transient and usually mild decrease in pulmonary function (77). The symptoms can be reproduced by inhalations of LPS (17, 18, 78, 109). It is not known if peptidoglycans or β-(1→3)-D-glucan can produce similar symptoms upon inhalation.

Chronic inflammatory changes in airways with symptoms of chest tightness and cough is characteristic of byssinosis, a disease seen in workers exposed to cotton dust. It is associated with over work-shift decrease in lung function of obstructive type (107, 116). Farmers also have an increased prevalence of chronic cough and expectoration. The symptoms have been reported from dairy farmers (74), in swine confinement building workers (26), and in grain dust exposed persons (50). The symptoms could be partly due to exposure to microorganisms (107, 110), but in view of the composite nature of the exposure, definite conclusions cannot yet be made regarding the role of microorganisms or microbial components such as LPS, peptidoglycan and glucans. Only a few reports have included studies of bronchial reactivity and the results are inconclusive (52, 111). In view of the composite nature of the exposure, it is difficult to identify a cause of such hyper-reactivity, but inhalation of large doses of LPS may cause a slight increase in bronchial responsiveness, probably transient (95, 108).

Allergic asthma and thus bronchial hyper-responsiveness has been reported from several of these environments, but the sensitizing agents are mites or animal dander rather than microorganisms (135, 138).
Mucous membrane irritation. Irritations of eyes and upper airways are commonly reported from various environments including "sick buildings" (125). The cause is not known and could be a result of a summation of subliminal effects from many sources. Very little data are available to support or refute a hypothetical role of microbial toxins.
Allergic asthma and rhinitis. Fungal spores commonly found in ambient air such as *Alternaria* and *Cladosporium* may cause or aggravate allergic asthma and rhinitis in a small proportion (<1%) of allergic subjects. Rhinitis is the most common form of allergy to common fungi (71). Asthma and rhinitis has also been reported following exposure to the fungi *Didymella exitialis* in a rural environment (45) and *Wallemia sebi* in house dust (117)

8. Physical/chemical data.

8.1 Spores/bacteria.

Fungal spores are in the size range of 2-100 µm, but normally between 2 and 8 µm, actinomycetes and bacteria 0.5-1.5 µm. Spores and bacteria can be single or be attached to other spores in strings or clusters. In air samples from the farming environment most spore aggregates were of respirable size as judged from the physical diameter (57). The aerodynamic size of aggregates have not been investigated. Actinomycetes are often to a large extent aggregated (31, 57).

8.2 LPS/Endotoxin

The outer part of the hydrophilic polysaccharide of LPS shows great variability but the inner part, the core oligosaccharide, varies less. The two innermost saccharide units are usually composed of components only found in LPS, (L-glycero-D-manno-heptose and 2-keto-3-deoxyoctonic acid, KDO). KDO is ketosidically linked to the disaccharide of Lipid A (see below) and seems always to be present in LPS (figure 2).

The lipophilic portion of LPS (Lipid A) in enterobacteria is composed of a disaccharide (β-1.6 linked *N*-acetyl-D-glucosamine, phosphorylated) which

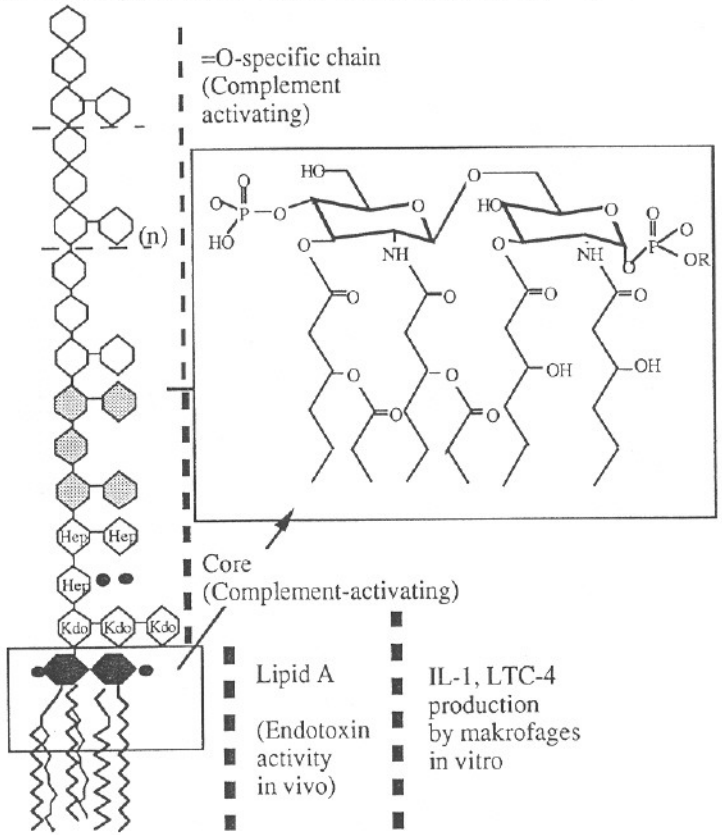

Figure 2. Schematic drawing of endotoxin redrawn from Sonesson (126), based on Rietschel (102). Insert shows part of the lipid A component of Escherichia coli, with the two phosphorylated N-acetyl-D-glucosamine sugars.

normally carries four fatty acids, which may be branched. In other Gram–negative species structural deviations may occur.The fatty acids are 3-hydroxy-substituted fatty acids and the most common is 3-hydroxymyristic acid (126). These are not found in Gram–positive bacteria or in fungi and have been used as chemical markers of Gram–negative bacteria (127, 129).

The endotoxin receptor on phagocytic cells interacts with free Lipid A, which is exposed in membrane fragments and possibly also in membrane "blebs" shed by Gram–negative bacteria (86). Most of the Lipid A in intact cell membranes probably does not contribute to the physiological effects caused by inhalation of Gram-negative bacteria (108). The minimal requirements for toxic effects of Lipid A are the phosphorylated disaccharide and hydroxy and acyloxyacyl groups in a defined structural arrangement (101). The composition and toxicity of lipopolysaccharides from different sources varies. Some deviant LPS do not appear to elicit typical endotoxin effects.

8.3 Peptidoglycan

The peptidoglycan cell wall is present in all bacteria (and thus also in actinomycetes). It is usually built up by repeating units of N-acetylglucoseamin-N-acetylmuramic acid, cross-linked by short peptide chains. In Gram–negative bacteria the cross-linking amino–acid is usually diaminopimelic acid (DAP) In Gram–positive bacteria lysine is more common (126). A disaccharide pentapeptid obtained by enzymatic digestion of peptidoglycan (figure 3) has immunomodulating and tumoricidal effects, but the disaccharide is inert (137).

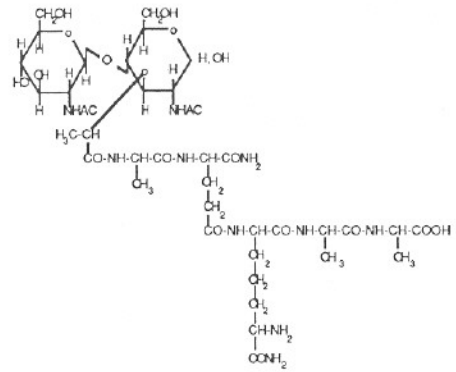

Figure 3 Disaccarid-pentapeptide GlcNAc-L-Ala-D-isoglutaminyl-*meso*-diaminopimelyl-D-Ala-D-Ala. from *Brevibacterium divaricatum*

8.4 β- (1→3)-D-glucan.

The effect of particulate glucan on phagocytic cells can be inhibited by poly- β-(1→3)-D-glucose strings with a minimum of 7 glucose units. Probably the ingestion of particulate glucan requires contact at multiple sites, and deformation associated with the contact may contribute to the effect. The requirement of 7 sugars for recognition and effect makes the receptor unusual and distinct from other receptors recognizing for example a galactose residue in a glycoprotein (23, 40).

9. Methods to collect, characterize and enumerate microorganisms in air samples.

9.1 Collection of samples, sampling strategy

Many methods have been used to quantify and characterize microorganisms in air. Collection have been done by filtersamplers, impingers, cyclones, slit samplers and multistage cascade impactors. Characterization can be based on morphology using light microscopy (41), fluorescence microscopy (94) or electron microscopy (32, 47, 57), or culture at different temperatures and on different media.

The choice of strategy for collection of the samples depends on the questions posed. Many Gram–negative bacteria are vulnerable to dessication and Gram–negative bacteria may dry out and die on filter samplers. If the prime object is to study Gram–negative bacteria, impingers or impactors are preferable. In environments where mouldy material is handled, spores of fungi and actinomycetes are usually predominant. They are resistant to drying and especially in environments with high spore concentrations, filter samplers are recommended.

Traditional impaction samplers where the collection plates are used for culture have several disadvantages. They become easily overloaded (61) and allow only one or a limited selection of growing conditions. The impaction method can be modified by solubilization of the gel where microorganisms have impacted, followed by incubation on different growing media (8). Filter casettes containing samples can be transported by mail without losses from the filter, provided that they are not overloaded (31). Polycarbonate membrane filters are often used since they provide a good background for microscopy.

The handling of mouldy material is associated with extreme exposures, that can account for most of a days exposure, even if it is of very brief duration (10 minutes). It is therefore important to try to localize and reproduce the exposure situation causing these peak exposures ("worst case" sample strategy). This may require some expertise in the field of the hygienist responsible for the measurements, since for example farmers may have difficulties in localizing the activity causing most of the exposure (61). Since the dust cloud generated by the

handling of mouldy material may be influenced by many local conditions, only personal sampling during a "natural" type of handling of the moulded material can be expected to give a reasonable estimate of the personal exposure.

9.2 Characterization, enumeration

Quantification of microorganisms may refer to "colony forming units" (cfu) based on cultivation, or to total spore count or spore containing units estimated by microscopy (31, 57).

Fungal spores can be counted directly on the collection filter in an optical microscope or using a scanning electron microscope. With these methods (and particularly the latter) a certain degree of characterization can be obtained. These methods also lend themselves to evaluate aggregates of spores, since a large fraction of the spores may be found in strings or lumps rather than as single spores, depending on the type of microorganism (31, 57). One disadvantage with electron microscopy is that bacteria (other than actinomycetes) and yeasts often collapse when preparing for electron microscopy. Microorganisms can be extracted from a collection filter, dyed with a fluorescent dye (acridine orange), refiltered and analyzed in fluorescent microscopy (94). This allows easier detection of small actinomycetes and bacteria compared to optical microscopy, especially if bacteria adhere to other particles. On the other hand the extraction procedure may break up aggregates and losses may occur during extraction (31).

Culture can be performed of material extracted from filters, impingers, cyclones or impacted on gels. Several types of media are needed to include the major types of microorganisms. Osmophilic fungi require media with low water activity (61). The media are often incubated at room temperature and at increased temperatures (45-55 °C).

9.3 Methods to analyze LPS, peptidoglycan and β- (1→3)-D-glucan.

The original method of analysis of LPS was described in 1964 (68). It was based on the clotting of an extract from blood cells of the *Limulus polyphemus* (horse-shoe) crab (limulus amebocyte lysate, LAL) method. The method replaced the rabbit pyrogenic test for detection of contaminations in infusion fluids and has subsequently been applied in many other fields. The "endotoxin" assay based on the LAL method measures lipid A activity rather than endotoxin activity. It is very sensitive, and in the more recent chromogenic versions (38) the dust-samples may have to be extremely diluted to obtain readable results. The test requires knowledge of many factors which may interfere with the test such as inhibitors, aggregation and adsorbtion phenomena (38, 97). The method is based on a biological principle and different batches may give different results. Therefore an internal standard must be used. Several such standards are in use.

The LAL assay was originally considered to be very specific. Peptidoglycans can influence the results, but only in concentrations 10^3-10^4 times higher than that of

LPS (79). One of the enzyme systems in the horseshoe crab extract is sensitive to nanogram concentrations of glucans (81). The enzyme reacting with glucan can be removed (89) and it has been claimed that by running both the original and the modified LAL-test an indirect estimate of glucan concentration can be obtained (35)

Thus in samples dominated by other microorganisms than Gram–negative bacteria the LAL-test may give falsely high values. Other methods have been described, which are based on the chemical identification of components unique for different classes of microorganisms (80, 126). Thus 3-hydroxy fatty acids from LPS were halogenated and detected with gas chromatography with mass spectrometric detection or two-dimensional gas chromatography with electron-capture detection.

Chemical methods result in about ten times higher estimates of LPS than the LAL assay when analyzing Gram–negative bacteria (108, 127), since chemical methods measure all Lipid A in the cell wall, while the LAL test presumably only reacts with "free" lipid A. By direct analyses of the filter rather than of an extract, data were obtained indicating that extraction of LPS containing dust may be very incomplete in the standard procedure.

Peptidoglycan can also be analyzed by gas chromatography using D-alanine and diaminopimelic acid as markers (127). The method of chemical analysis of both LPS and glucan requires further evaluation before general acceptance.

Glucan is essentially a polyglucose and can not be analyzed using the chemical method described above. The enzymatic method mentioned above also needs further evaluation before general acceptance.

10. Environmental levels.

10.1 Factors promoting exposure to microorganisms and endotoxin.

When organic material is stored wet with access to air a compost reaction may occur resulting in an enormous growth of microorganisms causing a rise in temperature. This process is aided if the material is composed of small particles and thus has a large surface area, such as chipped wood, grains or peasheap. Access to oxygen must not be impeded and the heap should be large enough to function as a thermal insulator. The necessary water is produced by the process, once it has started. The process is promoted by warm, humid ambient air. Thermotolerant or thermophilic microorganisms appear in large numbers in material that have undergone such a "compost reaction" (63). Several of the microorganisms are spore-forming and the spore production usually increases as the compost eventually dries up. When handling such dried mouldy material very high amounts of spores are released into the air (64). The dust is dominated by spores, and hyfal fragments (from the syncytium) and bacteria (other than actinomycetes) are usually less common (57).

Gram-negative bacteria are particularly common in wet environments, preferably with free water. They constitute only a small fraction of the microorganisms in dust from material which have undergone a compost reaction. Bacteria, both Gram-positive and Gram-negative are common in environments where dried fecal material is agitated.

10.2 Microorganisms

Types of microorganisms in different environments. Microorganisms such as *Cladosporium*, *Alternaria* and *Fusarium* grow saprophytically on living growing material (field flora) and are the the most common spores in normal ambient air (63, 85).

The most common fungal spores in the farming environment are storage fungi belonging to the *Aspergillus* (*A)* and *Penicillium* genera. The most common actinomycetes are *Streptomyces* spp., *Thermoactinomyces vulgaris* and *Faenia rectivirgula* (formerly *Micropolyspora faeni*) (31, 57, 61). In air samples from grain elevators the composition of fungi and actinomycetes is similar (85). In sawmills fungi like *Rhizopus rhizopodiformis* and *Paecilomyces variotii* are more common (31, 32). Often one or only a few types of spores dominate a sample (57). Some microorganisms such as the fungi *A. umbrosus and A fumigatus* and the actinomycetes *Thermoactinomyces vulgaris* and *Faenia rectivirgula* (47) have been implicated as particularly prone to cause allergic alveolitis (90).

In environments such as poultry and swine confinement buildings many microorganisms are derived from faeces. Common microorganisms in faeces are Gram-positive bacteria such as cocci, spore-forming bacilli and corynebacteria and Gram-negative enterobacteria.

In cotton-dust and grain dust from non-moulded material, Gram-negative bacteria are common and may dominate (27, 115). Gram–negative bacteria are often adherent, and may be found attached to other larger particles of organic origin. The bacteria *Enterobacter agglomerans* (syn: Erwinia herbicola) has been suggested as a possible source of disease, being rich in endotoxin and common, especially in grain dust (28)

Microorganism counts: Very high concentrations of microorganisms in air have been observed in environments where mouldy material has been handled (57, 63, 64, 76). Thus concentrations exceeding 10^{10} spores per m^3 have been recorded (57). Aerosols from mouldy material are dominated by spores from fungi and/or actinomycetes (63).

In studies from farms in Finland where the farmers had had farmer´s lung disease the concentration of fungal and actinomycete spores were higher during the handling of hay, straw or grain compared to reference farms. The levels were 10^4-10^7 cfu/m^3 (multistage impactor) and the difference was significant for *Thermoactinomycetes* (T) *vulgaris*, *Faenia rectivirgula* and *Aspergillus umbrosis* (61).

Air in normal barns, in swine confinement buildings or in poultry houses contain between 10^4 and 10^8 microorganisms/m^3 (25, 28). This is much higher than in outdoor or other types of indoor air, even in buildings where mould growth can be demonstrated. Thus air in "sick buildings" and ambient air typically contain between 10^1 and 10^4 cfu/m^3 (49). The total count of microorganisms may be ten to a hundred times higher than the cfu count in office buildings, with levels typically between 10^4 and 10^5 spores/m^3 (G. Blomquist, personal communication).

10.3 LPS activity

Measurements of LPS activity have been reported from many environments. These include pig farms, grain elevators, silos for wet "anaerobic" storage of farm products, dairy farms, poultry buildings, localities with defective humidifiers, sewage plants etc. Much of the LPS activity could reside in cell debris rather than in living gram negative cells, since these are susceptible to drying. Table 1 lists a number of studies. LPS adheres to different surfaces. In a study from a silo most (>75%) of the endotoxin activity was associated with particles having an aerodynamic diameter ≥9 µm (92).

Table 1.

	Endotoxin µg/m^3 mean (SD)(range)	number of sites (samples)	reference
Grain terminal	(0-0.008)	7	(24)
Silo, unloading of mouldy silage	(0.1-8.8)	5	(92)
Poultry processing plant	(0.6-0.9)[a]	2	(91)
Poultry processing plant	(0.03-0.8)[b]	3 (61)	(128)
Poultry conf.	0.3 (0.1-0.5)	7 (14)	(19)
Poultry conf. unloading cages	1.09 (0.8)	11	(136)
Poultry conf. div. activities	(0.13-0.45)[c]	4 (14)	(136)
Swine conf.	0.12 (0.04-0.28)	8 (18)	(19)
Swine conf	0.13 (0.002)(0.02-0.4)[d]	166	(3)
Swine conf.	0.24 (0.2)(0.02-1.1)	28	(25)
Swine conf.	(0.02-1.9)	8	(43)
Printing office, humidifier problem	(0.01-0.04)	3	(112)
Cotton mill, carding	0.46 (0.00)[e]	10	(113)
Cotton mill, spinning	0.20 (0.17)[e]	18	(113)

[a] Ten day time weighted work day averages from two sites. [b] Geometric means from three different sites, max value 2.8 µg. [c] Mean values from 4 sites. [d] Geometric means (GSD). [e] Vertical elutriators.

11. Dose response relationships

General considerations: Inhalation of microorganisms or LPS causes much smaller effects at a given dose level compared with parenteral administration. Other factors such as tobacco smoking may attenuate or augment responses. Many dose response evaluations have been made from environments with a mixed exposure and the results are therefore difficult to generalize. Whole or particulate fragments of

microorganisms is probably a more relevant exposure than extracts such as LPS, which may be handled in vivo in a different manner than particulate–bound LPS and does not necessarily reflect the natural exposure situation (see below). It cannot be ruled out that the viability of e.g. *Aspergillus fumigatus* spores influences the response, and there is evidence that individual strains may have different effects. At a crude level it can be fruitful to distinguish between exposure to endotoxin from Gram-negative bacteria, from fungi and actinomycetes and from Gram-positive bacteria.

Endotoxin: The effects of endotoxin have been evaluated in animal and human exposure studies, by inhalations of LPS or whole bacteria and by assessment of physiological responses to various environments with high levels of endotoxin.

Inhalation of 12 µg LPS (18) or 40 µg LPS (55) caused no change in forced expiratory volume in one second (FEV_1) or fever reactions in normal individuals, but FEV_1 decreased following inhalation of 80 µg (18). There is evidence that subjects with inflammatory changes in the airways are more sensitive to endotoxin challenge. Thus subjects with chronic bronchitis experienced symptoms at a lower dose (40 µg) of LPS than normal subjects (18). FEV_1 decreased by 5-11% in asthmatics following inhalation of 22 µg LPS, but this dose caused no change in FEV_1 in healthy subjects. LPS increased bronchial responsiveness in asthmatics and a slight but not significant decrease was found in normals. The change in FEV_1 was seen 45 minutes after challenge and continued for at least 5 hours (78).

Exposure to LPS (from *Enterobacter agglomerans*) gave no change in FEV_1 at 20 µg, a slight (-1.4%) nonsignificant change at 30 µg and a significant change (average -8%) at 200 µg of nebulized dose in naive subjects. Exposure to whole bacteria, corresponding to 300 µg LPS according to chemical analysis, (30 µg LPS according to LAL), resulted in slightly smaller changes in FEV_1 than following 200 µg of a LPS preparation (FEV_1 -6 %). Thus the biological dose was approximately 100 µg or three times higher than the LAL value. At the dose 30 µg LPS 20-30% of the exposed workers experienced fever, airway irritation and/or chest tightness. With 300 µg of LPS in the form of whole cells 40-80% experienced similar symptoms (108).

Most environmental LPS is bound to cell membranes resulting in underestimation of the total amount of LPS by the LAL- test. The biologically active LPS activity can be assumed to be three times the activity measured in environmental samples from the experiments described above (108). In an experimental cardroom, an exposure level of 1.5 µg LPS (LAL) for 4 hours caused a 5% decrease in FEV_1 (42). This was estimated to result in an inhaled dose of 14 µg of biologically active LPS assuming 50% deposition, and following multiplication of measured LPS activity by 3. This value can be compared to the lungdose of a pure LPS preparation required to produce a similar change in FEV1. Thus a lung dose of approximately 28 µg was required to cause a similar change following inhalation of pure LPS (correcting for 30% losses in the nebulizer and assuming that 50% of the remainder reach the lungs and lower airways). Thus the estimated dose effect relationship in the experimental cardroom with composite dust and following

provocation with pure LPS differs by a factor two. It has also been shown that endotoxin, but not total dust correlates with change in FEV_1 over workshift in cardrooms (17, 114). The relationship seems to be logarithmic rather than linear. In a compilation from several studies mostly from the United States, a relation between endotoxin exposure (range 0-0.8 µg) and FEV_1 change (range +0.5 to -9.1 %) was demonstrated (17). The latter report however suggests a much greater sensitivity to endotoxin activity in cardroom dust than the previously mentioned studies.

In a dose response study from a swine confinement building, endotoxin (range 0.1-1.1 $\mu g/m^3$) was correlated with workshift change in FEV_1 (range +4 to -6%). Since Gram-positive bacteria are predominant in this environment a problem with reliability of the LAL analysis exists, as indicated from comparisons of chemical analysis of LPS and LAL determinations of LPS in poultry confinement buildings (127). LPS determinations from environments with high levels of fungal spores also have shown erratic values suggesting interaction between fungal glucan and the LAL assay (100).

Spores of fungi and actinomycetes: Limited information concerning possible associations between exposure to fungal or actinomycete spores and toxic symptoms or allergic alveolitis are available. Exposure exceeding 10^9 spores per day for weeks was associated with allergic alvoelitis and exposure exceeding 10^{10} spores on a single day was associated with acute symptoms. This corresponds to spore concentrations in air in the order of $10^9/m^3$ or more.

Rats given 10^9 spores intratracheally showed high mortality 48 hours after administration of two out of five strains of *Aspergillus fumigatus* spores, with signs of invasive growth, neutrophil accumulation and necrosis. Three weeks after exposure there were chronic granulomatous changes with macrophages and giant cells (66). A dose of 10^5 - 10^6 spores given intratracheally did not much affect pulmonary function in cortisone treated mice (141). Four hour long exposures of an aerosol of 10^8 to 10^9 spores/m^3, 5 days a week for several weeks resulted in a lymphocytic granulomatous condition resembling allergic alveolitis in guinea pigs. Shorter exposure times (40 minutes) or lower aerosol concentrations (10^6 spores/m^3) did not cause such changes (36).

12. Discussion

The mechanisms causing noninfectious inflammatory disease from inhalation of microorganisms are not fully known. An acute inflammatory event and a more prolonged immune-mediated disease state (allergic alveolitis) can be recognized. They could be viewed as normal defence reactions to extreme exposures to potentially harmful microorganisms, as opposed to the development of allergic asthma or rhinitis to common environmental agents. The acute symptoms could be mediated by receptors directed against different classes of micro organisms which do not require immune recognition or by nonspecific mechanisms such as complement activation. So far the interaction between LPS and its receptor has been

most studied and it has been demonstrated that inhaled LPS in high quantities can produce the typical symptoms of acute reactions to inhaled dust in man. It has, however, not been shown that endotoxin in air is the sole or even major cause of the acute symptoms observed in all the environments where such symptoms have been observed. A possible role of β- (1→3)-D-glucan and peptidoglycan remains an interesting speculation in the absence of provocation studies and generally accepted methods to measure the concentrations of these agents in air samples.

Much has been speculated regarding the antigenic properties of dusts causing allergic alveolitis. It could be more fruitful to speculate about the adjuvans properties of the dust, and again the in vitro and experimental work would suggest a possible role for glucans, peptidoglycans and possibly LPS.

Many issues relating to the choice of strategies for monitoring the environment with regard to microorganisms remain to be clarified. Today it is not possible to state with certainty whether the microorganisms need to be alive or not in order to cause allergic alveolitis or toxic symptoms. It also remains to be shown whether certain types of fungi or actinomycetes are more dangerous than others. Finally the role of LPS, peptidoglycan or β- (1→3)-D-glucan alone or in combination as risk indicators for toxic or immune mediated diseases is still not fully clarified.

It is necessary to reduce exposure to microorganisms in many work environments where exposure levels are excessive today. A simple indicator of the effectiveness of preventive measures is to measure the changes in total spore count and/or LPS activity, depending on the environment. Especially in the cotton industry there is a good argument for using LPS activity as indicator of toxicity of the dust. LPS and total spore count in the dairy farming environment are clearly better risk indicators than total dust, but at present neither can be said with certainty to represent the causative factor (s) of disease in the occupational setting. Questions regarding the validity of LPS activity measured with LAL assay in dust with a large proportion of Gram–positive bacteria or fungal spores need to be resolved before the LPS-method can be recommended in such environments.

Inhalations of LPS in amounts corresponding to several tens of µg of free LPS may affect health adversely. Environmental samples may have a biological activity which is about three times higher than the measured LPS level compared to free LPS. Thus inhalation of dust with a LPS concentration of 1 $\mu g/m^3$ or more for a full working day could result in symptoms caused by inhaled endotoxin. The minimal level causing symptoms in sensitive persons should be clearly lower. Similarly even short exposures (ten minutes) to spore concentrations of $10^9/m^3$ or more may cause harm, but it is not known if this applies equally to all kinds of fungi and actinomycetes.

Summary

P. Malmberg. Microorganisms. Nordic Expert Group for Documentation of exposure limits. (available in Swedish in Arbete och Hälsa 1991:) Arbete och Hälsa 1991

Inhalation of microorganisms may cause non-infectious symptoms and diseases by activation of non-immune defence mechanisms and by causing immune responses. The symptoms include allergic alveolitis, febrile reactions (organic dust toxic syndrome, ODTS), chronic inflammatory changes in airways with chest oppression, cough and "monday exacerbation of symptoms", "mucous membrane irritation", allergic asthma and rhinitis. Inflammatory cells have receptors which recognize and respond to common components on microorganisms, such as endotoxin, peptidoglycan and "fmlp-like" peptides on bacteria and β-(1→3)-D-glucan on fungal spores. This results in a "toxic" inflammatory reaction which has been reported from many work environments and upon repeated intense exposure the reaction is compounded by immune-reactions causing allergic alveolitis. One or both of these reactions have been reported from the cotton industry, various aspects of farming, including swine and poultry production, from grain handling, some types of wood handling, composting and from offices with unclean humidifiers.

Keywords: microorganisms, endotoxin, febrile reactions, allergic alveolitis, occupational exposure limit.

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TRICHLOROETHENE

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CONTENTS

Abbreviations:

Dichloroacetic acid	= DCA
Dichlorovinylcysteine	= DCVC
N-(hydroxyacetyl)-aminoethanol	= HAAE
2,2,2-Trichloroacetic acid	= TCA
2,2,2-Trichloroethanol	= TCEOH
1,1,2-Trichloroethene	= TCE
Total trichlorocompounds (in urine)	= TTC

BACKGROUND

TCE is a colourless liquid with a slightly sweet chloroform-like odour. The compound was first synthesized by Fisher in 1864. TCE has been used industrially for the past 60 years, mainly to degrease metal. It is also widely used in textile cleaning, solvent extraction processes, as a carrier solvent, and as a solvent in adhesives and a variety of other products. It is no longer used as a grain fumigant. TCE is now used only occasionally in anaesthesia (309).

Persons may be exposed to both TCE vapour and TCE liquid at work. Liquid TCE causes skin problems due to its irritating properties, but exposure by inhalation is considered most important in an occupational context. The highest atmospheric concentrations occur in connection with open degreasing processes. TCE may be emitted to the environment from industrial plants as vapour and in aqueous effluent (309), and is degraded by the natural microflora (227,228). Although the exposure levels experienced by the general population as a result of accidental spillage and contamination of water are usually much lower than occupational exposure levels it is the former that has attracted attention in recent reviews (68,309). This document is an updated version of the Nordic Expert Group's document on trichloroethylene published in 1979 (233).

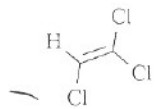
1 PHYSICO-CHEMICAL DATA

Chemical name (IUPAC): trichloroethene

Synonyms: acetylene trichloride, 1-chloro-2,2 dichloroetylene, 1,1-dichloro-2 chloroethylene, ethylene trichloride, ethinyl trichloride, TCE, TRI, 1,1,2-trichloroethene, trichloroethylenum, trichloroethylene, 1,1,2-trichloroethylene.

CAS registry number : 79-01-6
Molecular weight : 131.4
Chemical formula : C_2HCl_3

Structural formula



Physico-chemical properties:

boiling point	: 87° C
melting point	: -73° C
vapour pressure (20° C)	: 7.7 kPa (58 mm Hg)
vapour density (air=1)	: 4.54
specific gravity (25° C, water=1)	: 1.46
flash point	: non-inflammable

conversion factors at 25° C : 1 ppm= 5.38 mg m^{-3}
: 1mg m^{-3} = 0.19 ppm

Log partition coefficient
n-octanol-water : 2.42

Solubility: 0.1 part per 100 parts of water at 25° C. Mixes freely with alcohols, ethers and many other organic solvents.

Odour threshold 115 mg m^{-3} (21.4 ppm) (279).

Commercial TCE has a minimum purity of 99.85 % when used as a chemical reagent. The commercial product used in industry contains impurities and stabilizers. The amount of impurities, mostly other halogenated aliphatic hydrocarbons, depends on the manufacturing procedure. The total amount of impurities is usually less than one g/kg. The chemical lability of TCE is counteracted by adding antioxidants. Of the numerous additives used by different manufacturers the epoxides (e.g. epichlorhydrin) have attracted interest due to their chemical reactivity and mutagenic potential. The amount of additives normally ranges from 20 to 600 mg/kg in commercial TCE, but much larger concentrations have been reported. In the vicinity of an open flame (e.g. welding) or a glowing cigarette, TCE reacts to form phosgene and hydrogen chloride (309).

2 OCCURRENCE AND USES

2.1 Uses

Most of the industrially produced TCE is manufactured from 1,1,2,2-tetrachloroethane, derived from either ethylene or acetylene. The industrial processes for TCE production are well established and have changed little over the years (304).

In Western Europe, production of TCE amounted to approximately 250 000 tonnes in 1978. In the USA production was 130 000 tonnes in 1979 (309). In Sweden no TCE has been produced since 1988. Sweden imported 10 000 tonnes in 1988, and consumption (mainly for degreasing purposes) was 6 500 tonnes the same year (Institute of Occupational Health, Solna, personal communication). Import of TCE to Norway has decreased from 1 053 tonnes in 1975 to 835 tonnes in 1985 (34).

TCE is an industrial solvent used mainly for vapour degreasing of manufactured metal parts. It has also been used as a carrier solvent for insecticides and fungicides, as a solvent for waxes, fats, resins, and oils, as an anaesthetic in medicine and dentistry, and to extract oleoresins from spices and caffeine from coffee. TCE is a common solvent in printing inks, varnishes, adhesives, paints, lacquers, stain removers, rug cleaners, disinfectants, and cosmetic cleaning fluids. It may also be used as a chain terminator in polyvinyl chloride production and as an intermediate in the production of pentachloroethane (309).

By far the most prolific use of TCE has been as a fat solvent in vapour degreasing of metal parts prior to painting, anodizing, and electroplating. In the USA, as much as 90-95% of all TCE produced is used for degreasing (304).

2.2 Occupational exposure

Occupational exposure to TCE occurs in production of the compound itself, in handling and transporting liquid trichloroethene, in degreasing operations, etc. (309).

Exposure during production of TCE is fairly low and can be controlled (309). Users of TCE may be exposed to higher levels under relatively uncontrolled conditions, depending on the type of operation. Cleaning and maintenance of vapour degreasing equipment are critical factors for exposure levels. In Norway, approximately 1 000 industrial degreasing facilities used TCE in 1985, the majority were more than 10 years old (Directorate of Labour Inspection, Norway, unpublished).

A Danish study shows a decrease in mean urinary TCA levels from 1947 to 1986. The mean value was 82 mg per litre urine in the period 1947-1951 and 18 mg per litre in the period 1982-1986. The data are based on measurements among monitored workers exposed to TCE (37).

During the period 1985-1990 the National Institute of Occupational Health in Norway carried out a total of 7 021 measurements of solvents in ambient air. TCE was reported in 258 (3.7%) measurements, and the TCE in the air samples exceeded 108 mg m^{-3} (20 ppm) in seven percent of the cases (Per Fjeldstad, personal communication).

2.3 Methods for analyzing TCE concentration in air

TCE in air is determined by adsorption to charcoal tubes, followed by desorption with carbon disulphide (6,222) or N,N-dimethylformamide (Syvert Thorud, personal communication), and gas chromatographic analysis using a flame ionization detector. A method is available for direct determinations in the field based on spot sampling or sampling in Tedlar bags, followed by analysis using a portable gas chromatograph with a photoionization detector (223).

3 KINETICS

3.1 Uptake

3.1.1 Uptake by inhalation

Inhalation is the most important route of entry of TCE during occupational exposure. TCE is taken up fairly easily from the alveoli, as indicated by a medium high blood/air partition coefficient. In man this is reported to be 9.92 (256). In rat

there is some discrepancy in the reports of blood/air partition coefficients; 13.1 and 13.2 has been measured in pregnant and lactating Fischer 344 rats (79,80), while Sato et al. (256) report 25.82.

The amount and proportion absorbed were estimated for three male volunteers based on alveolar ventilation rates, ambient air and alveolar air concentrations of TCE. After exposure to 290-522 mg m^{-3} (54-97 ppm) for eight hours, the alveolar retention was found to be in the range of 70.8-78.0 % (77). Åstrand & Övrum (313) found that in male volunteers, the amount absorbed was 80 mg after exposure to 540 mg m^{-3} by inhalation for 30 minutes and was 170 mg after similar exposure to 1 080 mg m^{-3}. Thus the amount absorbed constituted about 55 % of the amount supplied. When volunteers exercised (50-150 W) during exposure, the uptake increased in absolute terms (mg h^{-1}), but declined in relative terms (e.g. 25 % retention while excercised (150 W) and exposed to 1080 mg m^{-3} for 1.5 h.). The latter result shows that during moderate exposure and exercise absorption to blood and tissues is slower than the rate of alveolar supply (313). Monster et al. (205) found approximately 70 % (uptake 6.6 mg per kg lean body mass) after repeated four-hour exposures of 377 mg m^{-3} to male volunteers.

Uptake by inhalation of TCE (160, 538, 5 380, 18 830, 43 040 mg m^{-3}) in male Fischer 344 rats has a mixted form, possessing both slow first order and saturable rate-curves (3). The first order curve reflects tissue loading, while the saturable component reflects enzymatic metabolism. With increasing concentration up to 18 830 mg m^{-3}, the first order rate constant decreased, and is estimated to be 0.062 $hr^{-1}kg^{-1}$ at higher concentrations. The exposure level at which uptake proceeds at 50 % of maximal velocity (K_m) was estimated to be 2 490 mg m^{-3} (463 ppm), and the estimate of maximum uptake (V_{max}) was 24.3 mg kg^{-1} hr^{-1} (3). Uptake is probably somewhat slower in pregnant and lactating Fischer 344 rats, since the blood/air partition coefficient, as well as the mixed function oxidase activity are slightly lower (79,80).

3.1.2 Uptake through the skin

Uptake through the skin has been assessed from human experiments where parts of limbs have been immersed in TCE, and excretion of TCE or its metabolites subsequently measured. No direct data on skin absorption rates have been reported.

Stewart and Dodd (280) found the peak concentration in exhaled air to be 2.7 mg m^{-3} (0.5 ppm) after immersion of a thumb in TCE for 30 minutes.

Concentrations of TCE in exhaled air and in blood, as well as excretion of TCE metabolites, were measured among volunteers after immersion of one hand in TCE for 30 minutes. TCE concentration in venous blood was monitored for 10 hours after end of exposure. Mean values were initially 15.5 µmol/l, decreasing to 0.24 µmol/l. These levels are approximately 80 % of the levels following inhalation exposure to 538 mg m^{-3} for four hours. The authors assess the uptake through the skin from immersion of a hand to be one third of the exposure by inhalation (257).

3.1.3 Uptake from the gastrointestinal tract

TCE passes easily across the gastrointestinal wall as illustrated by the numerous cases of poisoning following oral ingestion of TCE (304).

Data on excretion of TCE or its metabolites after oral administration clearly show that absorption from the gastrointestinal tract is almost complete. Single doses of ^{14}C-TCE (2.0, 20 and 200 mg/kg) in corn oil have been administrered by gavage to female Wistar rats and female NMRI mice. For both species 90-95 percent of the radioactivity was regained in exhaled air or urine 72 hours after cessation of exposure (54). After oral administration to rats, peak blood concentrations of TCE have been found after 6-10 minutes (277).

Absorption of TCE has been measured after administration of solutions of TCE (0.1, 0.25 and 0.5 %) to different intestinal segments of anaesthesized dogs. After two hours 50-70 % of the administrered amount had been absorbed, assessed from the TCE remaining in the segments. Administration to the colon gave higher blood concentrations of TCE than administration to the ileum or jejunum (125).

Exposure to TCE through the milk has been studied among nursing pups after different exposure regimens in lactating Fischer 344 rats. After inhalation exposure of the dams (3282 mg m^{-3} (610 ppm), 4 hr/day, 5 days/week) on days 3-14 of lactation the concentration of TCE in the milk (approx. 105 µg/ml directly after exposure) was much higher than concentrations in the blood (the milk/blood partition coefficient was 7.1). Nevertheless, no detectable levels of TCE were found in pup blood (detection limit <0.03 µg/ml blood). TCE was not detected either in dam milk or pup blood after oral administration to dams. Using a pharmacokinetic model the authors estimate that the exposure of the suckling pup is less than two percent of the exposure of the mother (80).

3.2 Distribution

TCE disappears rapidly from blood after absorption, due to distribution and metabolic transformation (205), or exhalation of unchanged TCE (46).

Distribution of TCE depends on tissue partition coefficients, blood perfusion rates and volume of compartments, and biotransformation/excretion of the parent compound. TCE is lipophilic, with a human fat/air partition coefficient of 674 and fat/blood partition coefficient of 26. The different tissue/blood partition coefficients in rats are estimated to range from 0.63 (muscle/blood) to 1.69 (liver/blood) (256). This corresponds well with the human erythrocyte/plasma partition coefficient of 1.59 (48).

There is evidence that distribution may depend on the route of uptake (see also chapter 3.3). Uptake from the intestines follows the portal circulation, leaving only a small proportion of TCE unchanged for further distribution. By contrast, TCE taken up by inhalation or through dermal exposure will be distributed into the systemic circulation, and will leave a larger part of the inhaled TCE unchanged (257).

Distribution of TCE and its metabolites in mice has been studied by means of low-temperature, whole-body autoradiography after inhalation of ^{14}C-labelled TCE. Immediately after inhalation, uptake of unchanged TCE occurs in the brain and other lipid-rich, well perfused tissues. Thirty minutes after inhalation, accumulation of TCE metabolites was found in the liver, kidney, and bronchi. After 30 minutes the brain was almost entirely cleared of unmetabolized TCE, while TCE was still present in adipose tissues (17). The latter finding concurs with kinetic modelling for TCE, where the predicted partial pressure of TCE in adipose tissue increases slowly during exposure and for several hours after an eight hour period of exposure. This makes a slight build-up of unchanged TCE likely in adipose tissue during long-term exposure (78).

Blood concentrations of TCEOH and TCA have also been monitored after experimental exposure of volunteers to 375-1 080 mg m^{-3} TCE for 2-4 hours (204,300). TCEOH concentration in blood reaches a maximum during the first hour after exposure, then declines exponentially with a half-life of 10-12 hours. The concentration of TCA in blood increases slowly during and after exposure, with a maximum concentration after 40 hours. After 60 hours the TCA concentration falls slowly with a half-life of 70-100 hours (204). These results are similar to those observed after inhalation exposure for 5-10 days, with estimated biological half-lives of 12.4-13.3 hours for TCEOH and 86-99 hours for TCA (213). One explanation of the long half-life of TCA is its high affinity for albumin in plasma (212). With daily exposures, TCEOH concentrations in blood increase sharply during exposure, and decline exponentially between exposures. The mean blood concentrations increase day by day (69,205). By contrast, TCA concentration in blood increases gradually when exposures are daily, with little diurnal variation (205).

Tissue concentrations of TCE (and TCA) have been measured in guinea pig following inhalation exposure (6 000-9 000 mg m^{-3}) for 5-23 days (20-115 hours). Comparatively high TCE concentrations (2-4 mg TCE/100 g fresh tissue) were found in adipose tissue, adrenals, ovaries and kidneys. 1-2 mg/100 g was found in blood and spleen. TCE concentrations in brain, lungs and liver were lower (71).

TCE passes easily from maternal to foetal blood in humans under anaesthesia. After 10-20 minutes exposure, the TCE levels in maternal and foetal blood TCE levels were equal, or slightly higher in the foetal blood (probably due to removal of TCE from maternal blood by metabolism) (175). Distribution of TCE and TCE metabolites has also been measured in pregnant animals and foetuses. The unmetabolized compound passes rapidly across the placenta to the foetus in rats (308), sheep and goats (115) and mice (94,95). Ghantous et al. (95) followed the distribution of TCE and its metabolites by means of whole body autoradiography after inhalation exposure. They found lower concentrations of TCE in the foetus and in the amniotic fluid than in maternal blood, while TCA concentrations were steadily higher in the amniotic fluid than in maternal serum (95). Unmetabolized TCE does not accumulate to any extent in foetal tissues (including brain). Foetal tissues are probably unable to form TCE metabolites, but these are transported from the mother (94). In another study (79) pregnant Fischer 344 rats were exposed to TCE by different routes (inhalation, gavage, drinking water) on day 3-20

of pregnancy. It was estimated that, per unit of body weight, foetal exposure to TCE was 67-76 % of maternal exposure (depending on the route of exposure in the pregnant rats), while foetal exposure to TCA was 63-64 % of maternal exposure. The estimated foetal tissue/blood partition coefficient was 0.51 (79).

3.3 Biotransformation

3.3.1 Hepatic biotransformation

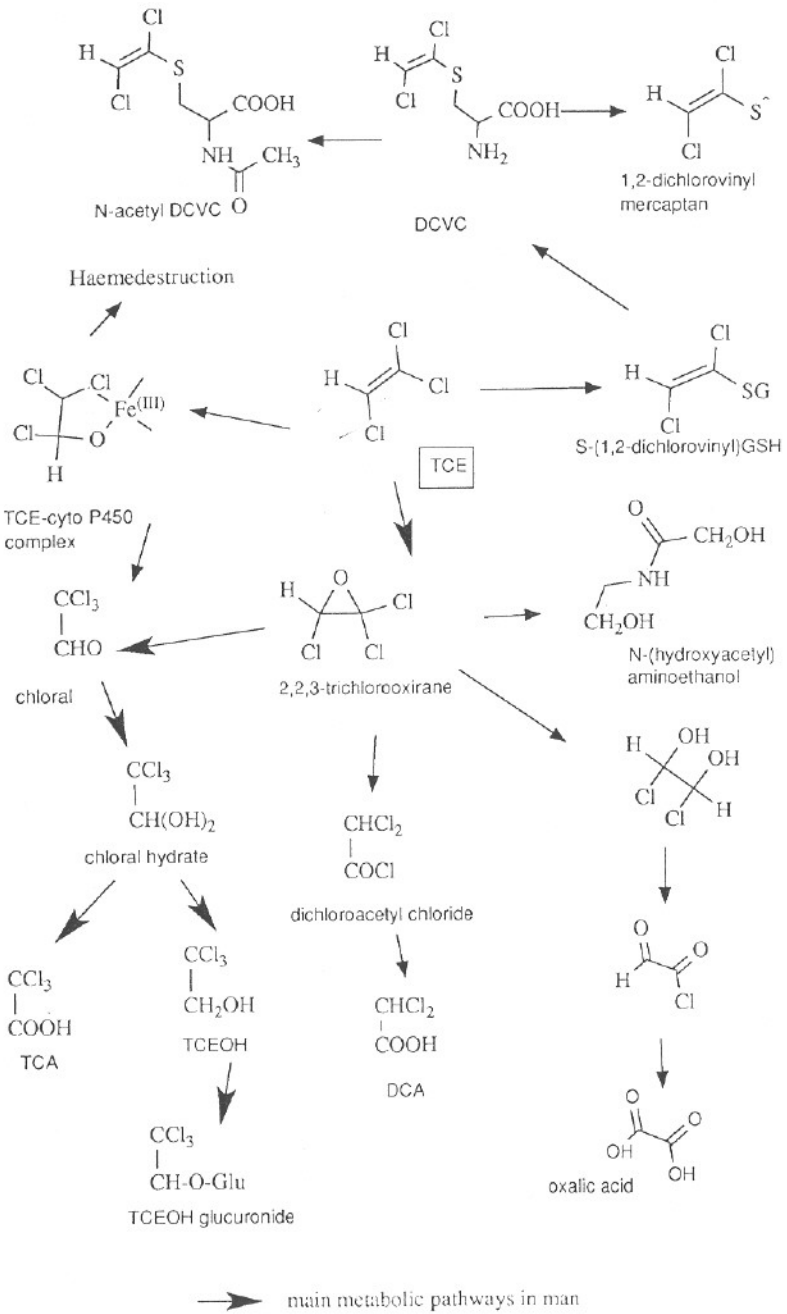
It is agreed that the main biotransformation of TCE takes place in the hepatocytes, and the liver is usually the only biotransforming organ taken into account in kinetic modelling (21,78). Biotransformation of TCE (in hepatic and extrahepatic tissus) is outlined in Figure 1.

Already in 1945, Powell found an oxidized TCE metabolite with three chlorines on one carbon in human urine. She suggested that TCE metabolism proceeds via an unstable epoxide intermediate (304). Subsequently Daniel (46) exposed rats to ^{36}Cl-labelled TCE and demonstrated that TCE oxidation to TCEOH and TCA proceeds without loss of labelled chloride, indicating an intramolecular rearrangement of chlorine and no exchange of chloride ions with the body's chloride-ion pool.

The initial biotransformation of TCE is carried out by the mixed function oxydase system and is dependent on cytochrome P-450 (Figure 1). The reactive epoxide intermediate 2,2,3-trichlorooxirane has not been identified, but there is indirect evidence of its formation. Uehleke et al. (296) have investigated spectral absorbance in vitro where TCE was incubated aerobically using rabbit liver microsomes and NADPH. The observed absorption spectra provide evidence of epoxide formation in the system. Kawamoto et al. (148) have shown by immunohistochemical staining of epoxide hydrolase in rat liver that this enzyme is induced by TCE. Oxidation of TCE by cytochrome P-450-dependent monoxygenase is also plausible, considering the subcellular location and cofactor requirements, and the response of the monoxygenase system to inducers and inhibitors (reference 2, see also chapter 3.5).

In the presence of NADPH and oxygen the major metabolites of TCE in liver microsomes are chloral hydrate, TCEOH and TCA. Under most conditions TCEOH is readily conjugated with glucuronic acid (213). An initial molecular rearrangement to chloral, and subsequent hydrolysis to chloral hydrate, occurs in the microsomal fraction. The reduction of chloral hydrate to TCEOH takes place in the cytosol, and the formation of TCA requires cytosol or mitochondria with NAD. The three major metabolites have been detected both in vitro (135) and in vivo (50). The formation of chloral hydrate is catalyzed by cytochromes P-450 (40,135). Different parts of the P-450 system seem to serve as catalysts (200), depending on the substrate concentration (219). The reduction of chloral hydrate to TCEOH is catalyzed by liver alcohol dehydrogenase and requires NADH (135). The oxidative formation of TCA is catalyzed by the NAD^+-dependent chloral hydrate dehydrogenase, or from oxidation of the TCEOH formed (258).

Figure 1. Simplified scheme of TCE biotransformation



Miller & Guengerich (199) have found experimental evidence of other ways of chlorine migration and formation of chloral from TCE than via the epoxide intermediate. In studies of in vitro systems using purified cytochrome P-450, liver microsomes or hepatocytes the kinetic data suggest the formation of a transitory complex of TCE and oxygenated cytochrome P-450. The chlorine migration occurs within this complex, forming chloral.

Alternative pathways of biotransformation other than the formation of TCA and TCEOH via chloral, as described above, have been extensively studied in the past 10 years. Although these pathways may be quantitatively of less importance compared with the formation of TCEOH and TCA, they may be significant from a toxicological aspect (see chapter 4.1).

Hathway (112) identified small amounts of dichloroacetic acid in urine of mice after intragastric administration of TCE. He proposed a "spill-over" theory, with formation of dichloroacetyl chloride, and subsequently dichloroacetic acid, from 2,2,3-trichlorooxirane when the haeme ferric ion favouring chloral formation became saturated. This agrees with earlier proposals (296) of chloral formation from the epoxide in the presence of electrophilic compounds (Lewis acids, e.g. ferric ion), and formation of dichloroacetyl chloride in the presence of nucleophilic compounds. The "spill-over" theory has later been confirmed by Dekant et al. (51,52,54) who administrered ^{14}C-TCE in single oral doses to mice and rats. Dichloroacetic acid constituted two percent of the urinary metabolites in rat urine 72 hours after exposure to 200 mg of TCE. This level was much lower after pretreatment of the rats with Arochlor or phenobarbital. The dichloroacetic acid level was much lower in mouse urine than in rat urine. The finding of $^{14}CO_2$ in exhaled air in dose-dependent amounts after administration of ^{14}C-TCE (2-200 mg) in mice indicates CO_2 as an end product of dichloroacetic acid biotransformation when the chloral pathway becomes saturated (54). $^{14}CO_2$ has also been found in exhaled air after administration of high doses (2 g/kg ip) of ^{14}C-TCE to mice and rats (237).

Formic acid and carbon monoxide have been found in vitro as breakdown products of TCE oxide (199), but carbon monoxide has not been observed in vivo after administration of ^{14}C-TCE (237).

Other metabolites found in the urine of mice and rats after oral administration of ^{14}C-TCE are oxalic acid (up to 1.8 % urinary metabolites) and N-(hydroxyacetyl)-aminoethanol (HAAE) (4.1-7.2% urinary metabolites) (52,54). The proposed transformation to oxalic acid and HAAE is via hydrolytic dechlorination of the intermediate oxirane. Glyoxylic acid may be an intermediate compound in oxalic acid formation, and HAAE formation may proceed via membrane lipid binding. HAAE has also been recovered in the urine of human volunteers exposed to 1 076 mg m^{-3} (200 ppm) TCE for six hours (52).

Miller and Guengerich (200) showed that the gluthathione (GSH) concentration decreased by 28 % when TCE oxide, GSH and phenobarbital-pretreated rat liver cytosol were incubated. Decrease of GSH has also been observed in rats in the initial stages of TCE anesthesia (248) and after inhalation exposure to 53 800 mg

m^{-3} for two hours (208). It has been shown that, as in the biotransformation of certain other haloalkenes, TCE forms an S-conjugate with GSH in vitro. The conjugate S-(1,2-dichlorovinyl) GSH is formed in the presence of GSH in rat liver microsomes and rat liver cytosol, but at a much slower rate than several other haloalkenes (57). The role of this conjugate in the renal metabolism of TCE is outlined in chapter 3.3.3.

3.3.2 Subcellular binding

Irreversible binding of TCE metabolites to macromolecules has been demonstrated in several studies, both in vivo and in vitro. Prerequisites for adduct binding are cytochrome P-450 and oxygen. Evidence that biotransformation is a necessary step is strengthened by the increased adduct formation after pretreatment with phenobarbital (54).

Several methods have been applied in the search for DNA adducts after exposure to TCE. Bergman (16) found radioactivity in RNA from kidney and liver and in DNA from kidney, testis, lung, pancreas and spleen of mice and rats after intraperitoneal injection of ^{14}C-TCE. This was due to metabolic incorporation of C_1, particularly into guanine and adenine, and not to adduct formation. In liver the dominant part of the DNA radioactivity was easily eluted, with no direct evidence of TCE-DNA-adducts. Parchman & Magee (237) measured DNA-labelling after intraperitoneal administration of ^{14}C-TCE to Sprague-Dawley rats and B6C3F1 mice. Very low DNA-labelling was detected in the liver, and no DNA-adduct formation was identified by high-performance liquid chromatography. In another in vivo study (284) B6C3F1 mice were treated orally or by inhalation to high doses of ^{14}C-TCE. Low alkylation of DNA was found in purified DNA from the livers. Trace amounts of protein adducts was not ruled out as an explanatory factor (284).

Searches for DNA adduct formation from TCE metabolites in vitro have consistently given other results than obtained from in vivo studies. Banerjee & Van Duuren (10) found covalent binding to DNA after incubation of ^{14}C-TCE with salmon sperm DNA in a cell-free system with addition of B6C3F1 mouse microsomes. The binding was enhanced when the mice were pretreated in vivo with phenobarbital pretreatment, or when an epoxide hydrase inhibitor was added to the system. In another study, covalent binding was observed after incubation of ^{14}C-TCE with calf thymus DNA in the presence of phenobarbital induced hepatic microsomes from rats (58). Miller and Guengerich (200) used liver microsomes from B6C3F1 mice, Osborne-Mendel rats and man in a system designed to observe covalent binding to calf thymus DNA. ^{14}C-TCE induced substantially higher DNA adducts in the presence of mice microsomes than in the presence of rat microsomes. DNA adduct levels in the presence of human microsomes were comparable to those found in the presence of untreated rat microsomes.

Irreversible binding of TCE metabolites to hepatic proteins from mice and/or rats has been found in several studies in vivo (22,237,295). In vitro, TCE has been shown to bind irreversible to microsomal proteins (10,295) and to intra- and extracellular proteins in isolated hepatocytes (200). In addition, the proposed TCE-

cytochrome P-450 complex (199) may result in irreversible binding to haeme ("suicidal haeme destruction"). Microsomal systems may be destroyed by this mechanism by up to 40 %. This reaction is enhanced by the deactivation of hepatic cytochrome P-450 under TCE anaesthesia of rats (248) and the in vitro reduction of cytochrome and haeme when TCE is administrered to a hepatic microsome-NADPH system (40).

Binding of TCE metabolites to RNA has also been demonstrated in vivo (16) and in vitro (200). Binding to lipids has been demonstrated in vivo and in vitro by Uehleke & Poplawski-Tabarelli (295) and in vitro by Leighty & Fentiman (181).

3.3.3 Extrahepatic biotransformation

The capacity of extrahepatic organs to biotransform TCE or major TCE metabolites has been assessed by comparing kinetics of anaesthesized dogs with or without circulatory bypass of the liver (124,126). Thirty minutes after inhalation of TCE for one hour (2 690-8 070 mg m^{-3}), the amounts of TCEOH, TCA and conjugated TCEOH formed in dogs with a bypass were estimated to be 50-80%, 10% and 10-20% of respective values in dogs without a liver bypass (124). Further biotransformation after intravenous administration of chloral hydrate, TCEOH and TCA was very limited in dogs with a bypass compared with dogs with hepatic circulation (126).

The gluthathione conjugate of the TCE metabolite 1,2-dichlorovinyl (S-(1,2-dichlorovinyl)GSH), identified in an in vitro rat liver system (57, see also chapter 3.3.1), is enzymatically metabolized in the kidney by sequential removal of glutamate and glycine to dichlorovinyl cysteine (DCVC), possibly in renal proximal tubules where the catalyzing enzymes are present in high concentrations (66,298). The cysteine conjugate can be N-acetylated by cysteine conjugate N-acetyl transferase to the mercapturic acid N-acetyl dichlorovinyl cysteine. Dekant et al. (53) found this metabolite in urine from male Sprague-Dawley rats. A possible alternative reaction to this deactivation reaction is metabolism to ammonia, pyruvate and the electrophilic thiol 1,2-dichlorovinyl mercaptan by cytosolic renal β-lyase (56). The suggested renal biotransformation of TCE is outlined in figure 1.

Isolated perfused lungs from rats and guinea pigs exposed to TCE vapour (160-240 mg m^{-3} up to 3 hours) seem able to metabolize the parent compound to TCEOH within 15-30 minutes. TCEOH formation was independent of TCE concentration and was increased pretreatment with phenobarbital (45). According to results obtained by Bergman (17), biotransformation of TCE by bronchus is also plausible. In mice, autoradiography after ^{14}C-TCE inhalation showed accumulation of non-extractable metabolites in the bronchi both 30 minutes and 8 hours after exposure. That lung cell microsomes are capable of metabolizing TCE has also been demonstrated using in vitro systems (10,200).

3.3.4 Quantitative aspects, interspecies variation

Qualitatively the metabolic pathways of TCE appear to be fairly similar in man,

mice and rats. There are considerable differences in the capacity of biotransformation, however. Mice have a much higher metabolic capacity than rats (54,237, 246,284). The amounts of metabolized TCE are probably proportional to body surface area rather than to body weight. Although there is only limited evidence on exposure levels that yield saturable metabolism in man it is reasonable to expect the same relation (28). This viewpoint is supported by in vitro kinetics, since rat hepatocytes metabolize four times more TCE than human hepatocytes do under the same experimental conditions (158).

In man, the metabolic clearance rate of the liver has been predicted from models that fit well with exposure and excretion data measured by Fernandez et al. (78). Following eight hours inhalation exposure (290 to 860 mg m^{-3}) the decay of alveolar TCE was observed for 50 hours and cumulative excretion of TCEOH and TCA for 200 hours (78). It is estimated that a fraction 0.86 of arterial blood flowing into the liver is metabolically cleared of TCE under sub-saturation conditions. Given a liver perfusion value of 96.3 liters per hour a reference man weighing 70 kg the predicted metabolic clearance rate is 83 liters per hour (21).

Nomiyama & Nomiyama (231) have compared metabolism in rat, rabbit and man based on kinetic measurements of urinary metabolites after intraperitoneal exposure. They conclude that the metabolic rate is lowest for man. There is a distinct difference between the species with regard to the relative amounts of excreted TCEOH and TCA. In the rabbit, TCA is a minor metabolite, with a high TCEOH:TCA ratio. In man the TCEOH:TCA ratio is much lower, while the ratio was between the two in the rat.

Results of experiments by Dekant et al. (54), where rats and mice were given oral doses of 2-200 mg/kg ^{14}C-TCE, confirm that mice have a greater capacity than rats for TCE biotransformation. The observed metabolic saturation seems to be confined to the first metabolic step, i.e. oxidative activation, since the saturation is manifested as increased exhalation of the parent compound. Further, some of the minor metabolites of oxidative activation (DCA in rats, and CO_2 (probably as an end product of DCA) in mice) increased at higher dose levels. This indicates that the capacity of the main deactivation pathway to TCEOH and TCA from chloral hydrate is saturated, and the alternative, minor pathways take over. This agrees with Hathway's "spill-over" theory (112). According to Dekant et al. (54) mice are most susceptible to these alternative pathways at high doses, due to the high capacity for oxidative activation in this species.

It has been demonstrated both in vivo (284) and in vitro (200) that mice have greater capability than rats to produce reactive metabolites that bind irreversibly to macromolecules.

3.4 Elimination

3.4.1 Elimination in humans

Several investigators have presented excretion data after exposing volunteers to

TCE by inhalation for a few hours (77,78,204,230,256,300) or on consecutive days (69, 205, 213). Data have also been reported referring to occupationally exposed populations (100,134,136) and experimental dermal (257) exposure to TCE.

Following inhalation exposure, most of the TCE is excreted as metabolites by the kidneys. The main metabolites excreted are TCEOH, conjugated TCEOH and TCA. A minor metabolite, N-(hydroxyacetyl)-aminoethanol, has also been recovered from the urine of volunteers exposed to 1080 mg m^{-3} TCE for four hours (52). Monster et al. (204) have reported exhalation of TCEOH from the lungs, but the amount is negligible compared with excretion of this compound in the urine. Exhalation of unchanged TCE is of next quantitative importance after urinary excretion of metabolites.

Fernandez et al. (77) exposed volunteers to 290-520 mg m^{-3} of TCE for eight hours, and monitored excretion for several weeks. Of the estimated absorbed dose, eight percent was exhaled as unchanged TCE, 32.7 % was excreted as TCEOH (conjugated and free) and 17.7 % was excreted as TCA in urine. These results agree well with those obtained by Monster et al. (204) where the exposure was 375-750 mg m^{-3} for four hours, and the excretion values of unchanged TCE, TCEOH and TCA were respectively 10 %, 39 % and 18 % of the retained dose. Monster et al. (205) obtained similar values after exposing volunteers after exposure to 375 mg m^{-3} TCE 4 hours daily for 5 days (exhaled TCE 11 %, TCEOH in urine 43 % and TCA in urine 24 % of retained dose). These elimination values do not agree with results obtained by Nomiyama and Nomiyama (230), who report a much lower retained total dose than demonstrated in other studies. As a consequence they find that excretion in exhaled air and as major urinary metabolites added up to 100 % of the retained dose (230). The discrepancy may partly be explained by the higher intensity of exposure in the latter study (1 345-2 045 mg m^{-3} TCE for 160 minutes).

The excretion kinetics of TCEOH (free and conjugated) and TCA in urine are closely related to the kinetics of these metabolites in blood (see chapter 3.2.)

Excretion of TCEOH (free and conjugated) in urine begins a few minutes after the start of inhalation. The excretion rate of TCEOH in urine increases during a single exposure lasting 4-8 hours, and reaches a maximum approximately one hour after termination of exposure (78,256,300). The TCEOH level in blood seems to be at peak for one hour (300). After a single 4 hours exposure (375-750 mg m^{-3}) TCEOH is almost totally eliminated from the urine after 3 days (204).

After a single inhalation exposure to 290-540 mg m^{-3} for 4-8 hours the rate of excretion of TCA in urine increases slowly to a maximum after approximately 48 hours (78,256). The very slow reduction in excretion rates in urine reflects the long half-life of TCA in plasma (see chapter 3.2.). After both single and multiple exposures to TCE, TCA will be excreted in urine for several weeks (204,205).

The relative importance of urinary excretion of TCEOH and TCA changes over time. During exposure to 270-540 mg m^{-3} of TCE for six hours daily for five days,

the TCEOH:TCA ratio was approximately 10 on day one, and decreased to between one and two on day five. For the five days of exposure accumulated, the TCEOH:TCA ratio was approximately two. After termination of exposure, the TCA excretion was highest and gradually increased its relative dominance (69). These results are consistent with those of Monster et al. (205). The kinetics of the exhaled parent compound will reflect the blood concentration of TCE, since alveolar and plasma concentration will follow each other closely. After end of exposure, decay of TCE in exhaled air is rapid, and exhaled air concentrations after five minutes are only 10 % of the concentration during exposure (205). Fernandez et al. (78) and Sato et al. (256) find a decay curve that can be resolved in three exponential components. Their results are not compatible with the biological half-life of the slowest component. Fiserova-Bergerova (81) suspects this discrepancy to be caused by differences in duration of measurements. While Fernandez et al. (78) monitored exhaled air concentrations for 72 hours, Sato et al. (256) made no measurements after 10 hours. Using kinetic modelling, Fiserova-Bergerova (81) estimates the half-lives of the three exponential components of TCE in exhaled air to be three minutes, 0.8 hours and 26 hours.

Monster et al. (205) have monitored TCE in exhaled air among subjects exposed for five consecutive days. They found no day-to-day differences in exhaled concentrations 100 minutes post exposure, but 18 hours post exposure the concentration in exhaled air was twice as high on day five than on day one.

Following dermal exposure to TCE, the concentrations of TCE in end-tidal air are higher than concentrations after inhalation exposure. Concentrations in exhaled air 0-2 hours post exposure were twice as high after immersion of one hand in TCE for 30 minutes than after inhalation of 540 mg m^{-3} for four hours, even though the absorbed dose was higher in the inhalation experiment (257).

3.4.2 Elimination in animals

Dekant et al. (54) have described elimination of TCE and its metabolites over a period of 72 hours after a single oral administration of ^{14}C-TCE (2, 20, 200 mg/kg) to rats and mice. The differences between species and variations with dose can be explained mainly by differences in capacity for biotransformation , as discussed in chapters 3.3.1. and 3.3.4.

93-96% of the administrered dose was recovered in urine, exhaled air, or faeces in both mice and rats. Most of the ^{14}C-activity was recovered in urine. In mice the amount recovered in urine was approximately 90 % in the low dose groups, decreasing gradually to approximately 75 % in the high dose group. In the high dose group the urinary elimination occurred mainly during the first 24 hours, but during the first 12 hours in the lower dose groups. In the rat, the total urinary elimination was approximately the same as for mice in the two lower dose groups, but was only 39 % of the administrered dose in the high dose group. The latter value was close to 50 % of the total dose for rats pretreated with Arochlor 1254 or phenobarbital. The rats exposed to low doses eliminated most of the urinary ^{14}C during the first 12 hours, while the elimination occurred mainly during the first 24

hours in the groups treated with 20 and 200 mg/kg of TCE (54).

The mice exhaled ^{14}C as CO_2 and TCE in increasing amounts with increasing doses, from two percent of administrered dose in the low dose group to 16 percent in the high dose group. The $^{14}CO_2$ exhaled was less than one percent in the low dose group, and approximately six percent in the high dose group. TCE exhalation that was approximately two percent of the total dose in the two lower dose groups, and increased to 9.5 % in the high dose group. The main reason for the low urinary elimination in the rats treated with high doses was a dramatic rise in elimination of TCE in exhaled air in this group. Exhaled TCE accounted for 3-4 % in the two and 20 mg/kg dose groups, compared with more than 50 % in the high dose group. The $^{14}CO_2$ exhaled increased in proportion to increasing dose, but not above two percent of total administered dose (54).

The dose recovered in faeces was approx. two percent in the low dose group of mice, and 4.5 % in the two higher dose groups. In all dose groups of rats, faecal recovery was close to that found in the low dose group of mice (54).

In the pooled 72 hours urine of mice, 93-94 % of the metabolites eliminated comprised 1,1,1-trichlorocompounds (TTC). The relative amount of TCA decreased with increasing dose from 20 % to eight %, while the relative amount of conjugated TCEOH increased with increasing dose, from 70 % to 86 % of the total urinary metabolites. Approximately five % of the urinary excretion was N-(Hydroxyacetyl)-aminoethanol, while dichloroacetic acid and oxalic acid constituted less than one percent. There was no apparent relative change with dose, other than in the relative distribution of TCEOH and TCA. In the pooled urine of rats the relative amounts of the minor metabolites HAAE, DCA, and oxalic acid were slightly higher than in the pooled urine of mice (54).

The characteristics of the elimination and variation between species in this gavage study (54) concur to a large degree with results from other studies where the same species were exposed orally to 10-2 000 mg/kg for 10 days (246), by inhalation (3 228 mg m^{-3} for 6 hours) (284), or by intraperitoneal injection of 2 g/kg TCE in corn oil (237).

The recovery of ^{14}C-activity in faeces indicates some biliary excretion of TCE metabolites. This agrees well with Bergman's (16) observation of nonvolatile radioactivity in the intestinal contents of mice 30 minutes to eight hours after inhalation exposure to ^{14}C-TCE. The cumulative amount of TCE and its metabolites excreted in bile has been monitored after intravenous injection of 25 mg/kg or inhalation of 3 765 mg m^{-3} in anaesthetized dogs (123). The cumulative biliary excretion two hours post exposure was approximately one percent of the administered dose (123). Studies of isolated perfused rat liver indicate that less than 10 % of the metabolites produced in the liver are excreted into the bile (147).

3.5 Factors affecting the metabolic model

As referred in chapter 3.3.1. the biotransformation of TCE is enhanced by inducers

of cytochrome P-450-dependent monoxygenases (54,200). Nakajima et al. have investigated the effects of different inducers in vivo (218) and in vitro (219). After pretreatment with ethanol (3 weeks) or phenobarbital (4 days) male Wistar rats were exposed to TCE vapour (2 690-43 040 mg m^{-3} for 2 or 8 hours). Both pretreated groups showed enhanced TCE metabolism, manifested by both increased TCE clearance from blood and increased urinary excretion of metabolites. There were significant differences depending on the inducers used. Ethanol-treated rats attained a maximum urinary excretion rate after exposure to 10 800 mg m^{-3} TCE, declining at exposures of more than 21 600 mg m^{-3}. By contrast, in the animals treated with phenobarbital excretion rates increased with increasing exposure, with no maximum rate attained at 43 040 mg m^{-3} (218). These results have been confirmed in an in vitro system using pretreated and control rat hepatic microsomes (219). The results of an in vitro study using rat liver microsomes from animals pretreated with phenobarbital, 3-methylcholanthrene and spironolactone indicates that TCE is metabolized by the phenobarbital-induceable form of cytochrome P-450, but not by cytochrome P-448. Addition of carbon monoxide to in vitro systems (40), or addition of cimetidine in vivo (178) (both are inhibitors of cytochrome P-450) lower the binding and metabolism of TCE.

Accelerated TCE metabolism after pretreatment with ethanol has also been observed, but without any increase in microsomal protein or cytochrome P-450 contents. The enhancement of TCE metabolism in male Wistar rats is most prominent when ethanol is administrered 16-18 hours prior to treatment with TCE (258). This may indicate that induction of TCE metabolizing enzymes by ethanol involves other systems than cytochrome P-450.

TCE metabolism is influenced by, and usually inhibited by, co-exposure to a number of other chemicals.

Like TCE, 1,1-dichloroethylene is a haloethene that is metabolized by microsomal oxidation. Different schemes of co-exposure by inhalation (TCE concentration 2 690 or 10 800 mg m^{-3}) of male Fischer 344 rats revealed competitive inhibition (4). The effect of co-exposure to TCE and another haloethene, tetrachloroethene, has been studied in a group of 38 Chinese workers. Assessment of the relation between individual TWA exposure intensities of TCE and urinary metabolite levels collected at the end of the shift shows distinct effects of co-exposure. For co-exposed workers the slope of the regression line between ambient air concentration and total trichloro-compound excretion was one-third of the slope for workers exposed to TCE alone. The reduction in excretion of metabolites was relatively more prominent for TCEOH than for TCA (264).

These findings are consistent with the results obtained after co-exposure of female Sprague-Dawley rats to tetrachloroethene and TCE (270-2,690 mg m^{-3} 2 or 6 hours). Much higher TCE concentrations were found in the blood after co-exposure than after exposure to TCE alone. The same inhibitory interaction was observed in the same experiment after co-exposure to TCE and aliphatic alcohols, possibly due to substrate competition, as well as pyrazole, which inhibits alcohol dehydrogenase activity, and disulfiram, which inhibits aldehyde dehydrogenase activity. By contrast,

coexposure to chloral hydrate and TCE had no influence on TCE metabolism (140).

Biotransformation of ethanol requires NAD^+, and increases the NADH/NAD^+ ratio in the hepatocytes. Therefore co-exposure to ethanol and TCE will shift the TCE biotransformation toward reduction of chloral hydrate to TCEOH (which requires NADH), and away from oxidation of chloral hydrate to TCA (which requires NAD^+). This has been shown experimentally in male Sprague-Dawley rats, but not markedly except with extreme doses of the two compounds (180). It has also been observed in rabbits that combined treatment with TCE and ethanol primarily reduces formation of TCA (307).

The complex interactive pattern between ethanol and TCE is probably the mechanism behind the well known intolerance to ethanol among workers exposed to TCE, often manifested as red flushes ("tri flush") on the face and other parts of the body (238,282,304).

The influence of host factors on TCE metabolism has been addressed in some studies, but there is little sound evidence. Nomiyama & Nomiyama (230) observed a marked sex difference in excretion of TCE metabolites after exposure of volunteers to 1 345-2 045 mg m^{-3} for 160 minutes. The urinary TCEOH:TCA ratio was 1.5 for males and 1.0 for women. This metabolic difference was even more pronounced the first 24 hours after exposure. The same effect, although less marked, has been observed in rats (231). Sex differences in excretion kinetics do not seem to be of practical significance in groups of workers that are occupationally exposed (133). Grandjean et al. (100) observed that the urinary excretion of TCE metabolites among workers exposed to TCE was dependent on age. Older workers excreted less TCA than younger ones did under equal exposure conditions. In rats, the maximum metabolic capacity has been shown to be moderately lower among pregnant and lactating animals than in the naïve female. The 21-day old rat pup (both sexes) showed a somewhat greater ability to metabolize TCE than adult animals did (79,80).

TCE metabolism may be influenced by circadian variations. The peak level values of TCEOH and TCA in serum of male Wistar rats were influenced by time of the day for administration of TCE and different lighting regimens. The highest TCEOH serum levels were obtained after intraperitoneal injection of TCE at 9 am, with a 12:12 hour light-dark cycle (209).

Little is known about whether long-term exposure to TCE may affect its own kinetics. There are reports of induction of the microsomal mixed function oxidase system after exposure to TCE (146,147,167). At the same time, TCE is capable of haeme destruction in the cytochrome P-450 (see chapter 3.3.4). Pessayre et al. (241) have shown that pretreatment with TCE increases microsomal mixed function oxidase activity with some substrates, while activity decreases with other substrates. It is difficult to compare quantitative kinetics among long-term exposed workers and among short-term experimentally exposed volunteers. The half-lives of TCE metabolites were estimated to be long in a TCE addict described in a case report (132).

3.6 Biologic monitoring

Amounts of TCA, TCEOH or total trichlorocompounds excreted in urine have been extensively used as biological indices of exposure and in biological monitoring of exposed workers. The validity of these methods is a matter of discussion, however.

Several investigators have found a linear relationship between urinary excretion of TCE metabolites and occupational exposure (133,134,136,235). As a consequence monitoring of total trichloro-compounds is suggested as an index of exposure to TCE at work (134,235). The validity of this conclusion may be questioned, mainly because the background for estimation of occupational exposure is not described satisfactorily in any of the reports. Exposure estimates, with no indication as to timing or duration (235), or exposure measurements performed on the days of urine collection (133,134,136), are of limited value, because urinary excretion may be influenced by variations in exposure over several preceding days, and by the time lapse between exposure and urine analyses,.

The limitations of the urinary metabolites as indices of exposure have been emphasized by several groups who have studied human TCE kinetics in experimental settings (69,204,205,213,300). Both production rates and excretion rates are very different for TCEOH and TCA, and the urinary excretion of total trichloro-compounds may therefore be misleading (213,300). Several studies (204,205,300) emphasize that the value of the methods is limited by large inter- and intraindividual variation in estimates of metabolites. Nevertheless, urinary TCA excretion is related to average exposure during the preceding days, and urinary TCEOH represents the most recent exposure (78,300). Given a stable long-term exposure, a TCA level of 100 mg per liter urine is approximately equivalent to a TWA exposure of 160 mg m^{-3}. Gubéran (105) has modelled excretion kinetics for subjects long-term exposed to steady TCE vapour concentrations of 538 mg m^{-3}, 8 h/d, 5 d/w. The estimated 24 hour urinary excretions of TCA are 243-322 mg, and of TCEOH are 288-429 mg (both increasing from Monday through Friday). The model fits well with the observed data from several studies using volunteers (105). Urine collection for 24 hours is of greater value than morning specimens or eight-hour collection, but is less feasible in practice (205).

Due to these limitations, other biological indices of exposure have been proposed, such as TCEOH in blood (300), TCA in blood (205), or a combination of blood and plasma TCEOH and TCA (213). These methods will present problems in regard to compliance and in connection with analytic methodology (99).

4 GENERAL TOXICOLOGY

4.1 Toxicologic mechanisms

When considering the mechanisms of TCE toxicity, several chemicals are potential agents of the toxic action. In principle, the parent compound, metabolite(s) from the major or minor biotransformation pathways, as well as stabilizers, impurities or

contaminants in technical quality TCE may play a role in the toxicity.

The major acute effects of TCE exposure in man are general narcotic depression of the central nervous system, and disturbances of cardiac rhythm. Both are probably caused by the parent compound. Intoxication due to TCE inhalation may induce an almost instantaneous loss of consciousness, narcosis and respiratory arrest from depression of the respiratory centre. This is likely to be the most common cause of fatality in cases of acute TCE poisoning (192).

There is sound evidence that TCE metabolites are responsible for chronic toxicity, in particular hepatotoxicity, nephrotoxicity, genotoxicity and carcinogenesis (28,44). However, the active metabolites that cause the different effects are not known. An attractive hypothesis is toxicity due to the electrophilic oxirane, which is more reactive in an assymmetrical substituted ethylene like TCE than in compounds with symmetrical substitution (2). Henschler et al. (117) have studied the transformation of 2,2,3-trichlorooxirane in an aqueous system, observing C-C and C-Cl fissions and reactive intermediates. The marked difference in this system compared with biological systems may be due to the presence of the haeme moiety with its trivalent iron. The binding of iron to the oxirane will protect biological systems from the direct reactivity of the oxirane that occurs spontaneously in aqeous systems (117).

When considering toxicity in different organs it is necessary to emphasize the impact of the route of exposure. In comparison with the filtering of metabolites in the liver after oral exposure, inhalation exposure will lead to greater accumulation of metabolites in the air passages (28).

The quantitative differences in rates of metabolism of different species offer several plausible explanations for the chronic toxicity and carcinogenicity of TCE. Both in vivo (54,237,246,284) and in vitro (10,200) studies report different rates of metabolism in different species. Thus treatment with high doses will lead to a much higher exposure to metabolites in mice than in rats (246). This interspecies difference in metabolic rates is correlated to the susceptibility to hepatocellular development of cancer.

Elcombe (64) showed that administration of TCE (50-2 000 mg/kg for 10 days by gavage) causes a dose-dependent proliferation of hepatic peroxisomes in mice, but not in rats. TCA administered by the same schedule induced proliferation of peroxisomes in both species. Therefore the interspecies difference as regards susceptibility to liver cancer may be a reflection of metabolic differences (65,98,101), for example the high formation of TCA in mice as suggested by Prout et al. (246). This may not be the whole explanation, however. TCA has been shown to inhibit gap junction-mediated intercellular communication in B6C3F1 mice hepatocytes, but not in Fischer 344 rat hepatocytes (157). This indicates an intrinsic species difference as well as the differing metabolic capacity between the two species (23,157). Indicators of peroxisome proliferation have also been found in a recent carcinogenicity study, where B6C3F1 mice received 1-2 g/l TCA in water for 52 weeks (30). In the same material, the TCA-treated mice showed

increased incorporation of tritiated thymidine in hepatic DNA, suggesting elevated DNA repair (255). Animals treated with similar doses of DCA in the same study showed liver necrosis and reparative hyperplasia (255). Therefore, the hepatic tumours in animals treated with DCA might depend on secondary cell proliferation (30). Although this study only reflects a small part of the evidence of TCE hepatotoxicity and hepatocarcinogenicity, it may indicate that different TCE metabolites may act through both epigenetic and genotoxic mechanisms. Considering the evidence as a whole, there is much to indicate that the mechanisms of TCE hepatocancerogenicity are epigenetic. However, some data suggest genotoxic mechanisms (28). There is little evidence in favour of DNA adduct formation in vivo. On the other hand, chloral hydrate disturbs chromosome segregation through direct interference with the spindle apparatus (see chapter 7.1.5.). Treatment with high doses of TCE shows no or only marginal promoting activity and no initiating activity in enzyme-altered rat liver assays (188,198,201,283).

Less is known about the mechanisms for extrahepatic chronic toxicity and carcinogenicity. Several studies show that extrahepatic cells can metabolize TCE to potential reactive metabolites (10,17,200).

Miller & Guengerich (200) have provided evidence in support of a theory of migration of reactive TCE metabolites from hepatocytes to other target cells, where they cause damage. They showed that in *in vitro* systems including isolated hepatocytes and exogenous DNA, the covalent binding of TCE metabolites to DNA and protein was mainly extracellular. The theory of migration of metabolites in particular offers an explanation of the mechanism of nephrotoxicity and nephrocarcinogenicity of TCE in rats. As outlined in chapter 3.3.1. and 3.3.3. GSH S-conjugates of 1,2-dichlorovinyl may be formed in the liver, and thereafter transported to the kidneys where further biotransformation takes place to either deactivated metabolites that are excreted or to reactive metabolites that are mutagenic and cytotoxic (57,66). In addition to the mutagenicity of some of the putative kidney metabolites (see chapter 7.1.5.), the metabolite DCVC is a kidney cortical tumour promoter in mice (195).

Pulmonary toxicity of TCE has been linked to TCE metabolism in situ. Bergman's autoradiography study (17) shows increasing accumulation of TCE metabolites in the bronchi for several hours after exposure. Forkert and co-workers (83-85) have shown selective damage to the bronchiolar Clara cells. This damage may be connected to high concentrations of cytochrome P-450 in Clara cells, as demonstrated in rabbits (266). It may also explain the increased incidence of lung adenocarcinomas (89) and adenomas (190) in rodent assays designed to demonstrate carcinogenicity by inhalation.

The mechanisms of the chronic neurotoxicity of TCE are not well understood, and nor is chronic toxicity in other organs and tissues. Studies indicate membrane effects (168,171,173,294), microtubular proteins (106), neurotransmitting systems (127,128,211,286), reduction of myelin in hippocampus (138,139), glial reactivity (106), and reduced glucose metabolism (229). One main problem is lack of

understanding of the potential neurotoxicological significance of these findings, and extrapolation to man.

4.2 Acute toxicity

Acute toxicity from inhalation of TCE has been experienced in individuals after industrial intoxication. Although the exposure concentrations in fatal accidents are unknown, reconstructions (involving other chlorinated solvents) indicate that exposure levels in the range of 50 000- 100 000 mg m^{-3} may produce brain stem depression and lead to death (234).

Acute toxicity in mice and rats is indicated in Table 1. The discrepancy between the results obtained by Parchman & Magee (237) and by Nomiyama et al. (232) is probably not due to a real difference in the susceptibility between strains. Nomiyama et al. (232) found that the LD_{50} value for ICR-JCL mice was lower when the animals were acclimatized to 8° C or 38° C compared with 22° C. In this experiment the low LD_{50} values after intraperitoneal treatment do not agree with toxicokinetic studies performed by Parchman & Magee (237) in the same species and using the same route of administration, or with other reported studies.

Table 1. Median lethal dose (LD_{50}) values for TCE in animals

Species/strain	Sex	Dose	Route	Ref
ICR-JCL mice	♂	40-75 mg/kg	ip	232
Sprague-Dawley rat/B6C3F1 mice	♂	2450 mg/kg	ip	237
CD-1 mice	♀	2443 mg/kg	oral	293
CD-1 mice	♂	2402 mg/kg	oral	293

The dose inducing a fifty percent inhibition of growth in cultured cells (ID_{50}) can be used as an index of acute toxicity. TCE and other chlorinated hydrocarbons have been investigated in two cell lines. The ID_{50} for TCE is 630 µg/ml in a human established cell line and 550 µg/ml in African green monkey kidney cells. According to this assay, TCE is less toxic than tetrachloroethene and 1,1,1-trichloroethane (203).

Various studies have shown that TCE is toxic to yeast cells. Cultures of *Saccharomyces* were treated with 0.05, 0.1 and 0.15 % concentrations of TCE. The result was a dose-dependent delay in growth of the culture (prolongation of the lag phase), but no inhibition. The growth rate curve of RNA was also retarded, but subsequently reached the levels obtained without treatment (184).

4.3 Subchronic and chronic toxicity

The subchronic and chronic toxicity of TCE in animals is indicated in Table 2. Tucker et al. (293) exposed CD-1 mice to TCE in water in concentrations of 0.1, 1.0, and 5.0 mg/ml for six months. The doses indicated in Table 2 are the mean daily intake values in the different exposure groups. Merrick et al. (197) administrered TCE by gavage both in corn oil and in 20 % aqueous Emulphor solution. The latter treatment induced increased mortality in the group exposed to the highest dose in both sexes (8/12 ♀ at 1 800 mg/kg, 12/12 ♂ at 2 400 mg/kg).

Kjellstrand and co-workers have carried out several studies in mice, rats, and gerbils to investigate the toxic effects of TCE inhalation (152-154). Animals were continuously exposed to TCE for 30 days (152). Some of the NMRI mice were allowed a recovery period of 30 days before examination. After 5 days recovery, 20 % of the increased weight of the liver still persisted, and almost the same value was obtained after 30 days without exposure. As far as effects on the liver are concerned, mice were more susceptible than Sprague-Dawley rats and mongolian gerbils (152). Kjellstrand et al. (153) exposed gerbil pups and young animals to TCE continuously (1 240 mg m^{-3}) during the first month of life. Mortality was increased among pups exposed from birth. Continuous exposure of NMRI mice to 200-1 615 mg m^{-3} for 30 days provided evidence of a dose-dependent increase in liver weight and plasma butyrylcholinesterase activity, with no apparent threshold level (154). Intermittent (1-16 hour/day, 7 days/week) exposure to higher concentrations (800-4 840 mg m^{-3}) did not seem to lead to any further increase in liver weight. Rehabilitation for 30 days led to partial normalization of liver weight, and after 120 days of non-exposure the liver weight had returned to normal (154).

Seven different strains of mouse that were exposed to 807 mg m^{-3} TCE for 30 days showed little inter-strain differences in increases in liver weight, but more marked sex differences in toxic effects (155).

Table 2. Subchronic/chronic toxicity assays for TCE in animals

species/strain/sex		dose/conc	duration	route	effect	ref
CD-1 mice	♂	24 mg/kg/d	14 d	oral	none observed	293
	♂	240 mg/kg/d		gav	liver weight ↑	
CD-1 mice	♂/♀	18 mg/kg/d	6 mo	oral	none observed	293
	♂	217 mg/kg/d		water	liver weight ↑	
	♂	660 mg/kg/d			weight gain ↓	
	♀	793 mg/kg/d			kidney weight ↑	
B6C3F1 mice	♂/♀	450 mg/kg/d	4 w	oral	liver weight ↑	197
	♀	1800 mg/kg/d		gav	mortality ↑	
	♂	2400 mg/kg/d			mortality ↑	
Wistar rat	♂	2000 mg/kg	15 w	sc	weight gain ↓	146
		twice weekly			liver weight ↑	
NMRI mice	♂/♀	807 mg m^{-3}	30 d	inhal	liver weight ↑	152
Sprague-Dawley rats	♂/♀	807 mg m^{-3}	30 d	inhal	weight gain[1] ↓	
					liver weight ↑	
gerbils	♂/♀	807 mg m^{-3}	30 d	inhal	liver weight ↑	
					kidney weight ↑	
gerbil pups	♂/♀	1240 mg m^{-3}	1-4 w	inhal	weight gain ↓	153
					mortality ↑	
NMRI mice	♂/♀	200 mg m^{-3}	30 d	inhal	liver weight ↑	154

[1] female rats only

5 ORGAN EFFECTS

5.1 Effects on skin, mucous membranes and eyes

TCE is a well known skin irritant, as experienced in hand immersion experiments, where the discomfort is significant after 30 minutes' immersion in liquid TCE (257).

Since 1974 several cases of generalized dermatitis connected with exposure to TCE have been reported from the United States, Spain, Singapore and Japan (15,39,96, 220,242). One of the characteristics of this severe dermatitis is that it ends in an intense desquamation with thin asteatotic scales. Most of the cases presented signs and symptoms of irritation of mucous membranes, such as conjunctivitis, sore throat, dysphagia, hoarseness, cough, corneal ulcers, oral ulcers, genital ulcers, otorrhea or rhinorrhea. It is suggested that the mechanism of reaction to TCE is delayed hypersensitivity (220).

Cases have been reported of scleroderma after prolonged and intensive exposure to 1,1,1-trichloroethane and/or TCE in connection with metal cleaning (82,108,253), some with a rapid and fatal outcome (186). It has been suggested that the epoxide of TCE might become incorporated in protein synthesis, creating a structural abnormal protein with an antigen property. The foreign protein might evoke a pathological immune response resulting in a scleroderma-like disease (186).

5.2 Respiratory effects

Intense haemorrhagic oedema of the lungs has been described in a teenager who was engaged for two years in metal degreasing involving exposure to TCE (129). Gases that are airways toxins (phosgene, hydrogen chloride) can be generated during thermal decomposition of TCE (309).

Male CD-mice were treated with 2 500 or 3 000 mg/kg TCE and killed 24 hours afterwards. The higher dose produced a significant decrease in the intracellular surfactant phospholipid. This finding has led to the suggestion that inhalation of TCE may damage the enzymes responsible for synthesising the pulmonary surfactant, thus resulting in lower amounts of surfactant being stored and available for secretion in the alveolus (263).

The effects of TCE on the microsomal mixed function oxidase system have been studied in mice. NADPH cytochrome c reductase activity was measured in microsomes isolated from lungs and livers of untreated animals and of animals exposed to TCE (3 injections each 0.33 g/kg ip). Pathological changes, such as formation of platelet thrombi and bronchiolar epithelial vacuolization were found in animals with reduced microsomal enzyme activities in the lungs. Thus decrease in pulmonary microsomal enzyme activity by TCE may reflect direct damage to the lungs (182).

TCE is toxic to Clara and alveolar Type II cells (83-85). Administration of TCE to mice causes severe morphological damage to Clara cells of the bronchiolar epithelium. This damage persists for at least 60 days after exposure. One hour after administration of a single dose of TCE (2 000 mg/kg) to CD-1 male mice, Clara cells of the bronchiolar epithelium exhibited necrotic changes involving the mitochondria and endoplasmatic reticulum. Dilatation of the endoplasmic reticulum became more severe two hours after administration of TCE and, after four hours, distended cisternae coalesced to form small vacuoles within the cytoplasmic matrix of the Clara cells. The severity of cellular damage increased progressively between

eight and 12 hours after exposure and, by 24 hours afterwards the majority of Clara cells within an air passage were severely vacuolated (85).

5.3 Gastrointestinal effects

The connection between pneumatosis cystoides intestinalis (PCI) and occupation was studied in 66 patients in Japan during a five year period from 1979-1983. Primary PCI occured more frequently in females (15/21) and predominantly affected the large intestine, with local involvement of the sigmoid colon. Most patients with primary PCI (16/21) were factory workers, of whom 15 (71.4 %) were engaged in degreasing manufacturing products with TCE. The high percentage of TCE-exposed workers among the patients with primary PCI suggests that occupational exposure to this agent is an etiological factor in the development of this disease (259).

Reactivation of a duodenal ulcer appears to represent a physical stress reaction secondary to exposure to TCE (193).

5.4 Hepatic effects

5.4.1 Hepatic effects in humans

According to Waters et al. (304) most of the evidence pointing to hepatotoxicity of TCE is inconclusive. Hepatitis and fatal hepatic necrosis from massive exposure to TCE in industrial settings is extremely rare (193) and may be caused by impurities. Cases of acute TCE poisoning, with a transient impairment of liver function, have been reported (93,287). TCE was not an inducer of liver monooxygenases in two groups of workers exposed to TCE (273).

Two hundred and eightyeight cases of industrial poisoning due to inhalation of TCE were reported to HM Inspectorate of Factories (UK) during the period 1961-1980. Five TCE poisonings were due to ingestion. One case was reported as having abnormal bilirubin concentrations, and another as having abnormal results in liver function tests, with a tender right hypochondrium. In two cases there were no signs of overt liver affection (192).

5.4.2 Hepatic effects in animals

Experiments to show the hepatotoxic effect of administration of TCE to mice and rats have provided little evidence of hepatotoxicity, even after inhalation of single doses up to 17 216 mg m^{-3} (3 200 ppm) (24,85,170). It is suggested that the hepatotoxic effects of TCE are caused by inadequate detoxification of its reactive intermediates (208). In isolated rat hepatocytes, however, TCEOH and TCA did not affect the viability of the hepatocytes (142).

In vitro and in vivo experiments during the past 10 years have shown that TCE has the ability to affect the enzyme activity of the liver. Kjellstrand et al.(154) showed that continuous exposure to TCE (200-1 615 mg m^{-3}) increased plasma

butyrylcholinesterase activity and liver weight in male mice in a manner dependent on concentration and duration of exposure. Significant effects on liver weight have been observed at 200 mg m^{-3} (154).

Koizumi et al. (160) exposed male Wistar rats to TCE continuously for 48 or 24 hours (2 150-4 300 mg m^{-3}). They found inhibition of δ-aminolevulinic acid dehydratase. Buben and O'Flaherty (29) administered TCE to male Swiss-Cox mice (0 to 3 200 mg/kg/day for 6 weeks). This affected liver weight and glucose-6-phosphatase activity. An in vitro assay demonstrated that, in isolated rat hepatocytes treated with TCE, viability was reduced in a dose-response relationship. Viability correlated with lactate dehydrogenase (LD) leakage at 1.9 and 5.7 mM TCE with a plateau level at 7.6 mM TCE. LD seemed to be more sensitive to TCE than aspartate transaminase (AST) and alanine transaminase (ALT) were, which suggests that TCE has an important effect on the plasma membrane due to formation of TCE epoxides (142).

In rats quite small doses of carbon tetrachloride produced severe liver injury when administered in a mixture with proportionately increasing doses of TCE than when administered alone. In all cases, pretreatment with acetone prior to the challenge led to more severe liver injury (36). TCE potentiates ethanol hepatotoxicity in male Wistar rats at TCE concentrations of 2 690 mg m^{-3} (218).

Elevations of serum bile acids at doses that have no effect on serum liver enzymes, bilirubin, or liver histology, have been observed in rats exposed to TCE (0.01, 0.1, 1.5 and 10 mmol/kg body wt) for three consecutive days by intraperitoneal injection or inhalation (302).

5.5 Renal effects

5.5.1 Renal effects in humans

Acute human exposure to certain organohalogenated compounds may sometimes result in proximal tubular injury. Only few studies have addressed renal effects among exposed workers, and these have provided little confirmation that exposure to these chemicals causes chronic renal damage (185).

David et al. (47) reported an unusual case of TCE poisoning where the patient developed acute renal failure but few of the other symptoms of TCE toxicity. The man was exposed to a 99% pure solution of TCE for eight hours (presumably by inhalation). A through-cut needle biopsy of the kidney was performed three days after admission to hospital, showing glomeruli within normal limits. The interstitium showed a patchy mononuclear cell infiltrate with large numbers of eosinophils. The infiltrate was related to tubules containing hard eosinophilic casts associated with damage to the tubular epithilial cells and rupture of the tubular basement membrane. In addition, there was mild diffuse interstitial oedema but no interstitial fibrosis (47).

The adverse effects of occupational exposure to TCE on kidneys are very mild and,

after measuring total protein and ß-2-microglobulin in urine from 104 male workers occupationally exposed to 80 mg m^{-3} TCE, are concluded to be glomerular rather than tubular (215).

5.5.2 Renal effects in animals

Conflicting results have been reported with respect to renal damage following exposure of animals to TCE (304). Abnormalities of the kidneys have been found, such as focal dystrophic changes in renal tubules and glomerular nephrosis (304).

The influence of continuous TCE inhalation (807 mg m^{-3} for 2-30 days) on kidney weights in rats, mice and mongolian gerbils has been tested. Increased kidney weight (15 %) was observed in gerbils exposed to TCE. This effect was less pronounced in mice and rats (152).

Male Fischer 344 rats pretreated with phenobarbital were exposed to TCE by intraperitoneal injection (5.5, 11.0 or 22.0 mmol/kg in corn oil) or by inhalation (5 380 or 10 760 mg m^{-3} for 6 hours). Urine was collected 24 hours after completed exposure and the animals were sacrificed. TCE exerts its acute nephrotoxic potential at a very high dose level and produces nephrotoxic insult at the proximal tubular and possibly glomerular regions of the rat kidney. An indicated threshold of nephrotoxic effects, assessed on the basis of biochemical effects, was found between the two highest injection doses (35).

Exposure of mice to a single dose of 125-1 000 mg/kg TCE and subsequent maintenance under hypoxic conditions induced lipid peroxidation and nephrotoxicity. It is suggested that the cause of the peroxidative changes and nephrotoxicity was caused by TCE-reactive metabolites and their reactive intermediates (38).

5.6 Haematologic effects

Twenty male humans were exposed to 510 mg m^{-3} TCE for 4 hours. The cytoenzymological activity in leucocytes increased during exposure (166).

Rats, guinea pigs, dogs, rabbits and monkeys were exposed to a concentration of 3 825 mg m^{-3} of TCE for 8 hours daily, 5 days a week over 90 days. No significant haematologic changes were noted (244). TCE was once a common animal poison when cattle, horses, and sheep ingested soya bean oil meal which had been defatted with TCE. Epidemics of fatal haemorrhagic disease, with aplastic anemia and marked decrease of leukocytes, were common (129).

Mature cross-bred dogs were acutely exposed to TCE. A marked but temporary decrease in all types of leukocytes was shown in a dose-response relationship between decreased leukocyte counts and exposure concentrations of TCE ranging from 0 to 700 ppm for one or four hours (122). Hobara et al: (124) has also demonstrated a transient decrease in leucocyte count in dogs with a hepatic bypass that were exposed for 1hour to 2 690-8 070 mg m^{-3}. TCE metabolites did

not decrease the leucocyte count in blood (126).

A pronounced and irreversible depression of the erythroid and liver δ-aminolevulinate dehydratase activity was observed in rats exposed to TCE vapour in concentrations ranging from 270-4 280 mg m^{-3} for 240 hours. This finding may indicate toxicity to TCE in erythroid cells (88).

5.7 Cardiovascular effects

5.7.1 Cardiovascular effects in humans

Hunter (131) reported disturbance of cardiac rhythm (mostly extrasystoles) 12 times in 50 cases anaesthezed with TCE. A suggested cause is dichloroacetylene contamination.

In an investigation of 75 degreasers, disturbances of cardiac rhythm due to the direct influence of TCE were rare, but could be demonstrated: In one case during the working shift (mean concentration was 470 mg m^{-3}), and in another case experimentally after exposure to 510 mg m^{-3} TCE for four hours (163).

TCE-associated deaths attributed to ventricular fibrillation and cases of cardiovascular irregularities, i.e. cardiac conduction, have been described (109,304).

Inhalation of large amounts of TCE for more than 20 years may have caused toxic myocarditis at some time during the period of exposure which subsequently led to the development of a congestive cardiomyopathy in a 49 year old metal degreaser (150). TCEOH is suggested as one of the most toxic agents with respect to the cardiac conduction system (150).

5.7.2 Cardivascular effects in animals

Like other halogenated hydrocarbons, TCE increases the sensitivity of the conduction system to epinephrine, and may consequently provoke cardiac arrhythmias. Rabbits treated with ethanol (1 g/kg po, 30 minutes prior to exposure) developed cardiac arrhythmias in response to epinephrine sooner and at lower doses during TCE exposure than control rabbits did (307). Disulfiram showed cardioprotective activity, reducing the percentage of rabbits that responded with arrhythmias after 60 min of exposure to 48 420 mg m^{-3} TCE (86).

The interaction of TCE combined with hypoxia and/or ethanol on the conduction system of the isolated perfused rat heart were investigated by recording the direct electrocardiogram. TCE has a direct, local effect on heart conduction. The co-administration of TCE (0.05 mM), hypoxia (45 % oxygen decrease), and ethanol (0.3 %) produced a significant ($p<0.01$) prolongation of the atrioventricular conduction time (PQ interval) ($P<0.01$) while TCE (0.05 mM) alone, hypoxia alone, ethanol alone, hypoxia with ethanol, TCE with hypoxia or TCE with ethanol caused no significant change in ECG findings. Hypoxia and ethanol synergistically enhance

the effect of TCE on the atrioventricular conduction system at the observed concentrations (145).

Myocardial damage due to TCE exposure has been demonstrated in animal experiments (150).

5.8 Effects on the nervous system

5.8.1 Effects on the nervous system in humans

5.8.1.1 Cranial nerve palsies

Barret et al. (12) reported impairment of the trigeminal and optic nerve in a group of 188 workers exposed to TCE. 50 % were exposed to more than 807 mg m^{-3} of TCE, 44 % to 269-807 mg m^{-3} and 6 % to less than 269 mg m^{-3} TCE. Symptoms were most severe in the heavily exposed group. These authors (13) also report disturbance of trigeminal somatosensory-evoked potentials in 40 out of 104 occupationally exposed workers (exposure levels not stated).

Abnormalities in the somatosensory-evoked potentials after stimulation of the trigeminal nerve were reported among nine workers suffering from TCE intoxication (duration and amount of exposure not stated) with clinical hypaesthesia in the fifth nerve distribution (11).

The latencies of the masseter and the blink reflex were determined in order to test the trigeminal nerve in 31 printing workers exposed to TCE (mean duration 16 yrs) at a mean concentration of 188 mg m^{-3}. An increase in the latency of the masseter reflex was reported (250).

A population of 21 persons were exposed to 63-400 ppb TCE through the public drinking water supply. A highly significant difference was observed in the conduction latency means of the blink reflex components (involving the trigeminal and facial cranial nerves) when the study population was compared with a control group (N=27) (75).

Plessner (243) described four patients with affection of the trigeminus nerve after occupational exposure to TCE. Humphrey and McClelland (130) have reported 13 cases of cranial nerve palsies following general anaesthesia. The nerves involved were mainly the fifth and seventh, but the third, fourth, sixth, tenth and twelfth were affected in some cases. The condition was associated with labial herpes in nine cases. The authors also present evidence of chemical reactions between soda-lime and TCE in the system, with production of dichloroacetylene (130).

Feldman et al. (73) describe a young man with acute industrial TCE intoxication. They report electrophysiologic evidence of a peripheral neuropathy, and present a clinical definition of the pattern of trigeminal sensory loss and recovery. The onion peel distribution, usually indicative of segmental or nuclear trigeminal lesions, is best explained in this case by disturbed nerve conduction, possibly due to the lipid

solvent effect of trichlorethylene on peripheral myelin sheets. Long-term residual oculomotor and ciliary reflex dysfunction, as well as impaired neurophysiological performance, have been reported in the same subject 18 years after the event (74).

Sections of the brain of a fatal case following extreme exposure to TCE for four hours showed striking alterations in the brain stem, the fifth nerve nuclei, spinal tracts and nerve roots. Extensive myelin and axon degeneration were found. The principal sensory and spinal tract nuclei of the fifth nerve showed severe loss of nerve cells, which was also observed in the motor nuclei of the fifth nerve. Somewhat similar, though less severe, damage was seen in the nuclei of the sixth and seventh nerves. Less severe damage was seen in ascending sections. The red nucleus, substantia nigra, mammillary bodies, as well as Sommer's sector in the hippocampus all showed some damage (31).

TCE can decompose to form dichloroacetylene, a compound with marked neurotoxic properties. This compound is believed to be the causative agent in TCE-induced trigeminal neuropathy (278). Several reports on trigeminal neuropathy and cranial nerve palsies have been published. This affection has usually appeared after massive exposure to TCE contaminated by dichloroacetylene. Contact of TCE with concrete or other alkaline material, with formation of dichloroacetylene, has been suggested as the cause of symptoms attributable to polyneuritis cranialis, for example, among the inhabitants of a private home (103). It has been suggested that TCE can reactivate latent herpes virus (33).

5.8.1.2 Central nervous system - experimental exposures

Ettema et al. (70) exposed groups of volunteers to 0, 807 and 1 615 mg m^{-3} TCE. Six out of 11 of the high exposure group showed decreased performances in a psychometric test battery, while no decrements were noted for the two other groups.

Vernon and Ferguson (299) used six standard tests to evaluate visual-motor performance in eight male volunteers exposed to 538, 1 615, and 5 380 mg m^{-3} TCE for two hours. The highest concentration had an adverse effect on performance in the Howard-Dolman, steadiness, and pegboard tests. No significant effects were noted at the two lower concentrations (299).

Konietzko et al. (162) examined three different groups to investigate the influence of TCE on psychomotor functions. No short-term impairment was observed after experimental exposure to 510 mg m^{-3} of TCE, or during a working shift.

The effect of exposure to TCE on performance in tests of numerical ability, reaction time, and short-term memory was studied in 15 healthy male subjects (92). The subjects were tested individually on 3 different occasions during exposure to 540 mg m^{-3} (100 ppm) and 1 080 mg m^{-3} (200 ppm) of TCE respectively in inhaled air and under control conditions. A comparison of the results of the reaction time test and the short-term memory test under control conditions and during exposure to TCE provided no indication of decrement of performance from exposure to TCE.

Table 3. Effects on the central nervous system reported among volunteers after short-term exposure to TCE vapour

Number of volunteers	Exposure level[1]	Duration[2]	Investigated effect	Result	Reference
10	176-429 mg m^{-3}	90 min	Vestibulo-ocular reflex	+	179
20	510 mg m^{-3}	4h	EEG-changes	-	161
20	510 mg m^{-3}	4h	Performance ability	-	162
4	538 mg m^{-3}	3h/d 4 days	Performance ability	-[3]	217
10	538 mg m^{-3}	1h/5d	Subjective complaints	-	281
6	592 mg m^{-3}	8 h	Performance ability	+	254
6	592 mg m^{-3}	8 h	Performance ability	-	282
16	807 mg m^{-3}	2.5h	Performance ability	-	70
10	1080 mg m^{-3}	1h/5d	Subjective complaints	+[4]	281
15	1080 mg m^{-3}	70 min	Performance ability	-[5]	92
15	1080 mg m^{-3}	2.5h	Performance ability	-[6]	310
8	1615 mg m^{-3}	2 h	Performance ability	-	299
15	1615 mg m^{-3}	2.5h	Performance ability	+[7]	70
8	1620 mg m^{-3}	2h	Performance ability	+[8]	76
8	5380 mg m^{-3}	2 h	Performance ability	+[8]	299

[1] Only highest no-effect level and/or lowest effect level indicated

[2] NS=not stated

[3] Nonsignificant decrement in exposed group

[4] Mild fatigue and sleepiness on day 4-5 of exposure

[5] Affected performance in numerical ability test

[6] Co-exposed to ethanol 0.4 ml/kg

[7] Borderline decrement in performance stated

[8] Co-exposed to ethanol 0.5 ml/kg

However, the test of numerical ability showed statistically significant decrement in performance during exposure. The results as a whole indicate that there should be no risk of acute effect on the functions of the central nervous at concentrations that do not considerably exceed the Swedish threshold limit in 1976 (160 mg m^{-3}) (92).

Salvini et al. (254) exposed six male students to TCE with an average vapour concentration of 592 mg m^{-3} for two four-hour periods, separated by an interval of 1 ½-hours. Each subject was examined on two different days. On one day they carried out a set of tests in an atmosphere contaminated with TCE vapour and on the other day they did the same tasks in a control atmosphere which did not contain TCE. Four tests were performed: a perception test with tachistoscopic presentation, Wechsler Memory Scale, complex reaction time test, and manual dexterity test. All the tests showed a statistically significant decrease in performance; the greatest decrement occurred during the more complex tests (254). In another study (282), three female and six male volunteers were exposed in three groups to TCE vapour concentrations of 269 and 592 mg m^{-3} for 8 hours. Behaviour performance tests were carried out twice on each day of exposure. The investigation failed to collaborate the results obtained by Salvini et al.(282).

Human subjects exposed to TCE vapour (538 mg m^{-3}) for three hours twice a day for four days showed only a slight decline in psychophysiological functions, and a low percentage reduction in performance (statistically non-significant) (217).

Ten healthy volunteers were subjected to a vestibulo-oculomotor test battery before, during and one hour after exposure to 176-429 mg m^{-3} (32-78 ppm) of TCE. The subjects exercised (50 W intensity) during most of the period of exposure. Decreased ability to visually suppress the vestibulo-oculomotor reflex during sinusoidal stimulation was noticed during exposure, and a decrease in the maximal velocity of the voluntary saccade and ability to follow a sinusoidally moving target was observed one hour after cessation of exposure. The authors do not specify inter-subject variation to exposure, and it is therefore impossible to assess a dose-response relationship (179).

The EEGs of 20 volunteers were read at hourly intervals telemetrically during exposure to 510 mg m^{-3} TCE for four hours. The same investigation was carried out without TCE. Compared with responses to other compounds the changes were minimal (161).

Ten volunteers were exposed to TCE vapour, 538 and 1 076 mg m^{-3}, for periods ranging from one hour to a five-day working week. At the high dose level, subjective responses were mild, occurred inconsistently, and were of doubtful clinical significance. However, five subjects experienced a sensation of mild fatigue and sleepiness during their fourth and fifth consecutive days of exposure to the high concentration (281).

5.8.1.3 Central nervous system - occupational exposure

Affection of the CNS following exposure to TCE has been described in the

literature. Most of the acute or subacute effects refer to exposure to high doses of TCE. There is little evidence of chronic effects or encephalopathia following long term exposure to TCE. No controlled epidemiological studies have been performed to elucidate the chronic CNS effects from long term exposures to TCE.

Grandjean et al. (100) investigated the effects of exposure to TCE among degreasers. In most cases concentrations of TCE near the tanks varied from 100 to 200 mg m^{-3} (20 to 40 ppm). On average, larger amounts of TCA were excreted in men using TCE all day than in men who only worked part-time at the TCE tanks. Fifty persons had been exposed for periods ranging from one month to 15 years, the average being 3¾ years. Fatigue, dizziness, and inability to tolerate alcohol was particularly high in this group. Neurological changes were found in 28 % of the workers. Disorders of the vegetative nervous system were found in 36 % of the workers. Clinical and neurological examinations showed that 17 workers (34 %) had a slight or moderate psycho-organic syndrome.

The mental effects on 130 subjects exposed to TCE has been investigated. Workers exposed to highly levels of TCE, with more than 60 mg/l TCA in the urine, complained of tiredness, dizziness and gastro-intestinal complaints to a greater extent than workers with less than 60 mg/l TCA in the urine (275).

Thirty-one workers exposed to TCE for periods of one week to 20 years (exposure levels not stated) were examined by means of a psychometric test battery. Significant chronic impairment of their psychomotor function was noted (162).

Psychological and biochemical examinations were carried out on eight persons who were long-term exposed to 260 mg m^{-3} (50 ppm) TCE. The purpose of the psychological examinations was to clarify changes in mental functioning in terms of "psycho-organic syndrome". The results of the psychological tests gave no hint of changes in the CNS (291).

Liu et al. (183) reported a dose-response relationship for symptoms such as nausea, heavy-headedness, forgetfulness, tremor in extremities, cramp in extremities and dry mouth in over 100 workers exposed to TCE, mainly in concentrations lower than 269 mg m^{-3} (duration of exposure not stated).

Barret et al. (12) reported asthenia, headache and dizzyness in a group of 188 workers exposed to TCE. Fifty percent of the group was exposed to more than 807 mg m^{-3} TCE.

The duration and amplitude of alpha waves increased significantly from control episodes to episodes involving exposure to TCE. This study was performed on factory workers monitored with EEG telemetering devices during one week of exposure. Duration and levels of exposure were not stated (164).

Table 4. Effects on the central nervous system reported among workers after long-term occupational exposure to TCE

Number of workers	Exposure level[1]	Duration[1]	Investigated effect	Result	Reference
50	108-216 mg m^{-3}	<15 y	Symptoms	+	100
			Neurologic affection	+	100
130	>60mg/l (TCA)	NS	Symptoms	+	275
31	NS	<20y	Performance ability	+	162
12	274 mg m^{-3}	NS	EEG-changes	+	164
8	260 mg m^{-3}	NS	Performance ability	-	291
94	>807 mg m^{-3}	7y	Trigeminal affection	+	12
83	269-807 mg m^{-3}		Symptoms	+	12
11	<269 mg m^{-3}		Symptoms	+	12
100	<269 mg m^{-3}	NS	Symptoms	+	183

[1] NS=not stated

Schiøtz (261) surveyed 18 dry cleaning facilities. He reported 29 workers (cases) with TCE poisoning. 22 had experienced mental confusion, 24 had experienced fatigue also after the end of the working shift, and 11 showed signs of nausea and vomiting.

Stracciari et al. (285) report a case with reversible neuropsychological impairment and persistent EEG paroxysms after exposure to TCE for 3-4 hours a day for one year.

5.8.1.4 Peripheral nervous system

There seems to be no large-scale involvement of the peripheral nervous system. A case of polyneuropathy has been reported, assumed to have been caused by chronic exposure to TCE (288). The most prominent symptoms were paraesthesia and pain in the extremities, paraesthesia around the mouth, and headache in the occipital region.

Sagawa et al. (252) describe a case in which complete loss of sensation in the trunk and the lower extremities developed after accidental exposure to an anaesthetic level of TCE. Causative factors may have been dichloroacetylene or other impurities.

The sural nerve conduction velocity (SNCV) response amplitude and refractory period (SRP) was measured in 31 printing workers exposed to TCE. A slight reduction (-1.1 m/s) in the SNCV and a prolongation (0.4 ms) of the SRP was found (250).

Serum dopamine-β-hydroxylase (DBH) activities were analysed in 84 male workers exposed to 59 mg m^{-3} TCE in the air (mean duration of employment was 6.1 years), in order to determine the influence of TCE on sympathetic nerve activity. No significant difference was found between mean serum DBH activities in the workers and in the corresponding controls (216).

5.8.2 Effects on the nervous system in animals

5.8.2.1 Effects on animal behaviour

Adult male rats were exposed to 7.9 µmol/l (200 ppm) TCE for six hours on four consecutive days. The motor behaviour of exposed animals was more active than that of the controls one hour after cessation of exposure on the fourth day. Trace effects of TCE on the emotional behaviour of the same rats could be seen 17 hours after cessation of the last exposure (260).

Mongolian gerbils were exposed to 1 720 mg m^{-3} TCE continuously for nine months. During this period the spatial memory of the animals was tested. No major differences were found between the exposed and control groups. Eleven days after cessation of exposure to TCE the animals were exposed to 2 300 ppm of 1,1,1- trichloroethane for 6 hours every second day. It was shown that the animals that had previously been exposed to TCE made more correct choices in the maze test on days when they were exposed to 1,1,1-trichloroethane than on days when not exposed to this substance. The choices of the control group that had not been exposed to TCE were less correct. The results are interpreted as indicating irreversible effects on the central nervous system from the exposure to TCE (151). Gade and Jensen (90) have discussed the interpretation of this study. The seemingly enigmatic data presented by Kjellstrand et al. conform closely to what would be predicted on the basis of a theory of state-dependent learning. Recollection of an event or behaviour learned under the influence of a drug may be facilitated by a similar drug state during the test of retention (90).

Female Sprague-Dawley rats were exposed to several nominal concentrations of TCE (312, 625 and 1 250 mg/l) in their drinking water. Experimental dams received TCE from 14 days prior to breeding, through gestation, until the pups were weaned at 21 days of age; control dams received destilled water. Exploratory behaviour was higher in 60 and 90 day old male rats which were exposed to any level of TCE. The effect exposure on locomotor activity (running wheel) was also

examined in 60 day old males (625 and 1 250 mg/l exposure groups). Locomotor activity was significantly higher in rats exposed to 1 250 mg/l TCE. These data suggest that TCE has long-term effects on behaviour (289).

Groups of rats were exposed by inhalation to either clean air (controls) or TCE concentrations of 2 690, 5 380 or 8 070 mg m^{-3} for 16 hr/day, 5 days/week for 18 weeks. At preselected intervals, the animals were evaluated for changes in: spontaneous activity, strength of grip, coordinated hind limb movement, performance of discret-trial operant two choice visual discrimination task, and peripheral nerve conduction velocity. Compared with the controls, TCE-treated rats showed no significant differences in open field behaviour, fore and hind limb movement and strength of grip or coordinated movement throughout the exposure period. Peripheral nerve conduction time was also unaffected. By contrast, TCE produced progressively marked changes in the speed and pattern of response in the two-choice visual discrimination tasks. Two-choice response latency, for example, was approximately four times higher in the highest dose group. In addition, a recurrent within-week functional tolerance developed in all exposed groups. However, tolerance was lost in the group exposed to the highest concentration as the exposure became long-term. Finally, after termination of exposure, there was no carry-over of TCE-related effects for any of the tasks, and performances quickly returned to baseline levels. This profile of effects argues for a primary involvement of the CNS with chronic exposure to TCE (169).

Thirty-two male rats were housed in pairs, eight pairs each in the experimental and control groups. They were isolated for 6-7 hours, five days a week, returned to their home cages early in the "dark" period, and observed for five minutes on selected days, starting within 30 seconds of removal from the exposure chambers. The experiments were performed at either 538-5 380 mg m^{-3} TCE (five levels), each lasting a month, or at 538 mg m^{-3} for 3 months. The results were consistent, with little or no change in the pattern of behaviour of the exposed groups, i.e., almost no selective change in any particular kind of behaviour. In every kind of behaviour, the exposed rats were slightly less active than the controls (270).

Rats were given 1.2 g/kg TCE two or four times daily under a light:dark = 12:12 hr cycle. The effect of TCE on the circadian rhythm of spontaneous locomotor activity was circadian-phase dependent, and treatment with TCE at 9:00 AM provoked greater impairment of circadian rhythm than did exposure at 9:00 PM (210).

The large amount of early studies of animal behaviour has been reviewed by Annau (5).

5.8.2.2 Structural and biochemical changes in the CNS

The reviewed studies are outlined in Table 5.

Table 5.Central nervous system biochemical and structural effects from TCE in animal studies

Species	Level/duration of exposure[1]	Route	Endpoint[1]	Result	Reference
Rats	1076 mg m^{-3} 1 mo 12 h/d, ? d/w	inhalation	Free amino acid level in midbrain	+	127
Rats	1076 mg m^{-3} 1 mo 12 h/d, ? d/w	inhalation	Neurotransmitter levels in brain	+	128
Gerbils	1720 mg m^{-3} 8 w	inhalation continuous	Soluble proteins in brain areas	+	106
Gerbils	322 mg m^{-3} 3 mo	inhalation continuous	Soluble proteins in brain areas	+	107
Gerbils	915 mg m^{-3} 5 mo	inhalation continuous	Soluble proteins in brain areas	+	172
Mice	915 mg m^{-3} 30 d	inhalation continuous	Neuronal enzyme levels in brain/blood	-	143
Gerbils	269 mg m^{-3} 12 mo 807 mg m^{-3}	inhalation continuous	Glutathion and amino acid levels	- +[2]	25
Gerbils	269 mg m^{-3} 12 mo 807 mg m^{-3}	inhalation continuous	Lipid composition and levels in brain	+ +	171
Rats	1720 mg m^{-3} 5 d 30 d 90 d	inhalation continuous	Fatty acid levels and composition of brain	- +[3] +[3]	173
Rats	1000 mg/kg 1 y daily, 5 d/w	oral	Inositol phospholipid levels	+	286
Rat pups	312 mg/l (dam)	oral[4] (water)	2-deoxyglucose uptake in brain	+	229
Gerbils	322 mg m^{-3} 3 mo 1722 mg m^{-3}	inhalation continuous	Ultrastructural neuronal changes	+ +	107
Rat pups	4-8.1 mg/l 6 w (dam)	oral[4] (water)	Ultrastructural myelin changes	+	138
Rat pups	5.5-8.5 6 w mg/day (dam)	oral[4] (water)	Ultrastructural myelin changes	+	139

[1] See details in text

[2] Glutathion levels only

[3] Partially normalized after 30 days rehabilitation

[4] Dams exposed in pregnancy and during lactation

Rats were exposed to TCE in concentrations of 1 076, 2 152 or 4 304 mg m^{-3} for one month. Effects of the exposure on free amino acid content of midbrain were investigated by high-performance liquid chromatography. Exposure of rats to trichlorothylene caused an increase in some inhibitory putative neurotransmitter substances and a decrease in some excitatory substances (127). In another study by the same group (128), rats were exposed to the same concentrations of TCE for the same length of time, to study the effects of inhalation exposure on neurotransmitter candidates of the rat brain. Changes in acetylcholine content of the striatum were investigated by pyrolysis gas chromatography. The dopamine in the striatum, norephinephrine in the hypothalamus, norephinephrine and serotonin in brain regions containing cortex and hippocampus were analysed by high-performance liquid chromatography. In this experiment, the long-term exposure to organic solvents caused some disturbance of the cholinergic neurons of the central nervous system (128).

Exposure of adult Mongolian gerbils to TCE (1 720 mg m^{-3} over a period of 8 weeks) caused a decrease of soluble proteins per wet weight in the frontal cerebral cortex, the cerebellar anterior part of the hemispheres, the posterior part of vermis, and the hippocampus. S-100 (a glial cytoplasmic protein), however, showed a transitional increase back to control levels, or a significant increase. In the sensory-motor cortex, a transient increase of soluble proteins (including S-100) was observed during the period of exposure. One of the major soluble polypeptides (MW 50 000-52 000) of the cerebral cortex, the cerebellar hemispheres and the brain stem decreased at the end of the exposure period. This polypeptide may be a subunit of microtubular protein or (NA^+ K^+)-ATP-ase (106).

Gerbil rats were exposed to a concentration of 2 745 mg m^{-3} intermittently (8 h/d) or to a concentration of 915 mg m^{-3} continuously for five months. The cerebellar content of S-100 protein increased, indicating delayed glial cell reactivity in the anterior cerebellar hemisphere. A decrease of S-100 protein was found in the posterior cerebellar vermis (172).

Acetylcholinesterase, glutamine synthetase, acid phosphatase, and glutamate dehydrogenase activity in brain and cholinesterase activity in blood were investigated in mice continuously exposed to 915 mg m^{-3} TCE for 30 days. The neuronal enzymes remained unaffected, which suggests that no general damage occurred to either the glia or the nerve cell populations (143).

Mongolian gerbils were exposed to 269 or 807 mg m^{-3} TCE continuously for 12 months. Glutathione was significantly elevated in the hippocampus of animals exposed to the highest concentration. Amino acids levels were not appreciably affected. After exposing the animals to TCE for 12 months, the accumulation of glutamate by the posterior part of cerebellar vermis increased in a dose-dependent manner, but no significant changes were observed in the hippocampus. The potassium-stimulated release of glutamate and GABA was unaffected in hippocampal tissue slices at both levels of exposure (25).

Exposure of Mongolian gerbils to TCE (269 or 807 mg m^{-3}) for 12 months caused

only small changes in the lipid composition of the cerebral cortex and hippocampus. Total protein content and lipid class distribution were almost unaffected. The cholesterol to phospholipid ratio, however, decreased in the cortex of animals exposed to TCE. This may have been due to changes in the relative proportions of different membranes or cellular types. The most interesting finding occurred among the long chain polyunsaturated fatty acids, where changed ratios were found between long-chain derivatives of the linoleic and the linolenic acid series, as well as a decrease in two minor fatty acids, 20:5 (n-3) and 22:5 (n-3). It is likely that such changes occur as a result of peroxidation of polyunsaturated fatty acids or in order to counteract the fluidizing properties of TCE (171).

Rats were exposed to a moderate level of TCE by continuous inhalation (1720 mg m^{-3}). The fatty acid pattern of brain ethanolamine phosphoglycerides was examined during exposure and after a subsequent exposure-free period. Alterations in the fatty acid pattern were noted after 30 days of exposure. An increased ratio of linoleic acid- derived (n-6) to linolenic acid-derived (n-3) fatty acids was observed in the cerebral cortex, the hippocampus, and the brain stem. Of the major fatty acids, arachidonic acid (20:4(n-6)) increased in the cerebral cortex and the brain stem, while docosahexenoic acid (22:6 (n-3)) decreased in the cerebral cortex and the hippocampus. A further change in these fatty acids was observed in the cerebral cortex following a longer exposure period of 90 days. The 22-carbon linoleic acid-derived fatty acids also increased after 90 days of exposure. A complete normalization of the fatty acid pattern was not observed during the 30-day solvent-free period. The smaller number of double bonds and shorter chain length detected after exposure to the solvent is consistent with the idea of a compensatory remodeling of membrane lipid composition based on membrane stability with regard to phase preference (173).

Data presented by Noland-Gerbec et al. (229) indicate that uptake of 2-deoxyglucose is affected throughout the brain. A low chronic dose (312 mg/l) of TCE in the dams' drinking water results in decreased 2-deoxyglucose uptake in the brains of developing rat pups. These results suggest that the relative uptake and/or metabolism of glucose is depressed by TCE treatment at all ages. The effect of TCE on 2-deoxyglucose uptake by the hippocampus, cerebellum and the whole brain is already evident in seven-day old pups and, except in the hippocampus, continues as the animals grow older.

Inositol phospholipids translate many hormonal and neurotransmitter signals at cell surface receptors into appropriate cellular responses. Repeated administration of TCE to rats (1 000 mg/kg) for one year markedly reduces the levels of phosphatidylinositol (52%), phosphatidylinositol-4-phosphate (23%) and phosphatidylinositol 4,5-biphosphate (45%) (286).

The exposure of adult Mongolian gerbils to 322 or 1 722 mg m^{-3} TCE by continuous inhalation for a period of 3 months, followed by a period of four months free of exposure, induces biochemical changes in the hippocampus, the posterior part of cerebellar vermis and the brain stem, compatible with alterations comprising astroglial hypertrophy and/or proliferation. Ultrastructural evidence of changes to

neuronal cells was observed, such as decreased amounts of mirotubules and increased content of lysosomes and myelin bodies (107).

Rat pups were exposed to TCE (in average doses of 4.0 or 8.1 mg/day, giving an exposure of rat pups to a daily dose of 0.003 mg/ml TCE and 0.012 mg/ml TCE) via their dams' drinking water while in utero and until they were sacrificed at 21 days of age. Frozen coronal sections through the dorsal hippocampus were stained for the presence of myelin. A significant decrease in myelinated fibres was found in the stratum lacunosum-moleculare, an area comprised of distal dendritic profiles of CA1 pyramidal neurons which receive input from the entorhinal cortex. The observed reduction in myelin in the hippocampus may be partly responsible for the behavioural effects observed after exposure (138).

Exposure of rat pups to an average daily load of 5.5 mg TCE first for four weeks, followed by exposure of the young adult rats to 8.5 mg for an additional two weeks caused an increased level of performance in spatial navigational tasks. Examination of the brains of these animals revealed a significant decrease in the amount of myelin in one layer of the hippocampus, the stratum lacunosum-moleculare. Learning latency times were shorter in the group exposed for six weeks, but not significantly in the four week exposure group (139).

A study by Westergren et al. (305) shows that the brain-specific gravity, which measures the water content and indicates the formation of myelin in the brain, was lower at birth and at the age of 10 days in litters from mice that had been exposed to 807 mg m^{-3} TCE for 30 days.

Cynomolgus monkeys were injected with TCE intravenously. 33 mg TCE/kg provoked a dramatic c-wave amplitude in the electroretinogram. The lowest dose of TCE that provoked clear-cut changes in c-waves was 11 mg/kg (141).

5.8.2.3 Peripheral nervous system

The literature contains little information on the peripheral nervous system and effects in animals.

Acute exposure to high concentrations of TCE (up to 92 000 mg m^{-3}) does not induce damage to the 8th cranial nerve of the guinea pig, according to measurements of cochlear reactions to high frequency sounds (311).

The effects of TCE on regeneration of the sciatic nerve after a crush lesion has been tested in mice and rats. A lesion was made on nerves in unexposed animals and in animals pre-exposed to TCE for 20 days. The experimental animals were then exposed to TCE for an additional four days. Exposures to concentrations of 807 or 1 614 mg m^{-3} were continuous. Regeneration was measured by pinching the outgrowing nerve fibres, and was retarded in the TCE-exposed animals compared with the controls (156).

5.9 Endocrinologic effects

Sagawa et al. (252) describe the case of a woman who was accidently exposed to high concentrations of TCE vapour. One year after the accident she menstruated only irregularly and her basal body temperature suggested lack of ovulation. Saihan et al. (253) describe a 42 year old man who became impotent and developed gynecomastia and peripheral neuropathy following prolonged exposure to TCE at work.

Male Long-Evans hooded rats were exposed to vehicle 10, 100 or 1 000 mg/kg /day by gavage for two weeks before mating and throughout mating to day 21 of pregnancy. TCE levels were uniformly high in fat, adrenals and ovaries, and uterine tissues contained high levels of TCA. Fertility was not influenced. Maternal toxicity had no influence on the outcome of the pregnancy (191).

Male rats were intubated with TCE (10, 100 or 1 000 mg/kg, 5 days/week for 6 weeks). TCE and its metabolites accumulateded in the reproductive organs, but no indications of spermatotoxicity or plasma testosterone effects were revealed (312).

6 IMMUNOTOXICITY AND ALLERGY

There is little information on the immunological or allergic effects of TCE. A case reported by Nakayama et al. (220) suggests that, according to a patch test, the patient was allergic to both trichlorethylene and trichloroethanol.

Putative TCE effects that may indicate immunological pathogenesis are outlined in chapters 5.1 and 5.3.

7 MUTAGENICITY AND GENOTOXIC EFFECTS

7.1 Mutagenicity

7.1.1 Bacterial assays

To a large extent, the differing results in bacterial mutagenicity assays using TCE (Table 6) can be explained by differing experimental procedures. Technical grade TCE with epoxide stabilizers are positive in different *Salmonella typhimurium* strains, with or without external metabolic activation (42,194,268).

Several investigators have found a reproducible weak mutagenic effect of epoxide-free TCE in assays using *Salmonella typhimurium* TA100 strain, in the presence of liver S9 from pretreated rodents (9,14,42,271). These results are contradicted by negative assays reported later by Shimada et al. (268) and McGregor et al. (194), but these assays may have been influenced by the bacterial toxicity of TCE and a possible masking of weak mutagenicity. The negative results reported in some assays where epoxide-free TCE was not applied in vapour phase (116,207) may have been biased by the high volatility of TCE and its low solubility in water (44).

Table 6. TCE - bacterial mutagenicity assays

Organism/Strain	Endpoint	Activation[1]	Purity of TCE[2]	Result[3]	LED[4]	Reference
S. typhimurium TA1535 TA100	Reversion	Pretreated	EF	+[5]	161400 mg m^{-3}	9
S. typhimurium TA100	Reversion	Pretreated	EF	+	269000 mg m^{-3}	14
S. typhimurium TA100	Reversion	Pretreated	EF	+	17750 mg m^{-3}	42
		Uninduced	EF	-		42
		Pretreated	Tech	+[8]	17750 mg m^{-3}	42
S. typhimurium TA100	Reversion	Pretreated	EF	-		116
S. Typhimurium TA98/100/1535	Reversion	Pretreated	EF	-		194
		Pretreated	Tech[7]	+[6]	33900 mg m^{-3}	194
S. typhimurium TA97/98/100/ 1535/1537	Reversion	Pretreated	99%	-		207
S. typhimurium TA100/1535	Reversion	Pretreated	99.98%	-		268
		Pretreated	Tech	+[6]	53800 mg m^{-3}	268
S. typhimurium TA100/1535	Reversion	Pretreated	EF	+[5]	53800 mg m^{-3}	271
S. typhimurium TA98/100	Reversion	Pretreated	NS	-		303
E. coli K12 343/113	Forward and back mutation	Pretreated	99.5%	+[8]	3.3mM	102

[1] External metabolic activation of mouse, rat or hamster liver S9

[2] EF:epoxide-free, Tech:technical grade, NS:not stated

[3] +:positive (reproducible,dose-related result), -:negative

[4] Lowest effective dose

[5] TA100 strain only

[6] Positive with or without metabolic activation

[7] Analytic grade TCE added 1,2-epoxybutane or epichlorhydrine

[8] arg$^+$ operon only

TCE has also been tested in an *Escerichia coli* assay, where revertants were increased in the *arg*$^+$ colonies, suggesting a weak induction of base-pair substitutions. The results were negative in other operons (102).

Table 7. TCE - Fungal mutagenicity assays

Organism, strain	Endpoint	Purity of TCE[1]	Result[2]	LED[3]	Reference
S. cerevisiae D7	Reversion, mitotic gene conversion	EF	+	30mM	27
S. cerevisiae D4	Mitotic gene conversion	EF	+	3 mmol/kg	27
S. cerevisiae D7	Reversion, mitotic gene conversion, mit. crossing-over	NS	+	15mM	32
S. cerevisiae D7	Reversion, mitotic gene conversion	EF	?	22.2 mM	159
S. cerevisiae D61.M	mitotic chromosomal malsegregation	EF	+	5.5 mM	159
S. cerevisiae XV185-14C	Reversion	Tech	?		267
A. nidulans 35	Forward mutation	EF	+	13450 mg m^{-3}	43
A. nidulans 35X17	Chromosome malsegregation	EF	+	40350 mg m^{-3}	43
S. pombe P1	Forward mutation	EF/Tech	-		249

[1] EF:epoxide-free, Tech:technical grade, NS:not stated

[2] +:positive (reproducible,dose-related result), -:negative, ?:inconclusive

[3] Lowest effective dose

7.1.2 *Fungal assays*

The fungal assays are summarized in Table 7. Several procedures have been applied in yeast assays, both in vitro incubation (27,32,43,159,249,267) and host-mediated assays where rodents were treated with TCE (27,43,249). To sum up, TCE without contamination of epoxides was mutagenic in most of the assays in the presence of endogenous or exogenous metabolic activation. Several endpoints were reported, such as reversion, mitotic gene conversion, forward mutation and chromosomal malsegregation (see Table 7). Rossi et al. (249) reported negative results in in vitro or host-mediated assays using *Schizosaccharomyces pombe*. This system is less sensitive than other fungal assays (44).

7.1.3 *In vitro assays with mammalian cells*

Results are summarized in Table 8.

Table 8. TCE - in vitro mammalian cell assays

Cell type	Endpoint[1]	Result	LED[2]	Reference
CHO	SCE	+	3.1 mM	91
Human lymphocytes	SCE	+	1.4 mM	104
CHO	SCE	-		306
Rat hepatocytes[3]	UDS	+	2.8 mM	41
Human lymphocytes[4]	UDS	+	0.02 mM	239
Rat hepatocytes	UDS	-		268
Syrian hamster embryo cells	Trans	+	NS[5]	1
Rat embryo cells	Trans	+	1.1 mM	245
BALB/c-3T3	Trans	+	0.15 mM	292
CHO	ChrAb	-		91

[1] SCE:sister chromatid exchanges, UDS:unsceduled DNA synthesis, Trans:transformation, ChrAb:chromosomal aberrations

[2] Lowest effective dose

[3] Phenobarbital-induced

[4] S9 mix from phenobarbital-induced rat liver added to system

[5] Not stated (range of dose 5-25 µg/ml)

The discrepancy of results regarding sister chromatid exchanges (SCE) may be explained by differences in protocol. White et al. (306) used exposure to vapour in contrast to liquid incubation, in studies which showed weak induction of SCE (91,104). None of the studies specify the purity of TCE used, and the effect could therefore have been induced by epoxide contaminants. Results are also conflicting in studies of unscheduled DNA synthesis (UDS). Both positive studies (41,239) used TCE of undefined purity. Shimada et al. (268) examined TCE both with and without stabilizers and in neither case did they observe unscheduled DNA synthesis. The discrepant effects may be due to the different activation systems used, since Costa and Ivanetich (41) used microsomes from pretreated rats, Perocco & Prodi (239) obtained positive results only in the presence of metabolic activiation, while Shimada et al. (268) used uninduced rat hepatocytes.

Table 9. TCE - in vivo mutagenicity assays

Species/strain/sex	Endpoint[1]	Purity of TCE[2]	Route	Result[3]	LED[4]	Reference
Rat/S-D ♂	SSB	EF	gavage	+	23 mmol/kg	226
Mouse/B6C3F1 ♂	SSB	EF	gavage	+	11 mmol/kg	226
Mouse/B6C3F1 ♂	SSB	99%	ip	-		237
Mouse/NMRI ♂	SSB	99.5%	ip	+	6 mmol/kg	301
Mouse/CD-1 ♂	UDS	>99%	gavage	-		59
Mouse/B6C3F1 ♂/♀	UDS	NS	gavage	-		202
Mouse/CD-1 ♂	Micronuclei	99.5%	gavage	+	2.9 mmol/kg	61
Mouse/C57B1xT embryos	Spot test	99.5%	ip	+	1 mmol/kg	72
Mouse/C57F1 ♂	Sperm abnormality	NS	inhal	+	10760 mg m^{-3}	177
Mouse/ NMRI/BGA ♂	Dominant lethals	99.5%	inhal	-		274

[1] SSB:single-strand breaks, UDS:unsceduled DNA synthesis

[2] EF:no epoxide stabilisers, NS:not stated

[3] +:positive, -:negative

[4] Lowest effective dose/concentration

7.1.4 Mammalian in vivo assays

The results are summarized in Table 9. Walles (301) reports a linear, increased rate of single-strand breaks in liver and kidney of NMRI mice. The author notes that the fragmentation is quickly repaired. Parchman & Magee (237), who did not report SSB in hepatic DNA, applied a longer interval between treatment and sacrifice than applied in the two positive studies (226,301)(six hours, compared with four hours and one hour). This difference in method may thus explain the discrepancy of results. Nelson & Bull (226) found that large doses of TCE induced SSB in the hepatic DNA of both mice and rats, the former species being the most sensitive. The observed dose-response relationships suggest differences in the mechanisms inducing strand breaks in the two species (226).

Both studies on DNA repair (UDS) indicate that doses capable of hepatic cell proliferation do not cause unscheduled DNA repair in liver. These findings should be compared with the results of Stott et al. (284) who found that gavage treatment in hepatocarcinogenic doses (1 200 mg/kg/day) induced increased DNA synthesis in the liver of B6C3F1 mice and to a lesser extent in the liver of Osmonde-Mendel rats. In the mice, the same doses induced severe hepatocellular changes, with low or no covalent binding to DNA. No such changes were observed in the rats (see chapter 3.3.1.).

Investigations of other endpoints (Table 9) indicate some in vivo genotoxic activity of TCE in mice as well. Duprat and Gradiski (61) observed micronuclei in bone marrow cells of CD-1 mice. TCE was administrered by inhalation (up to 2 420 mg m^{-3} for 24 hours) in the dominant lethal study (274). In this study the animals showed no sign of toxic effects, and the absence of dominant lethals may be regarded as inconclusive (44).

7.1.5 Mutagenicity of TCE metabolites

Chloral hydrate shows mutagenic activity in several in vitro assays, i.e. *Salmonella typhimurium* TA100 (303). Several studies report activity of chloral hydrate on mitotic spindle function in *Aspergillus nidulans* (43,174,196,272), *Saccharomyces cerevisiae* (276) and human lymphocytes (297). In vivo observations by Russo et al. (251) confirm these in vitro results. Significant increases in hyper-haploid metaphases in secondary spermatocytes have been observed after intraperitoneal administration of chloral hydrate to male mice (251).

TCEOH is weakly mutagenic in TA100 and TA1535 strains of *Salmonella typhimurium*, and induces forward mutation in *Aspergillus nidulans* (19). In the latter species TCEOH causes chromosome malsegregation (43). TCEOH in massive doses does not induce SSB in rat liver DNA (226).

TCA shows no mutagenic activity in bacterial assays with or without metabolic activation (206). By contrast, TCA has induced both chromosome aberrations and sperm abnormalities in mice after intraperitoneal or oral administration (18). Both TCA and DCA are capable of producing SSB in rat and mouse liver DNA, DCA

being most potent (226). It has also recently been shown that high oral doses of TCA, and to a lesser extent DCA, increase incorporation of tritiated hydrogen in mouse liver DNA, indicating unscheduled repair (255).

The cysteine conjugate S-1,2-dichlorovinylcysteine (DCVC), a possible kidney metabolite of TCE (see chapters 3.3.3. and 4.1.), is mutagenic in *Salmonella typhimurium* TA100, TA2638 and TA98 without addition of mammalian subcellular fractions. The activity increased when rat kidney cytosol was added to the system (55). The proposed mechanism is β-lyase catalysis of DCVC to pyruvate, ammonia and the putative active compound 1,2-dichlorovinyl mercaptan. Both the *Salmonella* strains and the rat kidney cytosol contain β-lyase (55,298). The precursor of DCVC, S-1,2-dichlorovinyl N-acetylcysteine, is strongly mutagenic in *Salmonella typhimurium* TA2638 in the presence of rat kidney cytosol (298).

7.1.6 Summary of mutagenicity assay data

TCE metabolites are capable of irreversible binding to macromolecules. Contrary to the results obtained in vitro, adduct formation to DNA has not been convincingly demonstrated in vivo (see chapter 3.3.1.).

TCE without epoxide stabilisers is weakly mutagenic after metabolic activation (endogenous or exogenous) in several assays in bacteria (reversion), fungi (several endpoints) and mammalian cells (transformation).

Epoxide-free TCE induces SSB and other genotoxic effects in rodents in vivo.

Several of the stable TCE metabolites are potential candidates for the documented TCE effects. Among those with greatest biological significance are the effects of TCA and DCA on rodent hepatocytes, the effects of chloral hydrate on mitotic spindle and the possible effects of S-diclorovinylcysteine in the presence of β-lyase.

7.2 Occupational studies of cytogenetic changes

On the basis of lymphocyte cultures, chromosome studies were carried out on 28 degreasers exposed to different loads of TCE. The degreasers had been exposed for between one and 21 years. No information was provided either on exposure dose or stabilizers. Nine degreasers had pathological numbers of hypodiploid cells, but otherwise normal karyotypes. In these nine cases the cell changes were more related to TCE load than to duration of exposure, alcohol consumption, or smoking (165).

In a survey of 11 males and four females, Gu et al. (104) found higher SCE frequencies in peripheral lymphocytes among the six subjects exposed to TCE than in the nine who were not so exposed. No adjustment is made for age, sex or smoking. In a more recent study of 22 workers exposed to TCE, no increase of SCE was observed in peripheral lymphocytes after stratification for smoking status (214). After adjustment for smoking, SCE frequencies did not increase in a study of 38 TCE synthesis production workers and degreasers (stated geometric mean of

exposure 40 mg m^{-3}), compared with 51 controls (265). The data may indicate an interaction between TCE and smoking (265).

Structural aberrations (breaks, gaps, translocations, deletions, inversions) as well as hyper-diploid peripheral lymphocytes, were observed in a group of 15 metal degreasers exposed to high levels of TCE. Lymphocytes from physicians and a sampled reference from the cytogenetic laboratory served as controls. Confounding factors (not smoking) were explored (247).

To sum up, these human data do not provide firm evidence in either direction, due to insufficient control of different sources of bias and insufficiently exact information on exposure.

8 CARCINOGENICITY

8.1 Carcinogenicity in humans

Axelson et al. (7) undertook a retrospective study of cancer mortality in a cohort of 518 male workers at a Swedish TCE production facility. All workers had been employed at the plant during the 1950s and 1960s and were followed from 1955 through 1975. The men were grouped into high-exposure and low-exposure groups by urinary levels of trichloroacetic acid (>100mg/l and <100 mg/l, respectively; 100 mg/l corresponds to exposure at 160 mg m^{-3}). The number of total deaths (n=49) was less than expected. Eleven deaths were from cancer (14.5 expected). As regards the types of tumours in the study, no particular type of cancer was found to be overrepresented, though there were two leukemias and two stomach cancers. The leukemias were found to belong to the low-exposure category, one having a latency time of less than four years, the other of more than ten years. This study (7) has been extended and includes 1 424 men, 65 % of whom were exposed in 1970 through 1975 (8). There was a deficit in total cancer mortality, with 22 versus 36.9 expected, but a significant excess in urinary tract (11 versus 4.85) and hematolymphatic (five versus 1.2) tumours. Specifically, for two years or more of exposure and 10 years of latency, there were three urinary bladder cancer cases (0.83 expected), four cancers of the prostate (2.35 expected) and two lymphomas (0.27 expected) (8).

In a retrospective cohort mortality study of dry-cleaning and laundry workers, Blair et al. (20) report an increase in the total number of cancer-related deaths (n=87) compared with the expected number (n=67.9) among a group of 330 deceased workers ($p<0.05$). Site-specific significant increases are reported in lower respiratory cancers (17 observed vs. 10 expected), uterine cervical cancer (10 observed vs. 4.8 expected) and skin cancer (3 observed vs. 0.7 expected). The increase in cancer was possibly due to exposure to dry-cleaning fluids (carbon tetrachloride, tetrachloroethylene and TCE). It is less plausible that the results are due to TCE alone, since TCE has only been used for dry cleaning to a minor extent in recent decades. The increased number of lung and cervical cancer may be explained by confounding due to socioeconomic factors.

The mortality pattern of 671 female laundry and dry-cleaning workers during the period 1963-1977 showed elevated risk of cancers of the kidney and genitals, along with a slight excess of bladder and skin cancer and lymphosarcoma. The laundry workers had been exposed to tetrachloroethylene, carbon tetrachloride, TCE, and other petroleum solvents (144).

In a study of 2 117 relatively young male and female workers exposed to TCE between 1963-1976, Tola et al. (290) report no increase in total mortality or cancer mortality (11 observed vs. 14.3 expected). Exposure to TCE was usually low to moderate, with 78 % of the measured urinary trichloroacetic acid levels less than 50 mg/l (corresponds to < 80 mg m^{-3} exposure). No increased cancer risk was demonstrated, but the latency time was short.

An association between primary liver cancer and occupational exposure to solvents has been demonstrated by Hernberg et al. (120). In a case-control study, six female cases compared with none of the controls had been exposed to solvents, but only one case had possibly been exposed to TCE.

Paddle (236) examined tumour registry records in Great Britain and attempted to match reported primary liver cancers in persons employed at Imperial Chemical Industries, where TCE was produced. Only two liver cancers occurred in the period from 1951 to 1977 in the group of 1 000 workers exposed to TCE. Both of these cancers were metastases from other sites. Thus no risk of primary liver cancer among trichlorethylene workers was identified.

No positive effects have been reported from two other studies, Malek, 1979, and Novotna, 1979, both dealing with exposure to TCE and liver cancer (8).

A prospective study was conducted among 2 646 employees who worked for three months or more during the period 1957-1983 in a manufacturing plant that used TCE as a degreasing agent. Comparison of the observed mortality within the study cohort with calculated expected values based on national mortality experience revealed fewer deaths overall than would be expected in comparable groups of similar size and age distribution. Statistically significant deficits of deaths from cancer were noted among the white males in the cohort, possibly explained as a healthy worker effect (269).

A case-control study of colon cancer was conducted encompassing 329 cases and 658 controls. Occupations and various exposures were assessed by questionnaire. As regards exposure to TCE in general, a slightly increased risk was found, whereas such exposure among dry cleaners indicated a risk increase (OR 7.4, 95% CI 1.1-47.0) (87).

A matched case-control study was performed to investigate cases of malignant lymphoma (Hodgkin's disease and non-Hogdkin's lymphoma). This study included 169 cases and 338 controls. Analysis of high-grade and low-grade exposure to organic solvents yielded relative risks of 2.8 and 1.2 respectively. Of the subjects who had experienced high-grade exposure, seven cases and three controls were

exposed to TCE (110).

The effects of potential risk factors for Hodgkin's disease and for non-Hodgkin lymphomas were evaluated in a case-referent study encompassing 54 cases of Hodgkin's disease, 106 cases of non-Hodgkins lymphoma, and 275 referents, all alive. Information on exposure was obtained from mailed questionnaires. Exposure to TCE was associated with an increased risk of Hodgkin's disease (OR=2.8). Exposure to TCE was non-significantly associated with non-Hodgkin lymphoma (OR=1.5) (240).

8.2 Carcinogenicity in animals

Results of four studies carried out in 1944, 1951, 1955, and 1967 in order to asses the carcinogenic potential of TCE revealed no evidence of carcinogenicity. The studies were performed on a variety of experimental animals and with different levels of TCE exposure (304).

The National Cancer Institute (221) conducted a chronic carcinogenicity bioassay of technical grade TCE in B6C3F1 mice and Osborne-Mendel rats. Two dose levels of TCE were administered by gavage to groups of 50 animals of each sex and species on five days per week. Eight groups of male mice (including two control groups) received 100-2 400 mg TCE/kg. Seven groups of females (including two control groups) received 700-1 800 mg TCE/kg daily during the 78-week period of exposure. Eight groups of rats received 650-1 000 mg TCE/kg daily over the 78-week period of exposure. Hepatocellular carcinomas were reported in mice, with a highly significant dose-related increase in incidence in both sexes. There was no increased incidence of neoplastic lesions in any group of treated rats compared with the controls (221). Technical grade TCE has been found to be carcinogenic in mice after high daily oral doses. A GC-MS analysis of this technical sample revealed the presence of large amounts of epichlorohydrin and 1,2-epoxibutane as stabilizers. These epoxides are highly mutagenic in the Ames test and may have been responsible for the carcinogenic effect found in the 1976 NCI study (116).

NTP (224) conducted a study using epoxide-free TCE and obtained similar results as in the 1976 NCI study. The number of hepatocellular carcinomas increased in both male and female mice. A statistically significant ($p=0.05$) increase in renal adenocarcinomas was observed in the male rats exposed to high doses (224).

High-purity, epoxide-free TCE was also used by Maltoni and Maroli (189). TCE was administered for 52 weeks in a dose four times lower than administered in the NCI and NTP bioassays in Sprague-Dawley rats. No tumourigenic effect was detected.

Swiss mice (ICR/HA) of both sexes were dosed daily with TCE by gavage (males: 2.4 g/kg, females: 1.8 g/kg) with or without addition of epichlorohydrin (EPC), 1.2-epoxybutane (BO) or both for 18 months. The ensuing observation period terminated at 106 weeks. Gross and microscopic examination of all organs revealed a statistically significant increase in the incidence of forestomach papillomas and carcinomas after EPC, BO and (EPC + BO)-stabilized samples of

TCE, but not after pure, amine base-stabilized TCE (119).

0, 2.5 or 10 µg/g bodyweight of ethylnitrosourea was adminstered intraperitoneally to male B6C3F1 mice 15 days old. At 28 days of age, the mice were put on drinking water containing either TCE (3 or 40 mg/l), TCA (2 or 5 g/l), or DCA (2 or 5 g/l). All drinking waters were neutralized with NaOH to a final pH of 6.5-7.5. The animals were killed after 61 weeks of exposure to the treated drinking water. Both DCA and TCA in concentrations of 5 g/liter were carcinogenic without prior initiation with ethylnitrosourea, and induced hepatocellular carcinomas in 81 % and 32 % of the animals, respectively (121). According to Crebelli and Carere (44) this study should be regarded as inconclusive concerning TCE due to the low dosage of TCE applied (6 mg/kg/day) explained by its low solubility in water.

Table 10. Trichloroethene in animal cancer assays

Species/strain/sex	Daily dose/conc/ duration	Purity of TCE[1]	Endpoint[2] Incidence	Reference
Osborne-Mendel rat	gavage 5d/w 78w	Epoxides		221
	549 mg/kg		-	
	1098 mg/kg		-	
B6C3F1 mouse	gavage 5d/w 78w	Epoxides	HCC	221
	0 mg/kg		0/18	
female	869 mg/kg		4/42	
	1739 mg/kg		11/37	
	0 mg/kg		1/20	
male	1169 mg/kg		26/48	
	2339 mg/kg		31/40	
F344 rat	gavage 5d/w 2y	EF	RAC[3]	224
	0 mg/kg		0/33	
	500 mg/kg		0/20	
	1000 mg/kg		3/16	
B6C3F1 mouse	gavage 5d/w 2y	EF	HCC	224
female	0 mg/kg		2/41	
	1000 mg/kg		13/41	
male	0 mg/kg		8/48	
	1000 mg/kg		30/50	
Ha:ICR mouse	gavage 5d/w 78w	EF	-	119
	1800-2400 mg/kg	Epoxides	FSC	

Table 10, continued. Trichloroethene in animal cancer assays

Species/strain/sex	Daily dose/conc/ duration	Purity of TCE[1]	Endpoint[2] Incidence	Reference
B6C3F1 mouse	oral (water) 61w 6 mg/kg	EF	-	121
NMRI mouse	inhal 6h/d 5d/w 78w 0 mg m^{-3} 538 mg m^{-3} 2690 mg m^{-3}	EF	ML[4] 9/29 17/30 18/28	118
Wistar rat	inhal 6h/d 5d/w 78w 2690 mg m^{-3}	EF	-	118
Syrian hamster	inhal 6h/d 5d/w 78 w 2690 mg m^{-3}	EF	-	118
ICR mouse female	inhal 7h/d 5d/w 0 mg m^{-3} 269 mg m^{-3} 807 mg m^{-3} 2420 mg m^{-3}	Trace	LAC 1/49 3/50 8/50 7/46	89
Sprague-Dawley rat	inhal 7h/d 5d/w 538-3228 mg m^{-3}	EF	RAC LCT	190
Swiss mouse	inhal 7h/d 5d/w 538-3228 mg m^{-3}	EF	LAC HCC[3]	190
B6C3F1 mouse	inhal 7h/d 5d/w 538-3228 mg m^{-3}	EF	HCC	190

[1] Epoxides:TCE stabilised with epoxides, Trace:TCE with traces of benzene/epichlorhydrin

[2] HCC:Hepatocellular carcinom, RAC:Renal adenocarcinoma, FSC:Forestomach carcinoma, ML:multiple myeloma, LAC:Lung adenocarcinoma, LCT:Leydig cell tumour,

[3] Males only

[4] Females only

Newborn rats were exposed for 10 weeks to 10 720 mg m^{-3} TCE (8 h/day, 5 days a week). TCE showed no detectable oncogenic potenial towards hepatocytes (176).

van Duuren (62) reported three benign forestomach tumours (no carcinomas) amongst 60 male and female (30 each sex) Ha:ICR Swiss mice intubated with TCE. The compound was adminstered in doses of 0.5 mg/mouse once a week for

622 days. TCE was clearly not carcinogenic in this strain of mouse at the dose levels concerned.

Pure TCE, stabilized by an amine base was administered by inhalation in doses of 538-2 690 mg m^{-3} for six hours/day, five days/week, for 18 months to mice, rats and Syrian hamsters of both sexes. With the exception of malignant lymphomas, which increased in female mice, no significant increase in tumour formation was observed in any species or dose group. It is not clear whether or not the high occurrence of lymphomas, which is peculiar to this strain of mice, has any connection with exposure to TCE. The authors conclude from these findings that it is impossible to deduce that pure TCE has the potential to induce cancer (118).

Female ICR mice and female SD rats were exposed to 269, 807 or 2 420 mg m^{-3} TCE by inhalation for seven hours a day, five days a week, for 104 weeks. The period of exposure was followed by an observation period of three weeks. Several tumours were noted. The incidence of pulmonary adenocarcinomas in mice exposed to 150 ppm was 16 %, and in mice exposed to 450 ppm was 15 %. These figures are significantly ($p<0.05$) higher than the incidence in the controls (2%) (89).

TCE was administered by inhalation, seven hours daily, five days a week, for eight weeks, at concentrations from 538 to 3 228 mg m^{-3} to Sprague-Dawley rats and Swiss and B6C3F1 mice. The rats were treated for 104 weeks while the mice had treatment a period of 78 weeks. The animals were kept under observation until death occurred spontaneously. The most relevant finding was the dose-related increased incidence of Leydig cell tumours in male rats, and the onset of a few renal tubuli adenocarcinomas at the highest dose, always in rats (4/130 males and 1/130 females). The renal tubuli adenocarcinomas were preceded by, and associated with, a characteristic lesion of the kidney: tubuli cell karyomegaly. Under the above experimental conditions TCE appears to be carcinogenic in rats and mice (190).

Four bioassays were used to test TCE for carcinogenicity in ICR/Ha Swiss female mice. This is a random-bred strain of mice with a relatively low incidence of spontaneous tumours. Four treatment regimens were applied. A single application of TCE followed by repeated applications of a tumour promoter (phorbol myristate acetate), repeated applications to mouse skin, weekly subcutaneous injections, or weekly intragastric intubations. The duration of the four tests ranged from 342 to 622 days. No increased incidence of tumours was found in the treated animals (62). van Duuren tested TCE oxide, a possible carcinogenic intermediate, as an initiating agent in a two-stage carcinogenesis assay. Here the result was negative (62-63). According to Crebelli and Carere, administration of TCE by application to the skin and subcutaneous injection do not provide an adequate experimental model for detecting TCE carcinogenicity (44).

9 REPRODUCTION AND TERATOGENICITY

9.1 Human data

Goldberg et al. (97) have reported a connection between human congenital cardiac malformations and drinking water containing contaminants, including TCE (97). The odds for congenital heart disease in children whose parents were in contact with contaminated water during the periode of active contamination was three times the odds for children born after closure of the contaminated wells ($p<0.005$).

9.2 Animal data

9.2.1 Mammalian data - reproduction

The epididymal spermatozoa of (C57B1/C3H)F1 mice were examined for morphologic abnormalities following exposure to near 0.1 MAC and higher concentrations of general anaesthetics. For TCE the exposure was 20 hours (4 hr/day for 5 days). The epididymal spermatozoa were examined 28 days after exposure. The examination showed significant increases in the percentage of abnormal spermatozoa (177).

Manson et al. (191) demonstrated that female fertility in rats was not affected in any group exposed to 10, 100 or 1 000 mg TCE/kg/day by gavage for two weeks prior to mating. Zenick et al. (312) showed a change in the copulatory behaviour of male rats exposed to 1 000 mg/kg TCE for five days/week for six weeks, possibly due to the narcotic properties of TCE. Rats exposed to lower levels (10-100 mg/kg) of TCE were not affected (312).

TCE (1 000 mg/kg gavage) four hours before testing had the effect of increased latency of ejaculation in male rats during copulation. Naltrexone (2.0 mg/kg ip) administered 15 minutes before testing blocked this TCE-induced effect. Cross-tolerance to morphine was demonstrated. These data suggest that the effects of TCE may be mediated via the endogenous opioid system at the CNS level (225).

9.2.2 Mammalian data - teratogenicity

The effect of maternally inhaled TCE on embryonal and foetal development in mice and rats has been reported by Schwetz et al. (262). Groups of pregnant Sprague-Dawley rats and Swiss Webster mice were exposed to TCE seven hours daily on days 6-15 of gestation in concentrations of 1 614-6 725 mg m^{-3}. TCE caused no significant maternal, embryonal or foetal toxicity and was not teratogenic in either species.

Female rats were exposed by inhalation at a concentration of approximately 9 700 mg m^{-3} TCE vapour to determine whether exposure before mating and during pregnancy is more detrimental to reproductive outcome than exposure either only before mating or only during pregnancy. Four treatment groups were utilized in a

two by two factorial design. Significant increases in skeletal and soft tissue anomalies, indicative of developmental delay in maturation rather than teratogenesis, were observed in the group exposed only during pregnancy. Behavioural evaluation of offspring effect in tests of general activity levels at 10, 20 and 100 days of age indicated no effect of treatment. However, a reduction in postnatal body weights was found in offspring of mothers who had been subjected to pregestational exposure. No results indicative of treatment-related maternal toxicity, embryotoxicity, severe teratogenicity, or significant behavioural deficits were obtained in any of the treatment groups (60).

Studies have been conducted where rats and rabbits were exposed to TCE by inhalation on days 1-19, respectively days 1-24, of gestation. There was no statistically significant change in malformation rates in either species, but four cases (2 foetuses in each of 2 litters) of external hydrocephalus were noted among exposed rabbits (111).

Thirty-two inbred Wistar rats (study group) were exposed to 538 mg m^{-3} TCE by inhalation for four hours daily from day eight to day 21 of pregnancy. A control group (31 animals) was exposed to the same experimental conditions but without administration of TCE. All the rats were sacrificed on the 21st day of pregnancy and the ovaries, uterus, liver, lungs, heart and the foetuses were examined. There is no evidence of teratogenesis, but significant reduction of foetal weight and increase in bipartite or absent skeletal ossification centres suggest a delay in foetal maturation (113).

1 500 ppm TCE or 15 ppm TCE was delivered through a catheter into the gravid uterus of rats. A total of 62 Sprague-Dawley maternal rats, with 0-16 foetuses each, yielded a total of 373 live foetuses. The foetuses were examined for cardiac anomaly. The control group demonstrated three percent defective hearts. Low dose (15 ppm) TCE produced nine percent defective hearts ($p=0.18$) and the high dose (1 500 ppm TCE) resulted in 14 % defective hearts ($p=0.03$) (49).

9.2.3 Avian data

Low dosages (1-25 µmol/egg) of TCE administered to chick embryos on the first two days of embryogenesis. Examination on day 14 showed growth defects and morphological anomalies. 50 % mortality and developmental defects such as evisceration, subcutaneous oedema, light pigmentation of the epidermis, beak malformations were reported, as well as club foot and patchy feathering (26).

Teratogenicity of TCE was studied in chick embryos injected at the developmental stages reached on the second, third and sixth days of incubation. LD_{50} values varied between 50 and 100 µmol/egg. TCE produced malformations in excess of the vehicle control. The malformation frequency was increased 4-6 fold (67).

Fertile White Leghorn chicken eggs were incubated under standard conditions. At different developmental stages TCE was injected into the air space of the egg (vol = 0.03 ml) in concentrations of 5 to 25 µM (2 to 28 µg/g body weight). Mineral oil

and saline served as control solutions in a double blind protocol. Embryonic hearts (n=1055) were examined. Gross examination was performed, followed by microdissection. Cardiac malformations were found in 7.3 % of chick embryos treated with TCE. By comparison, such malformations were found in 2.3 % of controls exposed to saline ($p<0.01$), and 1.5 % those exposed to mineral oil ($p<0.001$). The cardiac defects included septal defects, cor biloculare, conotruncal abnormalities, atrioventricular canal defects, and abnormal cardiac muscle. These data suggest that TCE is a cardiac teratogen in an avian model (187).

10 RELATION BETWEEN EXPOSURE, EFFECT AND RESPONSE

10.1 Effects of short term exposure

In man, the critical organ for mediating the effect of short-term exposure is the central nervous system. The reports providing the basis for dose-effect and dose-response assessment are outlined in Table 3. TCE seems to cause decreased performance in psychometric test batteries among volunteers exposed to concentrations in the 1 600 mg m^{-3} range and above. The results concerning a no-effect level in performance ability are not consistent. While several authors report no decrement in performance at 1 080 mg m^{-3} exposure under different exposure regimens (70,92,281,310), Salvini et al. (254), in a well-conducted experiment, found clear decrement in performance, especially in more complex tests, at an exposure level of 592 mg m^{-3} for eight hours. Other authors have found effects after lower exposure in single tests, e.g. Gamberale et al. (92) who reported a decrement in numerical ability after an exposure of 1 080 mg m^{-3} for 70 minutes. It should also be noted that tests of the vestibulo-ocular system were affected after 90 minutes of exposure to TCE in concentrations of 176-429 mg m^{-3} (179). It is impossible to make more detailed dose-response inferences, however, since different exposure levels were not used in this study.

10.2 Effects of long term exposure

The critical effects to be considered are those affecting the central nervous system in man and animals, as well as the genotoxic and carcinogenic potential of TCE.

10.2.1 Central nervous system effects

There are several reports claiming effects on the central nervous system in man, from uncontrolled cross-sectional studies or surveys among workers long-term exposed to rather low levels of TCE. The studies and surveys are outlined in Table 4. Several of these studies indicate that workers exposed long-term to levels of TCE below 270 mg m^{-3} show an increased prevalence of unspecific symptoms indicative of dysfunction of the central nervous system. Grandjean et al. (100) indicate that long-term exposure to concentrations of 108-216 mg m^{-3} causes a chronic psycho-organic syndrome, while Liu et al. (183) observed nausea, forgetfulness and tremor among 100 workers who had experienced eight-hours

TWA exposure of less than 270 mg m^{-3}. However, Triebig et al. (291) did not find objective decrements in performance test results in eight workers exposed to 260 mg m^{-3} TCE for long periods.

Judged from biochemical or histochemical examination in several animal species long-term treatment with rather low doses affects the central nervous system (Table 5). Long-term exposure of mongolian gerbils to 269-1 720 mg m^{-3} TCE induced changes in the composition of long-chain unsaturated fatty acids in the cerebral cortex and hippocampus (171,173). The changes were partly reversible, and may indicate a compensatory remodeling of membranes due to the fluidizing property of TCE (173). The results indicate a dose-response relationship, with effects at the lowest treatment concentration of 269 mg m^{-3}, although significant in the cerebral cortex only. In the same species, long-term inhalation exposure to 323-1 720 mg m^{-3} TCE induced other biochemical changes in the hippocampus, as well as in parts of cerebellum and cerebral cortex, after a four-month exposure-free rehabilitaion period (107). The reported amounts of soluble proteins in the various areas of the brain show significant effect from the concentration of 323 mg m^{-3}, but there is no clear dose-effect relationship. Developing rats receiving a cumulative TCE dose of 154 mg and 273 mg over four or six weeks respectively (administrered in drinking water), showed a dose-dependent reduction of myelin in the stratum lacunosum-moleculare of the hippocampus. The myelin was decreased in the 154 mg dose group, but results in spatial navigational tests indicate that this dose may be a no-effect level in regard to function (139).

10.2.2 Genotoxicity, carcinogenicity, teratogenicity

Few of the human epidemiological studies on cancer risk can be used in the evaluation of dose-response relationships. Axelson et al. (8) observed increased risk of urinary tract and haematolymphatic cancers, but due to low numbers it is speculative to make assumptions about dose-response relationships on the basis of cancer distribution in the low and high exposure groups (point of division approximately 160 mg m^{-3} TWA). Tola et al. (290) report no increase in cancer mortality in a comparatively young cohort exposed to low levels (TWA below 80 mg m^{-3} in 78 % of measurements based on urinary TCA monitoring). As stated by the authors this result may be explained by the relatively short period of follow-up, and be regarded as non-positive rather than negative (290).

Cytogenetic studies in worker populations add little to the dose-response evaluation. Seiji et al. (265) report a geometric exposure mean of 40 mg m^{-3} in a group of degreasers who did not show increased SCE in peripheral lymphocytes, while Rasmussen et al. (247) report several cytogenetic effects in a group of degreasers exposed to high concentrations (levels not stated).

The results of the animal carcinogenicity assays are summarized in Table 10. In the dose-response evaluation the data are difficult to assess, either because of very high rates of spontaneous tumour incidence (hepatocellular carcinoma in male B6C3F1 mice, malignant lymphomas in female NMRI mice), or small responses to the treatment at the doses applied (renal tubular-cell adenocarcinomas in Fischer

344 rats, malignant hepatoma in Swiss mice). However, the results indicate positive dose-response relationship in the reported studies (see Table 10), although, in the inhalation studies, this is non-significant or has a possible no-effect level in the lower dose groups (89,190).

According to Loeber et al. (187) none of the studies conducted before 1988 have evaluated the teratogenicity of TCE on cardiogenesis. An avian model (187), and recently a mammalian model (49), have demonstrated that TCE is a potential cardiac teratogen. These studies indicate that TCE may have teratogenic properties.

11 NEED FOR FURTHER RESEARCH

More knowledge of TCE metabolism is required. The identified minor metabolic pathways are known to operate when the initial oxydative activation is saturated. We do not know as yet to what extent these pathways are operative at lower dose levels. More knowledge of the role of these minor pathways in man is also required, as well as more detailed knowledge of the different roles of these pathways in different species. More knowledge about the extrahepatic biotransformation of TCE, especially in kidney, lung and the nervous system, could be important for our understanding of TCE cytotoxicity and carcinogenicity in these organ systems. Further development of studies that add to the knowledge concerning toxicologic mechanisms, as well as sites of action in brain, lung and kidney, is warranted. The reports of cardiac teratogenicity in rodent and avian models should be confirmed.

Well-conducted analytic epidemiological studies on several endpoints of toxicity are needed. Some of the effects that should be investigated further are cancers, cytogenetic effects, effects on the central nervous system, transplacental effects, and the relation of TCE to immunological and autoimmune disorders. In observational studies the potential toxic role of stabilizers in technical grade TCE used should ideally be controlled.

Experimental studies in humans involving controlled short-term inhalation exposure should also be performed among persons subjected to long-term exposure to TCE. Studies on neurophysiological effect indicators are essential.

12 DISCUSSION AND EVALUATION

Neurotoxicity is the critical effect which should be considered when establishing an occupational exposure limit for TCE, and when assessing its carcinogenic and genotoxic potential.

Acute TCE effects as measured by neurophysiological parameters, are induced by short-term exposures as low as 176-429 mg m^{-3} (179). The effects on the vestibulo-ocular reflex may be of biological significance, since they may explain the vertigo and dizziness reported by exposed workers (179). Reduced performance in

psychometric test batteries has been found after exposure to intensities higher than 1 600 mg m^{-3}. The finding of decremental function at lower exposure levels (254) is inconsistent with other results, also with those of Stewart et al. (282), who used the same intensity and duration of exposure (see Table 3). The results may indicate that routine psychomotor test batteries have rather low sensitivity, and that tests of other functions of the central nervous system may be of relevance. Another problem is that the reported experiments use young, healthy and mainly male volunteers. Short-term exposure experiments among workers that are long-term exposed to TCE, or semi-experimental field designs to test performance and other functions among workers under different exposure conditions have not been reported for TCE. Although the experimental studies apply sensitive designs, where the volunteer acts as his own control, small sample size in several studies tends to reduce their robustness. Nakaaki et al. (217) report reduced performance in a study of four volunteers exposed to 538 mg m^{-3} 6 hours daily, but the results were not statistically significant.

As regards the neurotoxic effects from long-term exposure to TCE the surveys and studies performed among occupationally exposed subjects (Table 4) are of limited value. Even if several of the studies may have been ahead of their time when they were carried out, they have critical shortcomings of design, measured by today's standards. Therefore, the reports of chronic subjective symptoms and neurological changes indicative of chronic neuropsychiatric effects among workers exposed long-term to rather low doses may be explained by other causative factors than TCE. Consequently, their value in dose-effect and dose-response evaluation is limited.

In animal studies irreversible or only partly reversible changes in proteins and fatty acids have been demonstrated after continuous inhalation of 269-323 mg m^{-3} for several weeks (107,171). Low doses of oral TCE for 4-6 weeks in developing rats reduces the myelin contents in specific layers of the hippocampus (139). The evidence of these chronic biochemical or histological changes induced by low doses in animals treated for weeks or months (Table 5) is probably of some biological significance, but their functional importance in regard to neurotoxicity is less certain. Only limited attempts have been made to extrapolate these results to man when assessing neurotoxicity.

The evidence of genotoxic or carcinogenic effects in man is inadequate to determine dose-response relationships. Some of the problems are related to uncertainty of historic exposure levels, others to difficulties of interpretation connected to shortcomings in design, or inconsistency of results concerning cancer sites in different studies. This is the case, for example, in regard to the suspected connection between TCE exposure and lymphatic malignancies.

TCE without epoxide additives should be considered a weak mutagen in vitro and in vivo. It is carcinogenic in the mouse, where it induces hepatocellular carcinomas and lung adenocarcinomas, and in the rat where it induces renal tubular adenomacarcinomas and testis tumours (Table 10). The mutagenic and carcinogenic effects should be considered caused by TCE metabolites, since

activating systems are necessary (see chapters 7 and 8). The carcinogenic potential of TCE is species-related. At least some of the carcinogenicity seems to be closely linked to the metabolic capacity of the species, and is demonstrated at very high treatment doses which also produce cytotoxic reactions. This connection has been demonstrated for hepatic tumours in mice, as well as renal tumours in rats (224). The same may not necessarily be true for lung adenocarcinomas in ICR mice, where there was a dose-dependent relationship from the lowest dose of 269 mg m^{-3} (7 h/d, 5 d/w, 104 w), although not statistically significant below 807 mg m^{-3} (89). Moreover, more than one mechanism may be involved in tumour development, as demonstrated in mice treated with the TCE metabolites TCA and DCA (30).

Rather than direct extrapolating cancer risk from animal species to man, one should take into account the important interspecies differences in toxicokinetics. EPA (68) considers the effective tumour-producing dose to be the total amount of TCE metabolized. On this basis, the dose could rather be related to surface area, since the amount of TCE metabolized per area unit is assumed to be equivalent in the different species (28). Such an assessment is most fully developed for the hepatocarcinogenic potential in the mouse, and to a less extent from kidney or lung tumour potential. Animal carcinogenicity assays have been used extensively to make assumptions about dose-response relationships in man (21). Tumour incidence data from animal studies (Table 10) can be related to the cumulated metabolized dose in the different treatment groups and studies, in order to assess the carcinogenic potency of TCE for the different tumours in man (21). Based on different stated assumptions, it is possible to predict the increased life-time cancer risk in man, and to apply this to environmental exposures (21).

Much of the recent evidence links TCE hepatocarcinogenicity to the stable metabolites TCA and DCA (30,121). It should be kept in mind, however, that in the reported carcinogenicity assays, the administered doses of TCA and DCA were very high. TCA is of interest for interpreting carcinogenicity in man. This metabolite is of much greater relative importance in man compared with several animal species (231), but an in vitro study indicates that human hepatocytes are only capable of transforming 1/20 the amount of TCA transformed by rat hepatocytes (158). TCA is a proven mutagen in rodents in vivo (18,226), but may also act through epigenetic mechanisms. TCA induces peroxisome β-oxidation in mouse, and to some extent in rat hepatocytes, but not in cultured human hepatocytes (64). This species specificity is of importance in the evaluation of TCE as a human carcinogen.

IARC evaluates the evidence of animal carcinogenicity of TCE as limited, while the evidence indicating carcinogenicity in humans is considered inadequate. TCE is not considered classifiable for carcinogenicity in humans (137).

13 SUMMARY

Kristensen P, Skogstad M: 98. Trichloroethene. Nordic Expert Group for Documentation of Occupational Exposure Limits. Arbete och Hälsa 1991

This document summarizes relevant data for the purpose of establishing permissible levels of occupational exposure to trichloroethene. The critical effect to be evaluated when establishing an occupational exposure limit of trichloroethene is neurotoxicity. Carcinogenic and genotoxic potential are also factors to be taken into account in this connection.

Key words: carcinogenicity, mutagenicity, neurotoxicity, occupational exposure limits, trichloroethylene.

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Appendix 1.

Permitted or recommended maximum levels of trichloroethene in air

country	ppm	mg/m^3	comments	year	ref.
Denmark	30	160		1988	1
Finland	-	-		1987	2
Iceland	30	160		1978	3
Netherlands	35	190	MAK-TGG	1989	4
	100	538			
Norway	20	110	C	1989	5
Sweden	10	50	TWA, C	1990	6
	25	140	STEL		
USA (ACGIH)	50	269	TWA	1990-91	7
	200	1070	STEL		
(NIOSH)	-	270	TWA	1990-91	8
	-	1080	STEL		

C: the compound is regarded as carcinogen

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DIMETHYLSULFOXIDE

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Table of Content

BACKGROUND

Dimethylsulfoxide (DMSO) is a colourless, odourless fluid with high skin penetration. Today DMSO is widely used as a solvent and earlier also as a vehicle for drugs for dermal application. DMSO in its own capacity is also used as a drug against certain musculoskeletal disorders and interstitial cystitis. DMSO has a very low acute toxicity. DMSO influences the refraction of the eye lens in experimental animals exposed to high doses. This observation provoked, in 1965, a halt in the use of DMSO as a vehicle for drugs in the USA, and restrictions in its use as a drug were set up. Today DMSO is used in treatment of arthritis and cystitis (46). DMSO has been found embryotoxic, and malformations have been reported in experimental animals exposed to high doses for which reason restrictions in the use of DMSO by pregnant women have been considered.
Two conferences on the pharmacology and toxicology of DMSO have been held in the USA (26, 65) and a series of reviews on the pharmacology and toxicology of DMSO have been elaborated (13, 54, 62, 88).

1. PHYSICO-CHEMICAL DATA

Chemical name	Dimethylsulfoxide
CAS number	67-68-5
Synonyms	DMSO, methylsulfoxide, sulfinylbis (methan), DMS-70, DMS-90
Molecular formula	C_2H_6OS
Structural formula	$CH_3\text{-}\overset{\overset{O}{\|}}{S}\text{-}CH_3$
Molecular weight	78.13
Melting point	18.45 °C
Boiling point	189 °C
Vapor pressure	0.0493 kPa (0.37 mm Hg) at 20 °C

Flash-point	95 °C
Density	1.100 g/ml
Refraction index	1.4767 at 25 °C
Viscosity	1.99 cP at 25 °C
Saturation concentration	1555 mg/m^3
Conversion factor for air concentrations	1 mg/m^3 = 0.313 ppm 1 ppm = 3.19 mg/m^3
Octanol/water partition coefficient	log P_{ow} = 1.35

At room temperature DMSO is a colourless, practically odourless fluid. DMSO has a bitter taste with a sweetish after-taste. DMSO is a solvent used within a broad spectrum. DMSO is soluble in water, ethanol, acetone, ether, benzene, and chloroform. DMSO belongs to the polar, but aprotic solvents which also comprises dimethylformamide, dimethylacetamide, N-methylpyrrolidone, acetonitrile and others. DMSO is very hygroscopic as the hydrogen binding between DMSO and water is 1 1/3 times stronger than the water-to-water hydrogen binding. The mixture with water is an exothermal process releasing 14.3 J/g (60 cal/g) DMSO (22). Pure DMSO absorbs water from the air and is rapidly diluted to 66-67% when in contact with the air. DMSO must therefore be kept under tight lock (13). DMSO forms stable complexes with metals. The dipolar and nucleophilic properties of DMSO is a result of accessible free electron pairs at both the sulphur and the oxygen atoms in the molecule.

2. USES AND OCCURRENCE

2.1 Uses and synthesis

DMSO is synthesized by oxidation of dimethylsulfide and produced from lignin, which is a byproduct in the production of paper pulp. DMSO can also be produced from coal and mineral oil (13). DMSO is used as an industrial solvent, as an inert solvent in laboratories, e.g. as a vehicle in biological research, and as a drug in the treatment of arthritis and other musculoskeletal disorders. DMSO has formerly

been widely used as a vehicle for pharmaceuticals in dermal preparations. RTECS reports exposure to DMSO in 1983 in the USA within 28 industries, 3489 workplaces, 39 professions with 43469 persons employed of which 16010 were women (79). The degree and the extent of the exposure are not mentioned.

2.2 Occupational exposure

No measurements of air concentrations in occupational environments has been found in the literature. In the intensive care ward of a hospital where patients were treated intravenously with DMSO against serious cerebral oedema, the staff administering the treatment were affected. The reported symptoms were unpleasant odour, dizziness, nausea (23) and accredited to the exposure to the expired air of the patient.
DMSO has a very low vapour pressure (0.0493 kPA at 20 °C) and a small saturation concentration in air (1555 mg/m^3 at 20 °C). The risk of inhalation of DMSO is therefore also dependent on the degree of atomization of DMSO into aerosol in working operations.

2.3 Methods for analysis of concentrations in air

DMSO can be detected using gas-chromatographical analysis. This can also be done in blood and urine (41,82).

2.4 Occurrence

Traces of DMSO occur in the water in the environment and traces of DMSO, as well as of the metabolites dimethylsulfide (DMS) and dimethylsulfon ($DMSO_2$), occur in meat and contribute to the taste of beer and milk (13).

3. KINETICS

3.1 Absorption

DMSO and 80-90% solutions of DMSO are easily absorbed through skin and other membranes (60). DMSO forms hydrogen bonds to proteins, displaces water and produces reversible changes of the conformation in the membranes enhancing the absorption (91). In concentrations of less than 67% DMSO molecules are already hydrated making the skin penetration of such solutions less effective than the penetration of solutions with higher DMSO concentration (13). At 100% DMSO

the penetration through the skin is less than the penetration of a 90% solution of DMSO in water due to the considerable hygroscopicity which induces a water flux in the opposite direction (44).
In all routes of administration a rapid absorption is seen, measured as the concentration of DMSO in blood. Within 5 minutes after skin application radioactively labelled DMSO was detected in the blood of a person (61). A maximal level of concentration was found after 4-6 hours. Hucker et al. reported maximum serum concentrations after 4-8 hours in persons after dermal application of 1000 mg/kg (51). DMSO-instillation has been used in the treatment of inflammations in the intestines and in the bladder. Systemic reactions have been reported indicating absorption of DMSO from these organs.
Skin absorption of a 90% solution of DMSO (61) showed, in dogs, that 80% of the dose had been absorbed after 4 hours and more than 90% after 24 hours.

3.2 Distribution

Brandt et al. carried out a whole-body autoradiography study in mice intravenously administered with 0.1 mmol/kg[^{14}C]-DMSO (12). One hour after the injection of DMSO radioactivity was measured in the eye lens and the skeleton while a high and selective accumulation of radioactivity in the intestinal mucous membranes was found after 24 hours and later. Kolb et al. found no specific accumulation in the organs of [^{23}S]-DMSO in rats after dermal application of 1000 mg DMSO/kg as 90% solution (61).
In measurements of the distribution of [^{23}S]-DMSO in rats using dermal application and intravenous injection higher concentrations of DMSO was found in soft tissues compared to cartilage and bone tissue (29). The transport of DMSO in the blood was through the albumine fraction of the serum.

3.3 Biotransformation

DMSO is reduced to the volatile dimethylsulfide (DMS) which has a characteristic garlic-like odour or is oxidized to dimethylsulfon ($DMSO_2$), which is excreted in the urine (42,51).
In figure 1 the possible metabolic routes of DMSO are shown (91). Only the metabolites DMS and $DMSO_2$ were identified in animals and humans, while the remaining substances shown in figure 1 were identified in microorganisms.
DMSO induces the microsomal oxidation system in the liver (25) and, thus, it is likely that oxidation and reduction takes place via this system. Gerhards and Gibian (42) have measured oxidation of DMSO to $DMSO_2$ in microsomes with $NADPH_2$ and in the presence of oxygen.

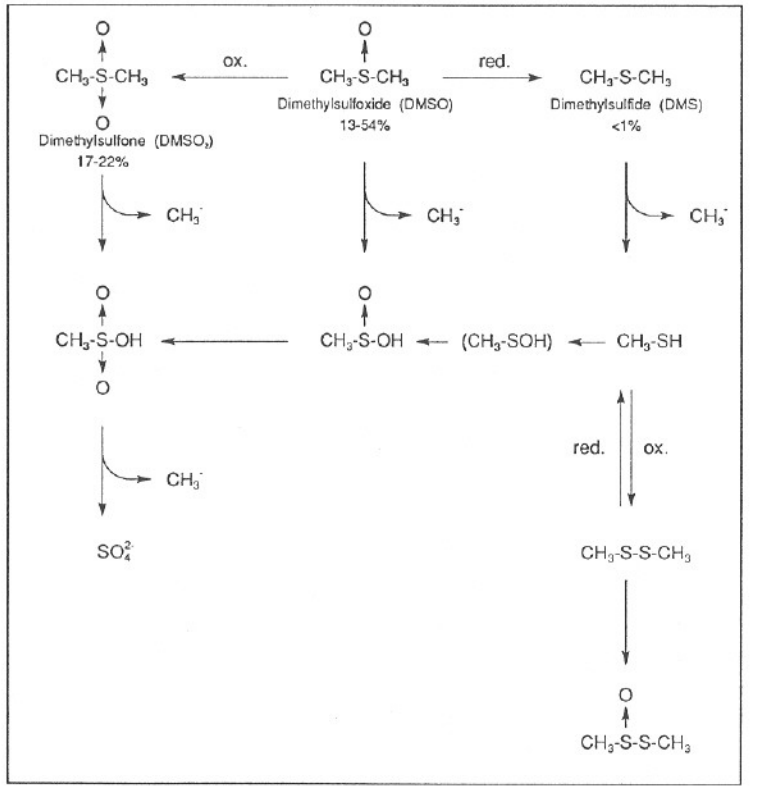

Figure 1. Possible metabolic pathways of DMSO (91). The percentages indicate relative excretion in humans.

DiStefano and Borgstedt discovered metabolism of DMSO in hepatectomized cats and indicated that metabolism was not by the microsomal enzymes (31). The observation may, however, indicate metabolization of DMSO in other tissues than the liver.
Induction of the microsomal oxidation system by e.g. other xenobiotica may be expected to influence the metabolism of DMSO.

3.4 Elimination

DMSO is primarily excreted through urine together with the oxidation product $DMSO_2$. Furthermore DMSO and the volatile reduction product DMS are excreted by exhalation. In two persons receiving skin application of 2000 mg DMSO in a

90% aqueous solution, the exhaled quantity of DMSO was measured to 1% after 6 hours and 1 % and 3% after 24 hours (61). In the exhaled air, a total of 4 mg and 9 mg DMS were measured 6 hours after application while no DMS was detected after 24 hours.

Less than half of the amount of DMSO given to persons intravenously or dermally was excreted in the urine unmetabolized (42). The un-metabolized DMSO was excreted during a period of a few days. Dimethylsulfon ($DMSO_2$) is also excreted in the urine (42, 51). Gerhards and Gibian found, in 4 persons, an increasing excretion of $DMSO_2$ 24 hours after intravenous or dermal administration of DMSO with an unknown amount of [^{35}S]-labelled DMSO. The amount excreted formed more than half of the total amount of DMSO and $DMSO_2$ excreted (42).

In volunteers given 1000 mg DMSO/kg in a 50% aqueous solution intravenously the half-life for the elimination of the administered dose was 4 days. After a week, 80% had been excreted. The half-life for the serum concentration of DMSO was 20 hours. Dermal application of 1000 mg [^{35}S]-DMSO/kg caused a maximum concentration of DMSO after 4-8 hours and a half-life of 11-14 hours. $DMSO_2$ had a half-life of 60-70 hours (61).

In a person to whom a dose of 21000 mg DMSO was administered in an unstated route of dosing, 3% of the dose was measured as dimethylsulfon in 24 hours' urine (50).

The excretion of DMSO and $DMSO_2$ in urine was 13% and 17.8%, respectively, of the total dose in two persons dermally administered 1000 mg [^{35}S]-DMSO/kg of a 70% solution in water (51). In 6 persons orally administered with 1000 mg DMSO/kg in a 70% solution in water, an average of 50.8% was excreted in the urine as DMSO. The excretion of $DMSO_2$ in the urine formed an average of 9.6% of the administered dose after 120 hours in all 6 persons. In 2 persons $DMSO_2$ in the urine was measured to 22% after 480 hours (51). In a person receiving 500 mg DMSO/kg orally for 14 days, 70.9% of the dose could be found in the urine after a total of 560 hours; 53.7% of the excreted amount was DMSO and 17.2% was $DMSO_2$ (51).

The excretion of DMS by exhalation in rats is reported to constitute between 1% and 6% of the administered dose (111). The elimination in faeces was 10% of the total dose after 24 hours in orally administered rats and 4% in intraperitoneally and dermally administered rats (111). Kolb et al. found that between 0.5% and 2% of the dose of radioactively labelled DMSO in rabbits (1000 mg/kg) and dogs (2000 mg/kg) was excreted via faeces independent of the route of administration (61).

Six horses were given 1000 mg/kg DMSO intravenously in a 10% solution in saline, and 6 other horses were given 100 mg/kg DMSO in a 1% solution in saline. The biological half-life was 8.6±0.3 and 9.8±2.2 hours respectively (9). The biological half-life of DMSO and $DMSO_2$ in rhesus monkeys orally administered

by gavage with 3000 mg DMSO/kg in a 50% solution for 14 days was 16 and 38 hours respectively (64).

Summarizing the excretion of DMSO in humans is estimated to totally constitute approximately 80% via urine as DMSO (13-54%) and $DMSO_2$ (17-22%). The rest is excreted via exhalation as DMSO (<5%) and DMS (<1%) and via faeces (figure 1).

3.5 Factors affecting the metabolic model

As DMSO is metabolized by the microsomal oxidation system (42) it is to be expected that other substances, which influence these enzymatic activities, e.g. by induction of the P450-system, will influence the metabolism of DMSO.

4. GENERAL TOXICOLOGY

4.1 Acute toxicity

Acute toxic effects of oliguria, hemolysis, convulsions and unconsciousness have been reported in a patient in an intravenous DMSO treatment against arthritis using three daily doses of 2000 mg DMSO in a 20% solution (113).

In an extensive study of the acute effects of dermal application of 1000 mg DMSO/kg to humans in Vacaville State Prison 1967 the following results were obtained 1) eosinophilia, probably as a result of the histamine releasing effect of DMSO, 2) irritation of the mucous membranes, 3) irritation of the skin with formation of blisters, flushing, drying, and scaling 4) systemic inconveniences as sedation, nausea, headache, and dizziness (15).

Rats survived the following concentrations of DMSO in the air (37):

8 animals exposed to 2900mg/m^3 for 24 hours
8 animals exposed to 2000mg/m^3 for 40 hours
32 animals exposed to 200mg/m^3 for 30 days with a daily exposure of 7 hours

In table 1 the LD_{50} determinations for experimental animals are summarized (72).

DMSO was cytotoxic to rabbit lymphocytes in a concentration of 0.128 mol/l (1%), but not in the concentration 0.064 mol/l (0.5%). The cytotoxicity was measured by tryphan blue colouring of dead cells (7).

Table 1. Single dose LD_{50} determinations, mg/kg (72).

Species	iv	ip	sc	po	Dermal
Mouse	3800-8900	14006-20600	13900-25600	16500-24600	44000
Rat	5200-8100	5500-13600	13700	17400-28300	40000-50000
Guinea pig	6000-8000	-	>14000	>14000	>MDA
Dog	2500-8000	-	-	>10000	>MDA
Monkey	4000-8000	-	-	>4000	>MDA

MDA: Maximally applicable dose

4.2 Mechanisms of effects

DMSO forms hydrogen bonds with organic molecules and water and the binding is reversible. Furthermore DMSO can substitute water in biological systems. DMSO acts among other things by changing protein and membrane conformations, thereby changing enzyme activities and membrane penetration. Hence DMSO can induce changes in the electrolyte balance as a result of an increase in the uptake or the excretion in the cells. This is shown e.g. in isolated hepatocytes from rats where DMSO influenced the absorption and flux of glucose (86).

Also, the respiration in rabbit liver homogenate, measured with a Warburg respirometer, was competitively inhibited by DMSO in concentrations of 1 mmol DMSO/l and more (8). The oxidative phosphorylation was uncoupled in vivo in the liver of rats intraperitoneally administered with about 2750 mg DMSO/kg (77). The mitochondrial cytochrom oxidase activity increased significantly to 4 times the control activity 2 hours after injection. The activity remained increased for 5 days after the intraperitoneal injection of about 2750 mg DMSO/kg (30). DMSO did not act as an uncoupler of the oxidative phosphorylation in vitro in liver mitochondria from untreated animals incubated with 0.1 mmol DMSO/l, whereas 0.1 mmol DMS/l uncoupled the oxidative phosphorylation in vitro (77). These results point to a toxic effect of the DMSO metabolite DMS. This assumption is supported by the observation of an increased cytochrom oxidase activity in mitochondria from rat liver incubated in vitro with DMSO as well as with DMS (30).

DMSO and DMS are "free radical scavengers" and as such have a protective effect on cells exposed to radioactive radiation and freezing. Even in cells exposed to radioactive radiation and the promotor TPA (to increase cell transformation),

DMSO was able to decrease cell transformation (58). The anti-inflammatory effect of DMSO is also due to DMSO being a "free radical scavenger"(13). DMSO reacts antagonistically on a number substances which are metabolized into free radicals, e.g. benzene (3), acetaminophen (87). As another example of the "free radical scavenger" properties of DMSO it can be mentioned that DMSO, in a concentration of 1 mmol/l, decreased the toxic effect of both Fe^{3+} and X-ray treatment of mouse macrophages from the peritoneal fluid (1). Both treatments are supposed to form free radicals.

The formation of superoxide radicals in air saturated aqueous DMSO with added OH^- has been reported (52), therefore the authors warn against alkaline solutions of DMSO as a consequence of the content of free radicals.

DMSO can induce the microsomal oxidation system (25) and thus can change the metabolism of other chemicals. Thus Hassan (48) found an increased cholesterol and bile acid metabolism in rats dosed with 0.26 mmol DMSO/l (2%) in drinking water. DMSO decreased the bioactivation of paracetamol in the liver and thus the liver toxicity of paracetamol (55).

Brodberg et al. found an inhibition of the mutagenic effect of N,N-dimethylnitrosamine in Drosophila melanogaster when the substance was dissolved in DMSO - probably as a result of the inhibition by DMSO of the metabolism of the substance into a mutagenic metabolite (16). Also in tests for aneuploidy in yeast with nocodazol and ethylacetate, which are positive in this test system, DMSO inhibited the effect (73). In Sprague Dawley rats and Swiss mice dosed with bleomycine and 5000 mg 50% DMSO/kg the number of bleomycine-caused lung tumours increased significantly, probably as a result of increased metabolism (47).

DMSO has anti-inflammatory activity in experimental models, in acute musculoskeletal diseases, acute traumatic or inflammatory injuries of the central nervous system, postoperatively where oedema are expected, in infectious or septic diseases (13).

DMSO stimulates the differentiation of a number of cancer cell lines, e.g. human melanoma cells (100), human myeloid leukemia cells HL60 (14) and mouse hepatoma cells (49).

DMSO increases the permeability of a number of other substances through the skin, e.g. hexopyrronium bromide, naphazoline hydrochloride, and fluocinolone acetonide (105), alcanoles (2), tetrachlorsalicylanilide (60), hydrocortisone and testosterone (69).

4.3 General findings

The following effects of DMSO after the administration of high doses have been summarized (54):

Increased membrane penetration and membrane transportation, effects in the

connective tissue, anti-inflammation, decreased nervous transmission (analgesia), bacteriostatic, diuretic, synergistic or antagonistic to other drugs, cholinesterase inhibitor, increased resistance against infection, vasodilation, muscle relaxation, decreased platelet aggregation, influence on serum cholesterol.
Albino rats in groups of 20 were given 400, 7050, 14100 mg DMSO/kg/day (2 ml of a 20% solution of DMSO in water, 8 ml of a 80% solution and 16 ml of a 80% solution) by gavage in 13 weeks (104). The highest dose was close to lethal as only 2 rats survived after 8 weeks. In these rats hyperemia was observed and in all groups a decrease in weight gain was observed.
In a cell culture system for toxicity determination, a good agreement was found between low in vitro toxicity in 5 cell lines, where the highest tolerated dose was approximately 300 mmol DMSO/l and a slight eye irritation measured in a Draize test (10).

5. ORGAN EFFECTS

5.1 Skin and mucous membranes

Effects on the skin as erythema, a burning sensation, blisters and pruritus have been reported after skin application of DMSO (60,88). Skin application of DMSO in prolonged periods caused dryness and scaling of the skin. As the mixture of DMSO and water is an exothermal process, the skin area to which DMSO is applied is heated.
A major study of the effects of DMSO in humans dosed with 1000 mg DMSO/kg dermally daily for 14 days and 90 days, respectively, has been performed on prisoners (15). The administration was accomplished by rubbing into the skin a 80% DMSO solution every evening after the person had washed. The absorption of the entire prescribed dose could last for up to 2 hours. In the group of 65 persons participating in the test, which lasted two weeks, 10 persons had eosinophilia which was not seen in the 33 control persons. Skin irritations appeared as blisters, erythema, drying and scaling of the skin. These effects were reversible as the skin reverted to its normal state after 3 weeks.
DMSO was applied dermally to dogs and rhesus monkeys in doses of 3300-33000 mg/kg weekly for 26 weeks. The only findings reported were formation of blisters and blushing of the skin at the site of application. The skin effect was reversible (103).
The effect of DMSO on permeability of human skin was measured in vitro on skin samples from thigh or lower leg. An increased penetration rate of tritiated water, which was dependent upon the time of exposure and the concentration of DMSO applied, was found. The permeability enhancement disappeared partially upon

removal of DMSO. The extent of reversibility varied with the concentration of DMSO applied. The reversibility was maximal (70%) at a 1:1 molar ratio of DMSO to water (4).
The dermal application of DMSO on nude mice and control mice using DMSO moistened gauze on 2 cm^2 of shaved skin for 5 minutes daily for 8 weeks showed significantly greater length of the claws (75). No information is available on the dose.

5.2 Respiratory system

Influenza-like symptoms, mucous membranes dryness, dyspnea, sore throat and coughing have been reported as secondary effects after DMSO treatment in humans (88).
In groups of 8 rats exposed to aerosols of DMSO with 2900 mg/m^3 for 24 hours, 2000 mg/m^3 for 40 hours and 1600 mg/m^3 for 4 hours spots were seen in areas of the alveoles with higher frequency than in the control animals (37). In rabbits exposed to a mist of DMSO oedemas were observed in the lungs (107), referred in (37).

5.3 The liver

Oral administration of 2500 mg DMSO/kg to mice daily for 6 weeks caused degeneration of the liver (19).

5.4 The kidneys

Tubular nephritis has been reported in mice administered intraperitoneally with 2500 mg DMSO/kg in 50% DMSO solution in 33 daily injections (20).
In a 28 day study of kidney toxicity, daily injections of 2000 and 4000 mg DMSO/kg were given intraperitoneally in a 40% solution in saline in groups of 10 male and 10 female Sprague-Dawley rats. A higher mortality and a significant inhibition of growth in the dosed groups was seen, compared to the control group, which was given saline. Serum concentrations of urea and creatinine and the in vitro absorption of p-aminohipputate and methylnicotinamide in tissue samples of the renal cortex of the dosed animals did not differ from those of the control group. The authors concluded that DMSO does not have major toxic effects on the kidneys in the tested species, i.e. rats (101). The diuresis increased four fold in monkeys intravenously dosed with 2000-3000 mg DMSO/kg in a 40% solution compared to control animals (27).
DMSO, in the range of 135-223 mmol/l, was osmotically active in perfused renal distal tubules from rat kidneys (33).

The osmotic activity of DMSO may be the explanation for the increased diuresis after DMSO exposure.

5.5 Gastrointestinal tract

Symptoms of breath odour, nausea, dry throat, vomiting, diarrhea, constipation , lack of appetite and intestinal hemorrhage are all reported secondary effects of exposure to DMSO by dermal application (15).

5.6 Cardiovascular system

Extravasations in patients in connection with injections of anthracycline in the arm could be relieved by local dermal application of 99% DMSO (84).
The frequency of pulse and respiration increased in monkeys immediately after the injection of 3000 mg DMSO/kg in a 40% solution. 15-25 minutes after administration the pulse and respiration were at the starting point (27).
Cats anaesthetized with pentobarbital (35 mg/kg, intravenously) and administered intravenously with 110-880 mg DMSO/kg (0.1-0.8 ml/kg) showed an increase in blood pressure dependent on the dose administered (108). The pulse was higher at 880 mg DMSO/kg compared to 110, 220 and 440 mg DMSO/kg. Blood pressure and pulse without an injection of DMSO have not been stated, and the authors conclude, contrary to the measured values set up in a table, that DMSO had a hypotensive effect in the cats together with a decrease in the pulse frequency. Thus the value of the study must be considered as being dubious.
A dose dependent decrease in the pressure of perfusion was caused by vasodilation in isolated rat hearts perfused with DMSO in the concentrations of 0, 22 and 128 mmol/l (0, 0.17, 1.0%) (39).
In rabbits with cholesterol induced atherosclerosis the effect of DMSO on aortic lesions was studied by the administration of cholesterol rich diet in parallel with drinking water containing DMSO. Lesions in the aorta were inhibited by 50% with a DMSO dose of 1500 mg/kg and were not seen at doses of 3500, 5500 and 9100 mg/kg (28).

5.7 The blood and the haematologic system

In a patient undergoing treatment against oedema of the brain by intravenous injection of 1000 mg DMSO/kg in a 20% solution a heavy hemolysis was seen (95). Eosinophilia was found in 10 out of 65 persons dermally applied with 1000 mg DMSO/kg for 14 days, but not in any of the 33 control persons (15). Hemolysis and eosinophilia have been reported as a secondary effect in less than 1% of patients treated with DMSO (88).

Infusion of 1000 mg DMSO/kg in 20% and 40% solutions in physiologic saline to horses caused hemolysis, whereas this was not observed using a 10% solution of DMSO (9).
DMSO inhibits the coagulation of the blood when it is added to blood in concentrations of 0.64 mol/l (5%) (13). Daily dermal applications on male and female Sprague-Dawley rats for 14 days of 0.11 mg and 0.055 mg (100 µl) DMSO in solutions of 100% and 50%, respectively, decreased the coagulation of blood (68). In the group of male rats dermally applied with a 50% solution of DMSO, a significantly longer coagulation time and a significantly smaller increase in weight were found compared to control animals dermally applied with water. In the group of male rats in which 100% DMSO was dermally applied the number of platelets was significantly increased. As the weight of the animals and the number of animals in each group are not stated, the relevance of the study is thus difficult to evaluate.

5.8 The central nervous system

Secondary effects as sedation, headache, dizziness and pain in the eyes have been reported in patients treated with DMSO (88) and in persons after dermal application of 1000 mg DMSO/kg daily for up to 90 days (15).
An analgetic effect of DMSO in tail-flick and hot-plate test was found in Sprague-Dawley rats intravenously and intraperitoneally administered with high doses of 5500 mg/kg in a 50% solution. The analgetic effect of DMSO is in accordance with the analgetic effect of morphine, but no inhibition takes place with naloxone administration. Thus, DMSO does not act through the opiate receptors (45).
A Russian study on rats regarding the anti-stress effects after intraperitoneal injections of 1000 mg DMSO/kg for 3 weeks did not show any significant changes in an open field test. DMSO neutralized the effect of simultaneous stress caused among other things by pain (66). This is in accordance with the above mentioned analgetic effect of DMSO.
Intraperitoneal administration of 5500-11000 mg DMSO/kg (5.0-10 ml/kg) to mice influenced CNS - decreased alertness, respiration, body and limb tone and rectal temperature (108).
DMSO in concentrations up to 128 mmol/l (1%) effects the neuromuscular transmission by inhibiting acetylcholinesterase in mouse diaphragm (76). In higher concentrations DMSO is supposed to exhibit an unspecified effect on the release of neurotransmitters as a result of a "fusogenic" effect, a reduction of the barrier of energy to be exceeded before a fusion of membranes and release of transmitters take place.
In isolated samples of muscle tissue incubated with DMSO in the range of 7 mmol/l-0.7 mol/l (0.5-50 ml/l) an inhibition of contractions induced by acetylcho-

line (1.10 x 10^7 mol/l), histamine (6.51 x 10^6 mol/l) and barium (7.38 x 10_4 mol/l) was measured at concentrations of DMSO greater than 56 mmol/l (4 ml/l).
DMSO in concentrations above 0.64 mol/l (5%) inhibited neurite development in chicken embryo dorsal root ganglions (92).
DMSO influenced the process of differentiation in hypothalamo-neurohypophysial neurones in primary cell culture with 0.128 mol DMSO/l (1%) added (97).

5.9 The peripheral nervous system

DMSO influences the peripheral nervous system via the above mentioned effect on neural transmission (76).

5.10 The bones

DMSO is absorbed into the bones (61), but no information on any harmful effects on bones has been found.

5.11 The eyes

Local administration of 125 mg DMSO daily in a 50% aqueous solution in each eye to patients with retinitis pigmentosa or with macular degeneration did not show any changes (40). No abnormal findings by slit lamp examinations, opthalmoscopy, determination of visual fields and refraction and tonometry were found in a big survey of persons dermally applied with 1000 mg DMSO/kg daily for 90 days.
In 55 patients with scleroderma wounds on their hands a randomized double blind study of the effects on the eyes when immersing the hands in 70% DMSO, 2% DMSO or saline 3 times daily for 15 minutes during 12 weeks was carried out. There were no differences in the vision, changes in the lens of the eye or the development of cataract between the three groups. The maximum dose of DMSO was calculated to 2600 mg/kg/day (99).
Repeated administration of DMSO to experimental animals caused changes in the lens. Changes in the refractive index of the eye lens affected the eyesight and caused myopia in the animals. The degree of harm done to the lens was directly dependent on the concentration and duration of DMSO administration (93).
Changes in the refractive index of the lens have been described in rhesus monkeys orally administered DMSO by gavage for 6 months. The dose was 16.5 mg or 49.5 mg/kg/day in 3 or 9 ml of a 50% aqueous solution to 4 male and 4 female monkeys in each dosing group (6).
Changes in the eye lens in a dog orally administered with an average of 3800 mg DMSO/kg/day for 48 days have been found (102).

6. IMMUNOTOXICOLOGY AND ALLERGY

Local skin reactions of varying severity are seen after skin application of DMSO: Erythema, itching and blistering, while systemic reactions are rare. Blistering was often seen after topical application of DMSO (60), while no specific sensibilization was observed. Systemic contact dermatitis occurred in a patient upon intravesical instillation of DMSO for the treatment of an interstitial cystitis (80).
In persons receiving daily dermal applications of 1000 mg DMSO/kg for 14 or 90 days an increase in peripheral eosinophil counts was observed. The eosinophilia was due to the cutaneous histamine-releasing effect of DMSO (15).
DMSO may enhance the penetration of various allergens and thereby promote allergic reactions. Inhibition of the allergic response has been reported for the metals of nickel, chromate and cobalt, which are apparently chelated by DMSO (44).
The addition of 0.385 mol DMSO/l (3%) to cultures of mouse lung carcinoma cells increased the expression of surface histocompatibility antigens. The authors suggest that the observed antitumour effect of DMSO may be explained by the increased antigen production (5).
Mice were injected intraperitoneally with 100% DMSO for 5 weeks and immunized twice (days 13 and 24) with sheep red blood cells. The mice received 55 mg (0.05 ml) DMSO daily for one week, 28 mg (0.025 ml) every other day for the second week and 55 mg (0.05 ml) daily for 3 more weeks. No effects of DMSO on the primary and secondary antibody response to sheep red blood cells were seen (18).

7. MUTAGENICITY AND GENOTOXICITY

DMSO was not mutagenic in Ames test in concentrations up to 0.64 - 1.92 mol/l (100-300 mg/plate). In the SOS chromotest, which is a bacterial test for the induction of SOS DNA-repair systems, DMSO in concentrations of 10^{-5} - 0.1 mol/l (7.8 ng/ml - 7.8 mg/ml) was not mutagenic (11).
DMSO was not mutagenic in tests for induction of sex-linked recessive lethals and loss of sex chromosome in Drosophila melanogaster. Males, 1-2 days old, were injected intraabdominally with 0.2 µl of solutions with concentrations of 2.5 - 641 mmol/l (0.02 - 5%) DMSO. DMSO was however toxic as demonstrated by dose-dependent sterility and mortality (78).
A test for induction of dominant lethals was performed with groups of 15 male Swiss mice injected intraperitoneally twice, with a 20 hour interval, with 5000, 7500 or 10000 mg/kg DMSO (21). In females, mated with males exposed to 7500 or 10000 mg DMSO/kg the day after exposure, an increase in the preimplantation

loss was observed. No significant increase in the preimplantation loss was observed in females mated with males 1, 2, 3 and 4 weeks after exposure. The mortality after 4 weeks was high in the two highest exposure groups: 20% at 7500 mg DMSO/kg and 73% at 10000 mg DMSO/kg. The authors suggest that the increased preimplantation loss for the first matings was a result of toxic effects and conclude that the study does not indicate any mutagenic potential of DMSO in male Swiss mice.

An in vivo cytogenetics study in the Sprague-Dawley rat receiving DMSO by intraperitoneal injection showed increasing number of chromosome aberrations with increasing dose (57). Each group of 10 rats was administered intraperitoneally with DMSO at a volume of 5 ml/kg body weight at doses of 55 mg, 550 mg, 2750 mg and 5500 mg/kg (1%, 10%, 50% and 100%) DMSO for five consecutive days. The number of chromatid breaks, exchanges, rings and dicentrics was significantly elevated in all treated groups compared with the control group. The highest dose was toxic resulting in a mean percent of aberrant cells of 68.7% compared with 4% in the control group.The authors conclude that the cytogenetic effects are dose dependent and the observations suggest that DMSO effectively disrupts the integrity of the chromosome structure. A need for further investigation of the genetic activity of DMSO in mammalian cells is stated.

The mutagenic effect of 1.03 - 1.18 mol/l (8-10%) DMSO on yeast cells (112) was demonstrated in the conversion of wild type yeast cultures to respiratory deficient petites. The mutation is suggested to be of a cytoplasmic nature as the metabolism shifted form aerobic to anaerobic.

In studies of Caenorhabditis elegans (a worm) DMSO concentrations higher than 0.64 mol/l (5%) inhibited the formation of synaptonemal complexes thus making effective pairing and segregation of homologous chromosomes not possible (43). These concentrations were lethal as well.

DMSO induced single strand breaks in kidneys from male NMRI mice injected intraperitoneally with 1950-7800 mg DMSO/kg (25-100 mmol DMSO/kg) (110), but not in other tissues. Also in leukemia cells in vitro the DNA single strand breaks increased after incubation with 0.24 mol/l (1.9%) DMSO (96).

The effects of DMSO upon interchalator-induced DNA single strand breaks in mouse leukemia cells (L1210) was investigated (90). DMSO concentrations of 0.64 mol/l are suggested to destabilize the DNA-chromatin protein interaction making the DNA more 'open' and susceptible to damage by DNA damaging agents. The nuclear proto-oncogene c-myc is believed to play a regulatory role in eucaryotic cellular growth and differentiation. DMSO concentrations of 0.16 mol/l (1.25%) induced a rapid but transient effect on the c-myc mRNA level in several cell lines (24). It is suggested that the initiation of transcription is inhibited.

In summary: DMSO reacts with electrophilic centers in e.g. DNA and chromatin. DMSO also has an effect on the permeability of the cell membranes affecting the

organization of the chromatid. These effects have been detected in test systems: In vivo cytogenetics in mice (57), yeast conversion (112), effects on the synaptonemal complexes in worms (43). The positive findings are all at toxic, practically lethal doses and should correctly be characterized as cytotoxic and not genotoxic effects. Studies of genotoxic effects of DMSO in lower doses and sensitive test systems are needed for an evaluation of the genotoxic potency of DMSO.

8. CARCINOGENICITY

A Russian long term toxicity study in rats and mice involving administration of DMSO by epicutaneous application and by gavage is reported (70). A number of rats, not specified, were given 3000 mg DMSO/kg/week by gavage in 243 single injections. The first tumours were observed after 11.5 months and the total number of tumours appeared in 17 out of 65 rats (26.1%). In 7 out of 34 female rats the tumours were breast tumours. No significant differences were found in the total number of tumours and breast tumours in exposed rats compared with control rats. These negative results were found in the rats given DMSO by gavage and in the rats receiving 1000 mg DMSO/kg weekly in 82 injections. By both routes of administration a significant increase in hemoblastomaes was reported.

In mice administered with 330 mg DMSO/kg weekly in 198 administrations by gavage, tumours were observed in 18 out of 54 surviving animals (33.3%). This number of tumours was significantly increased as compared with the total number of tumours in 13 out of 82 control animals (16.2%). No significant differences were seen in the number of tumours in mice administered DMSO by epicutaneous application compared with the number of tumours in control animals.

The life-time of mice administered DMSO was significantly longer than the life-time of control animals. Also rats administered DMSO and with tumours survived longer than control animals.

The tumours were most typically localized in the lungs, the liver, the kidneys and the lymph nodules. The authors conclude that DMSO has a low carcinogenic potency and workers engaged in DMSO production must be protected from direct contact with DMSO. The study is not described sufficiently to make a detailed evaluation feasible. Consequently the study may be evaluated as insufficient referring to the criterias for evaluation of carcinogenicity established by IARC.

DMSO was injected subcutaneously in rats twice weekly for 5 weeks with H_2O_2 or $TiCl_3$ or a combination. 10 rats in each group were observed for 1 year and no carcinogenicity was found (67).

Female Sprague Dawley rats were orally administered with 20 mg dimethylbenz-anthracene (DMBA) by gavage on the 48th day of life and the effect of DMSO on the induction of breast cancer was tested in three groups of 50 rats each. Group

1 was given 50 ppm DMSO in the drinking water three days prior to tumour induction by DMBA and the following 18 months. Group 2 was given 50 ppm DMSO in the drinking water three days after tumour induction and the following 18 months. Group 3 only received DMBA. The results showed that DMSO administration resulted in a decrease in tumours, though not significantly (38).
A significant inhibition of skin carcinogenesis in hairless mice receiving methylcholanthrene by topical application was observed with 50% DMSO in the solvent (53). Synergistic cytotoxicity between 0.64 and 1.28 mol DMSO/l (5 and 10%) and four antineoplastic agents was demonstrated in five human tumour reference cell lines (89).
Tatematsu et al. surveyed various chemicals for promoting activities based on generation of hyperplastic liver nodules in rats. DMSO was not found positive (106).
In conclusion, the available studies for evaluating the carcinogenic effects of DMSO are insufficient.

9. REPRODUCTION AND TERATOGENICITY

Swiss mice were treated with 50% DMSO or physiological saline given orally or intraperitoneally from day 6 to day 12 of gestation. A total of 102 mice were treated with DMSO in concentrations ranging from 5000 mg/kg to 12000 mg/kg. DMSO administered orally did not cause malformations, but with intraperitoneal administration 7 out of 100 surviving foetuses showed malformations: 4% had malformed limbs, 2 showed anencephalia and 1 had celosomia.
The quality of the investigation is doubtful as less than 50% of the animals administered DMSO or saline in the gestational period survived full term (20).
Ninetyone pregnant Wistar AG rats were given 5000-10000 mg DMSO/kg orally in 50% saline or by intraperitoneal administration day 6 to 12 of the gestational period. After intraperitoneal injection of 8000-10000 mg DMSO/kg a higher percentage of aborted foetuses and a reduction in weight in the live foetuses compared with the control animals were found. Eleven out of 729 live foetuses from the rats injected intraperitoneally showed malformations while only one of the 558 foetuses from control rats showed celosomia (20).
In 5 rabbits given 5000 mg DMSO/kg in a 50% solution orally and in 5 rabbits given 4000 mg DMSO/kg in 50% solution by subcutaneous injection from day 6 to day 14 of gestation, no remarkable differences in the weight and the number of aborted embryos were found compared with controls. Of the 86 foetuses examined only 1 had celosomia (20).
In a pilot teratogenicity study by Ferm golden hamsters were injected with single doses of 550 mg (0.5 ml) on the 8th day of gestation various degrees of

exencephaly and anencephaly were found in those embryos surviving (35). The study was followed by a new study of congenital malformations by DMSO in golden hamsters injected with DMSO intraperitoneally ranging from 50 to 8250 mg/kg on day 8 of gestation (36). In 11-day old embryos, effects on embryonic development were seen at levels of 2550 mg DMSO/kg and higher.
The observed effects were exencephaly, rib fusions, microphthalmia, limb abnormalities and cleft lip. No appreciable effects were seen of DMSO on maternal weight gain or health during the experimental period. Histologic examinations of the placentas revealed no obvious lesions.
Thirtyone Sprague-Dawley rats were given 10250 mg DMSO/kg/day as a 90% aqueous solution by subcutaneous injections on day 8, days 8 and 9 and days 8,9 and 10 of gestation. Rats with resorptions were found in all 3 groups. The litter size and the corresponding number of live foetuses were significantly decreased in the group receiving 3 injections. No gross or skeletal malformations were found among the 338 live foetuses from the mothers treated with DMSO (56).
Intraperitoneal administration of 5500 mg DMSO/kg to 12 pregnant hamsters on day 8 of gestation resulted in delayed closure of the neural tube (71).
On gestation day 9 the lower torso of pregnant ICR mice were dipped for 20 seconds into 0.04, 0.4 and 4% solutions of DMSO in water (98). Litter size decreased significantly in the DMSO treated mice compared to the distilled water treated controls. Abnormalities in the embryos including hemorrhaging of the embryo from head to tail, open head folds were seen in rates of 60, 68, 87 and 4% in the foetuses from the treated mothers and the control group, respectively. The authors claim "that 20 seconds external application of 0.04% DMSO duplicates a blood concentration of 19 ppm found in whole embryo research". It is also stated that the data indicate a need to study the effects of even smaller concentrations of DMSO to determine a non-teratogenic concentration. No information is given about the health status of the pregnant animals. Consequently it is not possible to evaluate whether the dose-related increase in abnormal foetuses is a foetotoxic or specific teratogenic effect.
DMSO antagonized the teratogenic effects of secalonic acid D in mice by simultaneous intraperitoneal injection of 30 mg secalonic acid/ kg in buffer with 1.28 or 2.56 mol DMSO/l (10 or 20%) on day 11 of gestation (32). DMSO apparently affords protection against secalonic acid induced cleft palate at these concentrations, while 3.85 mol DMSO/l (30%) enhanced foetal resorption with no reduction in the incidence of cleft palate.
Swelling and blister formation were associated with abnormal development in 4 days old chicken embryos injected with 22 mg (0.02 ml) 50% DMSO in saline into the allantois (17). The potassium and sodium concentrations and the osmotic pressure in the fluids were measured leading to the conclusion that some of the DMSO induced abnormalities apparently are related to physiological disturbances

in the ion balances. In chicken embryos the injection of 0.001 ml of 90% DMSO in saline affected the skeletal development when injected in stage 17-20 of the 23 stages of the limb development (63). The effects were scored in 10 days old embryos. The authors conclude that DMSO appears to cause somitic damage which is expressed as scapular and vertebral effects.

Cajoulle et al. reported teratogenic effects in 72 and 96 hours old chicken embryos injected with concentrations close to LD_{50}: 10.3 mg/embryo in 72 hours old embryos and 12.2 mg/embryo in 96 hours old embryos. The typical malformations in embryos treated at 72 hours were in beak and eyes. In 96 hours old embryos limb malformations were also seen (20).

Cajoulle et al. concluded that DMSO is teratogenic in chicken embryos. In mammalian foetuses the teratogenic action of DMSO is only seen when relatively high and repeated doses are given and there is a certain homology (uniformity) in the qualitative nature of the teratological lesions caused by DMSO in all the tested experimental species (20).

Day 10.5 rat embryos were cultured for 2 days in rat serum containing 0.013, 0.064 or 0.32 mol DMSO/l (0.1, 0.5 or 2.5%). DMSO was embryotoxic and embryolethal in the concentration of 0.32 mol/l, but not in lower (59).

In conclusion, the reported teratogenicity studies do not distinguish between foetotoxic and specific teratogenic effects.

10. RELATION BETWEEN EXPOSURE, EFFECT AND RESPONSE

An extensive study of the human toxicology of DMSO was performed on prisoners receiving daily dermal applications of 1000 mg DMSO/kg with 80% DMSO gel for 14 days and for 90 days respectively (15). The entire dose was applied in multiple layers and rubbed into the skin until it was completely absorbed. The daily dose was administered in the evening after washing. Complete absorption required up to 2 hours.

Blood and urine samples were obtained from all of the 65 persons participating in the 14 days experiment. Ten of the 65 subjects receiving DMSO exhibited an eosinophilia. No such eosinophilia occurred in the control group consisting of 33 persons. Skin irritation was observed as blisters and erythema, drying and scaling. These effects were reversible as the irritated skin returned to normal within 3 weeks of treatment. Systemic side effects were recorded by percent incidence: Sedation (52%), headache (42%), nausea (32%), dizziness (18%) burning or aching eyes (9%), vomiting (6%).

The 90-day use of 80% DMSO gel was completed by 40 of 54 persons. Twelve persons stopped in the first few days because of skin reactions, two dropped out

at days 31 and 52 respectively for breath odour and personal reasons. Ophthalmological examinations were conducted weekly during the 90 days dosing period and monthly up to the 8 month of the study. Blood and urine samples were obtained from all subjects at 1, 2, 4, 6, 8 and 13 weeks for laboratory analysis of a battery of biochemical parameters. All subjects received a physical examination prior to the study and at the end of the study. In conclusion, as in the 14 days study, no significant abnormalities were observed in the large battery of analyses, with the exception of eosinophilia,. The skin reaction and breath odour were anticipated and did occur. The other effects were sedation, occasional insomnia, nausea, dizziness and diarrhea.

Fishman et al. (37) exposed 32 Sprague-Dawley rats to 200 mg DMSO/m^3 7 hours per day, 5 days per week in 6 weeks. The characteristic garlic like odour was detected in the breath of each of the rats after the first day of exposure and the hair began to appear slightly yellow after the first week. No biochemical or hematologic abnormalities were seen. Non specific inflammatory changes of the lungs and liver was reported in nearly all the animals, including controls. In groups of 8 rats exposed to DMSO aerosols with 2900 mg/m^3 for 24 hours, 2000 mg/m^3 for 40 hours and 1600 mg/m^3 for 4 hours, areas of pulmonary oedema was seen in some of the animals and focal and diffuse collections of clear pneumocytes within the lung alveoli. The concentrations of DMSO in the air in these short term exposures were exceeding the saturated air concentration of 1555 mg/m^3 at 20 °C, and consequently the effects of DMSO-aerosols were tested. Fishman et al. refer to a Japanese study from 1960 by Uranuma (107) with exposure of rabbits to mists of DMSO resulting in edematous changes in lung tissue. As the non specific inflammatory changes in the lungs observed by Fishman et al. were present in exposed as well as control animals these changes are not related to DMSO exposure. At high levels of exposure a significant 'wetting out' of DMSO on the animals was seen and a significant contribution to the total dose of DMSO may derive from skin absorption.

The relations between dose and effects from DMSO at different routes of exposure are summarized in table 2.

Table 2: Relations between exposure and effect of DMSO

Species	Dose	Duration	Effect	
Inhalation				
Rabbit	Unknown	Unknown	Oedemas in the lungs	(107)
Rat	200 mg/m^3	7 h/day, 5 days/weeks in 6 weeks	Garlic like breath, yellow fur	(37)
Human	Unknown	Unknown	Garlic like breath, dizziness, nausea	(23)
Oral administration				
Mouse	2500 mg/kg/day	6 weeks	Growth retardation, effects on liver and kidneys	(19)
Rat	400-14100 mg/kg/day	13 weeks	Increased mortality, decreased weight gain, hyperemia	(104)
Dog	3800 mg/kg/day	48 days	Changes in the eye lens	(102)
Dog	3300-9900 mg/kg/day	2 years	Increased diuresis, changes in the eye lens	(81)
Rabbit	3000-4000 mg/kg/day	2-34 weeks	Increased mortality due to dehydration, decreased ascorbic acid concentration of the anterior aqueous humour	(34)
Monkey	3300 and 9900 mg/kg/day	6 months	Changes in the refractive index of the eye	(6)
Monkey	8900 mg/kg/day	18 months	Lethal dose	(109)
Mouse	330 mg/kg/week	1 year (?)	Tumours, longer lifetime	(70)
Dermal application				
Rabbit	4400 mg/kg/day	90 days	Changes in the eye lens	(94)
Dog	3300-33000 mg/kg/week	26 weeks	Breath odour, erythema	(103)
Monkey	3300-33000 mg/kg/week	26 weeks	Breath odour, erythema	(103)
Pig	4950 mg/kg/day	90 days	Changes in the eye lens	(94)
Pig	1650 mg/kg/day	123 days	Changes in the eye lens	(94)
Human	1000 mg/kg/day	14 days	Skin irritation, eosinophilia	(15)
Human	1000 mg/kg/day	90 days	Skin irritation, sedation, headache, nausea	(15)
Intraperitoneal injection				
Rat	2000-4000 mg/kg/day	28 days	Increased mortality, decreased growth	(101)
Rat	1000 mg/kg/day	3 weeks	No changes in open field test	(66)

11. NEEDS FOR FURTHER RESEARCH

DMSO has a low vapor pressure and a low concentration in saturated air. Information about actual exposure levels in occupational situations is missing. Also studies on the uptake from the lungs and systemic effects by inhalation are missing. As the reported studies of teratogenic effects are old, a clearer discrimination between general toxic effects and effects on the reproduction is missing. Studies of genotoxicity and promoting characteristics of DMSO in sensitive test systems are recommended.

12. DISCUSSION AND EVALUATION

DMSO has been widely used as a vehicle to medicaments due to the promotion of penetration of other substances through the skin. DMSO has also been widely used in treatment of arthritis and certain inflammatory diseases. The route of administration is normally dermal when DMSO is used as a vehicle for pharmaceuticals. Consequently most of the reported toxicological investigations involve dermal application. This route of administration is relevant in relation to occupational health, as DMSO is used as a solvent in working processes where skin contact may not always be avoidable. As DMSO promotes the skin penetration of a variety of substances, a considerable risk of increased uptake of toxic substances is expected when these substances are dissolved in DMSO or DMSO solutions e.g. in toxicity studies regarding cancer and allergy. Groups at risk are persons employed at laboratories. Also personnel handling chemotherapeutics dissolved in DMSO are at risk of increased uptake of chemotherapeutics. DMSO is labelled 'skin permeable' in the German series of documentation of occupational exposure levels (62) and this labelling is also recommended here.

The effects of irritation of mucous membranes, effects on the central nervous system and eosinophilia by DMSO applied dermally or orally are believed to be present also after DMSO inhalation. However only one study on the toxicology of DMSO by inhalation has been reported in English (37). The rats were exposed to 200 mg DMSO/m^3 (60 ppm) for 6 weeks and inflammatory changes in the lungs were seen. Studies at lower exposure levels are recommended.

Two studies of human exposure have been reported. The staff at the intensive care unit at a hospital reported headaches, dizziness and nausea when exposed to DMSO given intravenously to a patient. Industrial hygiene monitoring or biological monitoring of DMSO were not performed. The symptoms were not observed when ventilation was used during DMSO treatment (23).

In a comprehensive study in an American prison with prisoners receiving daily

dermal doses of 1000 mg DMSO/kg for 14 or 90 days eosinophilia, effects on the central nervous system such as dizziness, nausea and skin reactions were reported (15).

DMSO has a low vapour pressure (0.0493 kPa at 20 C) and a low concentration in saturated air. The uptake by inhalation is consequently considered low while exposure by skin contact is most probably due to the extreme skin permeability.

The critical effects of DMSO are irritation and effects on the central nervous system such as nausea and dizziness and other symptoms. Due to the biological activity of DMSO towards membrane stability and integrity of the chromosomes, DMSO in high doses may contribute to long term effects such as cancer and reproductive effects. These effects were however, observed at DMSO levels much higher than the exposures in the occupational environment. Well conducted and published, toxicity studies of mammalian genotoxicity, teratogenicity and carcinogenicity are requested for the toxicity evaluation of DMSO with respect to these effects.

The observation of longer life-time in DMSO treated animals compared with control animals seen in the only published long term study is important (70). A comparable observation, i.e. a longer life-time, has been reported in rats receiving the antioxidant butylated hydroxytoluene (BHT) (83). A significant increase in tumours was only seen in the old surviving animals receiving BHT. BHT and DMSO are both 'free radical scavengers'.

DMSO is metabolized to the volatile metabolite dimethylsulfide (DMS) with a very characteristic sulfur smell or garlic like smell as reported by persons exposed to DMSO. DMS is by far more toxic compared with DMSO. The LD_{50} in rats administered DMS orally was in the range of 535-3700 mg/kg (85) compared with LD_{50} of DMSO in the range of 17400-28300 mg/kg (72). DMS is irritative to the skin and eyes and acts as a 'free radical scavenger'. No reports were found on the teratogenicity and carcinogenicity of DMS.

13. SUMMARY

Lisbeth E. Knudsen. Dimethylsulfoxide. Nordic Expert Group for Documentation of Occupational Exposure Limits. Arbete och Hälsa 1991

A critical survey and evaluation of the relevant literature to be used as a basis for establishing an occupational exposure limit for dimethylsulfoxide (DMSO), is presented.

DMSO has a very low acute toxicity and chronic effects are only seen at high exposure levels. The critical toxicological effects are irritation (skin and mucous membranes), CNS effects (dizziness and nausea) and disturbance of normal cell-function, contributing to development of cancer and teratogenic effects. The metabolite dimethylsulfide has an unpleasant garlic sweet odour. DMSO has a low vapor pressure (0.0493 kPa at 20°C). Occupational exposure is most likely by skin-contact and DMSO also greatly enhances the permeability of skin to other substances. Special precautions must be taken to prevent skin-contact.

Further studies on genotoxic, teratogenic and carcinogenic effects are needed for evaluation of the hazards of DMSO associated with these endpoints.

Keywords: Dimethylsulfoxide, DMSO, skin penetration, genotoxicology, teratogenicity, carcinogenicity, irritation, CNS, occupational exposure limit, documentation.

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Appendix 1.

Occupational exposure limits for dimethylsulfoxide

Country	mg/m^3	ppm	Year	Ref.
Denmark	-	100[A]	1988	(1)
Finland	-		1987	(2)
Iceland	-		1978	(3)
Netherlands	-		1989	(4)
Norway	-		1989	(5)
Sweden	-		1990	(6)
USA (ACGIH)	-		1990-91	(7)
(NIOSH)	-		1990-91	(8)

A = Recommended exposure limit

References to Appendix 1

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INORGANIC ARSENIC

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CONTENTS

1. PHYSICAL AND CHEMICAL DATA

Formula	As
CAS–number	7440–38–2
Atomic number	33
Atomic weight	74,92
Melting point	812°C (28 atm.)
Sublimation	613°C
Density	5,72 g/cm^3
Conversion factors	1 μg/l = 0,013 μmol/l
	1 μmol/l = 75 μg/l

Arsenic (As) is a metalloid belonging to group $\underline{V}$a (N, P, As, Sb, Bi) of the periodic table. The main oxidation states are –3, 0, +3 and +5. There are many different arsenic compounds present in the environment, some of the more common ones are shown in table 1. It should be noted that the physical, chemical and toxicological properties may vary considerably between the various arsenic compounds. In general, inorganic arsenic compounds are more toxic than organic, and compounds containing trivalent arsenic more toxic than pentavalent (165).

Certain microorganisms are able to methylate trivalent arsenic (As(III)) to methyl arsonic acid (MMA), dimethyl arsinic acid (DMA, cacodylic acid) and trimethyl arsine oxide (TMAO), via the corresponding arsines (117). Arsenate may be absorbed by algae and converted to organic arsenic compounds, such as arsenic–containing ribofuranosides (40, 192). These are directed into the food web of marine animals and transformed into arsenocholine and arsenobetaine. Especially arsenobetaine may occur in very high concentrations, 1 mmol/kg (75 mg As/kg) or more, in certain fish and shellfish.

Arsenic was known as a therapeutic agent as early as 400 B.C. From the 19th century onwards, Fowler's solution, which contains potassium arsenite, has been used for the treatment of leukemia, psoriasis, chronic bronchial asthma, and as a tonic, often in daily doses as high as 3 mg As (191). In most countries Fowler's solution is no longer in use. However, various homeopathic medicines have been found to contain arsenic.

Table 1. Some commonly occurring arsenic compounds

Arsenous oxide (arsenic trioxide)	As_2O_3, As_4O_6 below 800° C
Arsenic pentoxide	As_2O_5
Arsenite	AsO_3^{3-}, AsO_2^-
Arsenate	AsO_4^{3-}, $HAsO_4^{2-}$, $H_2AsO_4^-$
Arsenic trisulphide	As_2S_3
Gallium arsenide	GaAs
Methyl arsonic acid	$CH_3AsO(OH)_2$
Dimethyl arsinic acid	$(CH_3)_2AsO(OH)$
Arsenobetaine	$(CH_3)_3As^+CH_2COO^-$
Arsenocholine	$(CH_3)_3As^+CH_2CH_2OH\ X^-$
Arsine	AsH_3
Trimethyl arsine	$(CH_3)_3As$
Trimethyl arsine oxide	$(CH_3)_3As{=}O$

The most common arsenic compounds in the working environment are arsenous oxide, arsenic pentoxide, arsenic acid and its salts, gallium arsenide, arsenic trisulphide and elemental arsenic. Arsenous oxide is formed by heating arsenic or arsenic compounds in the presence of air. The solubility in water is 0.2 mol/l (28 g As/l) at 20 °C, somewhat higher in warm water, HCl and alkali. A water solution of arsenic trioxide contains the weak acid arsenous acid (H_3AsO_3).

Arsenic trisulphide is less soluble than arsenous oxide, only about 2 μmol/l (0.3 mg As/l) in water. The solubility decreases with decreasing pH. Gallium arsenide is formed by passing arsenic vapor and hydrogen over gallium(III)oxide at 600 °C. The solubility in 0.1 M phosphate buffer, pH 7.4, is 1.3–2.6 mmol/l (100–200 mg As/l) at 37 °C, considerably less in distilled water and HCl–KCl buffer, pH 2.0 (189, 198).

2. OCCURRENCE AND USE

2.1 Use

Arsenopyrite (FeAsS), realgar (As_4S_4) and orpiment (As_4S_6) are the most common arsenic-containing minerals. Arsenous oxide is obtained as a by-product in the smelting of copper, lead, zinc and gold ores. During the smelting process arsenous oxide is vaporized, collected on electrostatic filters and purified. A large number of arsenic compounds are produced from arsenous oxide. The major current use of arsenic is in insecticides, e.g. lead arsenate, calcium arsenate, and sodium arsenite; herbicides, e.g. arsenic acid, monosodium arsenate and cacodylic acid (dimethyl arsinic acid); cotton desiccants, e.g. arsenic acid; and wood preservatives, e.g. copper and chromium arsenate (so called CCA products) (195). Arsenic containing wood preservatives are very common in the Nordic countries. Most of the about 200 wood preservations plants in Sweden use water soluble preservatives, mainly CCA-products (166). Since long arsenic has also been used to treat animal skins and bird feathers in taxidermy (36).

Arsenic is also used as a bronzing or decolorizing agent in the manufacture of glass, in the fabrication of opal glass and enamels (7) and in lead-acid batteries (34). In some countries substituted phenyl arsonic acids are used in the livestock industry as growth-promoting agents for swine and poultry. Elemental arsenic is produced by reduction of arsenic trioxide and is used mainly in alloys to increase hardness and heat resistance.

Recently, arsenic has become an important element in the electronic industry, e.g. to "dope" silicon semiconductors and to form important semiconductor compounds with group III and group IV metals, especially gallium and indium (154). Gallium arsenide is used in e.g. microwave devices, solar cells, lasers and light emitting diodes (194). According to Harrison (60) there is a potential risk of exposure to arsenic in several of the operations.

2.2 Occupational air levels

The concentration of arsenic in outdoor air may range from less than 0.1 ng/m^3 to a few ng/m^3 (61, 191). Much higher levels may occur near point sources. In the vicinity of a Swedish smelter, annual average air levels of 0.06-0.12 μg/m^3 were recorded in the mid 1980-ties (19).

In non-ferrous smelters and industries producing arsenic containing pesticides, the concentrations of arsenic in the working room air have decreased considerably during recent years. In the 1940-ies and 1950-ies the concentrations often exceeded 1 mg/m^3 (106, 140, 142). In the mid 1980-ies, measurements of airborne arsenic (8-h averages) using personal monitors in a Swedish smelter (18 subjects, 2-3 days each) showed 1-194 μg As/m^3 (186). In general, the air levels were higher for workers in the arsenic refinery plant (average 27 μg/m^3) than for workers in the arsenic metal plant (average 8 μg/m^3). Cant and Lederle (29) reported average air levels of up to 5300 μg/m^3 in the breathing zone air in various workplaces in a copper smelter in Western USA. About 70% of the measurements exceeded the occupational exposure limit of 10 μg/m^3.

The Swedish Occupational Health Board has carried out measurements of airborne arsenic (personal monitors) at various work places within a wood preservation plant using CCA products (155). During two days the air levels varied from <1 to 20 μg/m^3 for workers engaged in the preservation. Stationary monitors placed near the door of the cylinder gave up to 2 mg As/m^3 at the end of the operation. Measurements of airborne arsenic using personal monitors at three different plants, which carried out wood preservation using CCA products, showed concentrations between 2 and 6 μg As/m^3 (5 measurements, sampling periods of 1.5-5 hours) for the workers carrying out the preservation (3). Stationary monitors placed just above the cylinder openings and near the chemical mixing showed <1 to 8 μg As/m^3. Lindroos (103) has studied the exposure to arsenic among workers in an industry producing wooden houses in Finland. Air concentrations of arsenic were in general below the occupational exposure limit, 0.1 mg/m^3, but in one work place levels up to 0.23 mg/m^3 were recorded.

Investigations of arsenic and lead in respirable dust in 30 work places at three different glass factories in Sweden showed only minor amounts of arsenic, corresponding to <6 μg/m^3, on some of the filters used (2). At the biggest of three factories producing crystal glass in Norway the arsenic levels in air varied between 3 μg/m^3 in June to 48 μg/m^3 in February (51). Foà and coworkers (49) found 5-619 μg/m^3 among glass mixers and 0.6-3 μg/m^3 among glass blowers and casters in an Italian glass factory.

The exposure situation in the electronic industry is not well described. Thirteen samples from two plants producing gallium arsenide in USA showed short time exposure levels of 2.5 μg/m^3

during beadblasting and 289 $\mu g/m^3$ during vacuum servicing (60).

2.3 Methods for measuring airborne arsenic

The major part of airborne arsenic consists of inorganic As(III) and As(V) oxides, often associated with particles. Usually, sampling is carried out using traditional air samplers with cellulose acetate or glass fiber filters (6, 34, 47, 123). In work places with elevated air temperatures, these methods may underestimate the air concentrations. The equilibrium vapor pressure for arsenous oxide is about 0.5 $\mu m/m^3$ at 25 °C, and the As_4O_6 vapor passes the conventional filters (34, 91). More efficient sampling may be obtained with filters treated with Na_2CO_3 glycerol (107, 125) or tetra-n-butylammonium hydroxide (5).

Nordic interlaboratory comparison of metals, including arsenic, in dust has been conducted by the Occupational Health Institute in Norway (48). Standard reference samples for arsenic in air filters, outdoor dust and fly ash are commercially available.

3 KINETICS

3.1 Absorption

After inhalation of soluble arsenic compounds, e.g. arsenous oxide, most of the deposited arsenic is rapidly absorbed from either the respiratory or the gastrointestinal tract following clearance from the respiratory tract (111, 145). However, particles of low solubility, e.g. arsenic trisulphide, calcium arsenate, lead arsenate, and gallium arsenide are retained to a great extent in the lungs (111, 145, 156, 189). Dissolution of certain arsenic-containing particles, e.g. lead arsenate, by alveolar macrophages may increase the rate of absorption in the lungs (114).

Brune et al. (23) found that the concentration of arsenic in the lungs of deceased smelter workers who had been retired for 2 – 19 years was about as high as that in deceased workers who had been retired for 0–1.5 years only. This indicates that part of the inhaled arsenic was firmly bound in the lungs and/or that exposure to insoluble arsenic compounds had occurred in the smelter. Leffler et al. (98) reported that arsenic containing dust from a smelter had longer

retention time in the hamster lung than arsenic trioxide.

When ingested in dissolved form, inorganic arsenic compounds are readily absorbed, about 80–90% of a single dose, in the gastrointestinal tract (152). A much lower degree of absorption has been reported for powdered arsenic selenide (110), and particles of arsenic trisulphide, lead arsenate (111) and gallium arsenide (189, 198). There is a lack of information about dermal absorption of arsenic compounds (191).

3.2 Biotransformation

Inorganic arsenic is methylated in the body to methyl arsonic acid (MMA) and dimethyl arsinic acid (DMA) (for review see 183). The methylation takes place mainly in the liver and may be considered a detoxification mechanism for inorganic arsenic, since the methylated metabolites have a lower affinity for tissue constituents and lower toxicity than inorganic arsenic, especially the trivalent form (26, 180). Pentavalent arsenic (arsenate) is rapidly reduced (50–70%) in the blood to trivalent, part of which is methylated in the liver (113, 181).

The methylation efficiency decreases with increasing dose level and with low protein intake (182).

In cases of acute arsenic intoxication it may take a couple of days before methylated arsenic may be detected in the urine (104). In human volunteers (one person per dose), ingesting arsenite in daily doses of 125, 250, 500 or 1000 μg As in the form of arsenite for five days, the urinary excretion of DMA levelled off at the highest dose level (25). However, Swedish smelter workers with 16–328 μg As/g creatinine in the urine had about the same methylation efficiency as the general population, i.e. 10–20% inorganic arsenic, 10–15% MMA and 60–80% DMA (177).

3.3 Tissue distribution

Trivalent arsenic is bound mainly to SH-groups. In human subjects exposed to normal environmental levels of arsenic, hair and nails show the highest tissue concentrations of arsenic (0.3–40 μmol/kg or 0.02–3 mg As/kg dry weight), while fairly high concentrations are found in skin and lungs (0.1–13 μmol/kg or 0.01–1 mg As/kg dry weight) (36, 100). Experimental studies on

mammals exposed to inorganic arsenic have shown that the tissues with the longest retention of arsenic are skin, hair, squamous epithelium of the upper gastrointestinal tract (oral cavity, tongue, oesophagus and stomach wall), epididymis, thyroid, skeleton and lens (101, 184). The significance of the retention in the epididymis is not known. All the tissues mentioned, except the skeleton, contained higher concentrations following administration of arsenite than after administration of arsenate. The half-time in skin seems to be longer than one month (39).

There are very few data on the accumulation of arsenic after prolonged exposure. Data from experiments on animals indicate that continuous exposure to arsenic via drinking water or inhalation cause an increase in tissue levels for a couple of weeks, after which they may decrease in spite of ongoing exposure (14). It has also been shown that the concentrations of arsenic in liver and kidney from deceased smelter workers were only slightly higher than those in non-occupationally exposed subjects (22).

Inorganic arsenic, trivalent as well as pentavalent, and the methylated metabolites, pass the placenta during the entire pregnancy (58, 69). The concentration of arsenic in cord blood was found to be about the same (average 0.03–0.04 μmol/l, 2–3 μg/l) as that in the mother's blood (81). Human breast milk normally contains 3–80 nmol/kg (0.2–6 μg As/kg) (38, 53). The chemical form of arsenic in breast milk is not known.

Dimercapto compounds, especially such with adjacent SH groups, form stable complexes with arsenic and may be used to increase the urinary excretion. Animal experiments have shown that 2,3-dimercapto-1-propanol (BAL), which traditionally has been used in the treatment of arsenic poisoning, increases the uptake of arsenic in the brain (4). Other dithiols, such as dimercapto succinic acid (DMSA) and 2,3-dimercapto-1-propanesulphonic acid (DMPS) did not increase the uptake of arsenic in the brain. Both compounds were more efficient than BAL in the treatment of arsenic poisoning, had a lower toxicity and were, in contrast to BAL, efficient following peroral administration.

3.4 Elimination

The major route of excretion of most arsenic compounds is via the kidneys. However, the rate of excretion varies with the chemical form of arsenic. In six human subjects who ingested ^{74}As-

arsenate (0.01 μg As(V)/person), 38% of the dose was excreted in the urine within 48 hours and 58% within 5 days (169). The excretion followed a three component exponential function with 66% being eliminated with a half-time of 2.1 days, 30% with a half-time of 9.5 days and 4% with a half-time of 38.4 days. In three subjects, each of whom ingested 500 μg arsenic in the form of arsenite (As(III)), about 33% of the dose was excreted in the urine within 48 hours and 45% within 4 days (26). Following ingestion of the same amount of arsenic in the form of MMA or DMA, about 75% of the dose was excreted in the urine within 4 days.

Organic arsenic compounds present in fish and crustacea are rapidly excreted in the urine, about 70% of the ingested arsenic within 3 days (170, 185). The biological half-time is shorter than for inorganic arsenic.

3.5 Biological indicators of exposure

Due to the fast elimination the concentration of arsenic in blood can seldom be used for evaluation of the exposure to inorganic arsenic (178). Furthermore, there is no simple method for the speciation of arsenic compounds in blood. Such a speciation is of particular importance since intake of seafood may greatly increase the blood arsenic level. People living in coastal areas of Norway, where the consumption of fish is high, have considerably higher concentrations of arsenic in serum (0.09–0.24 μmol/l or 7–18 μg/l As/l) than people in the inland (0.01–0.04 μmol/l or 0.8–3.1 μg/l As/l) where there is a lower consumption of fish (16).

Inorganic arsenic, but not organic arsenic compounds from seafood, is accumulated in hair. Therefore, the concentration of arsenic in hair may be used as an indicator of exposure to inorganic arsenic. The concentration of arsenic in hair in subjects without known exposure to arsenic is normally less than 5 μmol/kg (0.4 mg As/kg) (9, 36, 61), while in subjects exposed to arsenic occupationally, via medicines or drinking water, the hair concentrations may be several mmol/kg (several hundred mg As/kg) (36). It should be noted that arsenic in hair may originate from external contamination from e.g. dust and hair dyes, and that there may be quite a large variation in the concentration of arsenic over the head of the same person (33).

Due to the short biological half-time of the major part of the absorbed arsenic, arsenic in urine will reflect the exposure (uptake) during the previous days. On a group basis, total urinary

arsenic may give an estimate of exposure to inorganic arsenic, provided that intake of seafood can be excluded. However, since one single meal of seafood can give rise to more than 10 μmol/l (more than 1000 μg As/l), and the intake of fish products cannot always be avoided, total urinary arsenic may overestimate the occupational exposure to inorganic arsenic (133).

Methods for the determination of metabolites of inorganic arsenic, without interference from the seafood arsenic compounds, have recently been developed (24, 35, 133). Treatment of acidified urine with sodium borohydride will produce arsines of inorganic arsenic, MMA and DMA, which may be quantified using e.g. atomic absorption spectrophotometry. The various urinary metabolites may be separated by gas chromatography (50, 136, 167), ion exchange chromatography (119, 168), HPLC (30) or temperature selective separation of arsines formed by treatment of the samples with sodium borohydride (21, 24).

The concentration of metabolites of inorganic arsenic in the urine of people not occupationally exposed to arsenic in Sweden and Finland is about 0.1 μmol/g creatinine (5–10 μg As/g creatinine) on average (179, 187). Intake of seafood may increase the concentration somewhat, probably due to the presence of small amounts of inorganic arsenic, DMA or trimethyl arsine oxide in seafood (177, 192).

The relationship between airborne concentrations of arsenic and urinary excretion of inorganic arsenic metabolites among smelter workers exposed to inorganic arsenic, mainly in the form of arsenous oxide, has been studied (186). On a group basis, there was a good correlation between the concentrations of metabolites of inorganic arsenic in urine and the concentrations of airborne arsenic measured by using personal monitors ($y = 2.0x + 29$ μg As/g creatinine; coefficient of correlation 0.92; $p<0.001$). According to those data, a concentration of 30 μg As/m^3 would correspond to 89 μg/g creatinine in urine (1.2 μmol/g creatinine; 138 μg/l at a specific gravity of 1.019). The results indicated a large variation in the exposure (uptake of arsenic) at the same air level. For example, the urinary concentrations of arsenic metabolites in workers with 10 μg As/m^3 in the breathing zone air could range from 0.5 to 2.6 μmol/g creatinine (40 to 200 μg As/g creatinine). The high urinary levels indicated peroral exposure from e.g. contaminated hands, cigarettes and snuff.

The relationship between airborne arsenic and urinary excretion of inorganic arsenic metabolites

discussed above, relates to exposure to arsenous oxide. Corresponding data for exposure to arsenic compounds of lower solubility have not been reported.

Interlaboratory comparison of total urinary arsenic has been conducted by the Centre de Toxicologie du Quebec, Canada (162, 190) and within the CEC countries (200). Standard reference samples for total arsenic in urine are available from IAEA, NIST and Nycomed, Oslo, but there is a need for reference samples for the arsenic metabolites in urine. In the above mentioned study on arsenic exposure in smelter workers, human urine spiked with As_2O_5, MMA and DMA (20:20:60) at three different levels were used for analytical quality control purposes (186).

4. GENERAL TOXICITY

4.1 Mechanisms of toxicity

At physiological pH inorganic trivalent arsenic is in the form of undissociated arsenous acid, and pentavalent arsenic in the form of $H_2AsO_4^-$ and $HAsO_4^{2-}$. This may influence the uptake through cellular membranes. Experimental studies have shown that As(III), but not As(V), is easily taken up by the liver (99).

As(III) is highly reactive and binds to various tissue components, mainly SH groups (180). This is the most important mechanism by which trivalent arsenic inhibits the activity of many enzymes in for example the mitochondrial oxidative phosphorylation (165). In many cases the enzymatic activity can be restored by excess glutathione. However, complexes with adjacent SH groups are very stable and dithiol containing enzymes, e.g. lipoic acid, are particularly sensitive to arsenic and not reactivated by simple monothiols. Therefore, dithiols such as 2,3-dimercapto propanol are used in the treatment of arsenic poisoning (4).

As(V) is less reactive with tissue constituents, but the arsenate ions can substitute phosphate ions in various enzyme catalyzed reactions (165). One effect based on such a reaction is the arsenate induced uncoupling of the mitochondrial succinate respiration. As(V) has been shown to accumulate in the mitochondria, especially in the kidney (112). Due to the reduction of As(V) to

As(III) (183), exposure to As(V) will give rise to the same toxic effects as As(III), although to somewhat lesser extent.

4.2 Factors influencing the toxicity

Since the methylation of inorganic arsenic in the body functions as a detoxification process, inhibition of the methylation by, e.g. excess As(III), Hg^{2+} (27) and low intake of protein (182), is likely to increase the toxicity. However, whether methylation of inhaled arsenic takes place in the lungs and protects against lung cancer, is not known. Probably there is also a large variation in the methylation between individuals.

In some experimental systems selenium has been found to protect against toxic effects of arsenic (191), but the mechanism is unclear.

4.3 Acute toxicity

The acute toxicity of arsenic is to a great extent dependent on the physical and chemical form. In general, trivalent inorganic arsenic is more toxic than pentavalent. LD_{50} for arsenite in mice is about 0.13 mmol/kg (10 mg As/kg) body weight, while that for arsenate is about 0.5 mmol/kg (35 mg As/kg) body weight (178). Human subjects are more sensitive to arsenic than are most experimental animals, due to a lower methylation efficiency (183). The lethal dose of arsenic trioxide in an adult person is about 1–2 mmol (75–150 mg As), corresponding to 0.01–0.03 mmol/kg or 1–2 mg As/kg body weight (191).

5. EFFECTS ON CERTAIN ORGANS AND TISSUES

5.1 Skin and mucous membranes

Inorganic arsenic compounds can induce acute dermatitis. Sensitization has been observed among smelter workers exposed mainly to arsenic trioxide (66). Increased skin pigmentation and palmoplantar hyperkeratosis can occur after occupational exposure to airborne inorganic arsenic (56, 142), as well as following intake via food, drinking water or drugs (46, 57, 174). The

increased pigmentation may develop already within a few months, while hyperkeratosis normally does not occur until after a few years, which indicates that a total dose of about 0.5 – 1 g of arsenic is required.

5.2 Respiratory tract

Effects in the respiratory tract among smelter workers have been described in many reports (15, 65, 73, 106, 150). Smelter workers exposed to airborne arsenic concentrations, which normally did not exceed 0.5 mg/m^3 (occasional levels up to 7 mg/m^3), experienced symptoms resulting from inflammatory reactions in the nose and throat as well as perforation of the nasal septum (106). Bronchitis and decreased pulmonary function also developed. It should be noted that the smelter workers were not only exposed to arsenic; high exposures to sulphur dioxide may also have played a role.

5.3 Liver

Occupational exposure to arsenic among smelter workers and vineyard workers has been associated with an increased mortality due to liver cirrhosis (8, 85, 92). This has not been observed in other occupations where high arsenic exposure has occurred, such as among workers producing arsenical pesticides. Alcohol consumption was not controlled in any of the investigations. The vineyard workers probably had an excessive alcohol consumption (85).

There are a few case reports of portal hypertension without liver cirrhosis after exposure to trivalent inorganic arsenic in drugs (often as Fowler's solution) in doses of several milligrams daily (70, 84, 120). Similar effects have been reported following intake of arsenic in drinking water (55).

5.4 Kidney

A transient effect on the kidney manifested by red and white blood cells in urine has been noted among Japanese infants who were poisoned unintentionally by pentavalent inorganic arsenic in dry milk (57). During one month about 3.5 mg of arsenic was ingested daily and 130 children died out of a total of about 12000 exposed. Kidney effects have not been reported among arsenic

exposed workers, except secondary to hemolysis following arsine poisoning.

5.5 Gastrointestinal tract

Acute poisoning following ingestion of inorganic arsenic compounds in doses of several tens of milligrams of arsenic is initially characterized by symptoms from the gastrointestinal tract, including colic, vomiting and diarrhoea (52). Severe poisoning may result in shock due to dehydration.

5.6 Cardiovascular system

A study of German vineyard workers, exposed to arsenic containing pesticides about 30 years earlier, described signs of peripheral vascular effects such as endangitis obliterans with skin atrophy in over 60% of the subjects (54). Swedish smelter workers exposed to inorganic arsenic have shown an increased vasospastic reactivity indicated by low finger blood pressure following cooling and an increased prevalence of Raynaud's phenomenon (88). The vasospastic tendency was unchanged after 4 to 8 weeks break in the exposure, but appeared to decrease after several years of lower exposure (89). The arsenic concentration in air was estimated to about 0.5 mg/m^3 before 1975 and about 0.05 mg/m^3 from then on.

Peripheral vascular disease, in some cases leading to gangrene of toes and feet ("black foot disease"), has been reported from Taiwan among persons drinking water containing about 0.1 – 1 mg/l of arsenic (175). In Chile arsenic containing drinking water (about 0.8 mg/l) was consumed during a considerably shorter period than in Taiwan and gave rise to Raynaud's phenomenon, primarily in children (20). A poor nutritional status in parts of the populations in Chile and Taiwan may have contributed to the observed effects.

An increased mortality from cardiovascular disease has been reported in some epidemiologic studies among smelter workers exposed to high levels of airborne arsenic (8, 95), but the results are not consistent (80, 151). It should be noted that even if an increased risk of cardiovascular disease in occupational cohorts has not been observed in comparison with the general population, there could be a real risk increase which is masked by the "healthy worker" effect.

5.7 Hematopoietic system

Inorganic arsenic can affect the hematopoietic system following both short and long term exposure. In subjects who had ingested arsenic in contaminated drinking water (0.03 – 3 mg As/l) reversible anaemia and granulocytopenia has been observed (172, 176). The bone marrow in subjects poisoned with arsenic has shown a disturbed development of red blood cells and megaloblastic changes (87, 153).

5.8 Central nervous system

Effects in the central nervous system have been reported in investigations of Japanese children who had been poisoned by arsenic contaminated dry milk 15 years earlier (cf. section 5.4). Severe hearing loss and EEG changes were reported (137, 197). Hearing loss has also been associated with exposure to arsenic among children near a coal fired plant in Czechoslovakia using coal with a high content of arsenic (13). Such effects were not observed in another study of arsenic exposed children living near a smelter in the US (118).

5.9 Peripheral nervous system

Effects in the peripheral nervous system have been observed among workers exposed to inorganic arsenic, for example using arsenic containing pesticides and in coal desulphurization (59, 62). The workers displayed symptoms from both sensoric and motoric nerves. In a study of arsenic exposed smelter workers a correlation was observed between urinary arsenic excretion and subclinical/clinical neuropathy (43). The average urinary arsenic concentration in the most heavily exposed group was 0.38 mg/l. In Swedish smelter workers exposed to arsenic no increased prevalence of neurologic symptoms was observed (17). There was, however, an inverse relationship between cumulative arsenic exposure and nerve conduction velocity. In the arsenic exposed group there was an average urinary arsenic concentration of 0.07 mg/l. A follow up 5 years later indicated increased differences in nerve conduction velocity between exposed and controls, in spite of a reduction in the arsenic exposure (90).

Intake of arsenic in drinking water has also been associated with disturbances in the function of peripheral nerves (64). In the population of an area in Canada with arsenic levels in drinking

water of 0.05 – 1.4 mg/l there was a higher frequency of pathological EMGs than in a group with lower arsenic levels. No neurophysiological effects associated with arsenic intake in drinking water were observed in a study from Alaska, with an average arsenic concentration of 0.35 mg/l, and the highest levels up to 5 mg/l (86).

6. IMMUNOTOXICITY AND ALLERGY

Exposure to inorganic arsenic compounds has resulted in skin sensitization in smelter workers (66). Trivalent arsenic compounds, such as arsenic trioxide, appeared more potent than pentavalent. There are no data on allergic effects in the respiratory system among arsenic exposed workers.

7. MUTAGENICITY AND GENOTOXICITY

Most studies on mutagenicity of inorganic trivalent and pentavalent arsenic compounds have given negative results, such as in bacterial and mammalian cell systems (108, 115, 158), although the results are not entirely consistent (83, 126, 135). Many experimental studies show that both trivalent and pentavalent inorganic arsenic compounds can induce different types of chromosomal damage (1, 37, 129, 149, 188).

An increased frequency of chromosomal aberrations in peripheral lymphocytes has been observed among arsenic exposed smelter workers (127, 130), however, the correlation to estimated individual arsenic exposure was low. An increased frequency of chromosomal aberrations has also been observed among vineyard workers and patients treated with inorganic trivalent arsenic (128, 149). One study showed an increased frequency of sister chromatid exchange in arsenic exposed psoriasis patients (28), which could not be confirmed in another similar study (128).

Several studies indicate that inorganic arsenic can affect DNA-repair. This could result in a cocarcinogenic effect. Tri- and pentavalent inorganic arsenic compounds have been shown to inhibit DNA-repair activity following UV-irradiation in human and mammalian cells as well as

in bacteria (78, 138, 157, 159). In different experimental systems inorganic arsenic compounds have induced mutations and chromosomal damage in combination with other agents, such as UV-irradiation and different alkylating agents (93, 94, 171). In other studies inorganic arsenic have shown an antagonistic interaction in combination with genotoxic agents (12, 134, 141).

8. CARCINOGENICITY

8.1 Human studies

A large number of epidemiologic investigations have shown that occupational exposure to inorganic arsenic increases the risk of lung cancer. The most extensive evidence comes from workers in copper smelters exposed primarily to trivalent inorganic arsenic in the form of arsenic trioxide. The studies come from Japan (173), Sweden (80) and the US (41, 96). Positive exposure-response relationships were generally observed using different kinds of exposure measures. A description of quantitative exposure-response relationships is given in chapter 10.

Increased lung cancer risks have also been observed following exposure to inorganic arsenic in other occupational environments, for example in the manufacture and use of arsenic containing pesticides (109, 140, 160). An increased lung cancer risk has also been reported in populations living near point sources for arsenic emissions, primarily copper smelters (18, 144) and pesticide manufacturing industries (116). It is difficult to assess the role of inorganic arsenic exposure for these findings.

A few studies have investigated interactions between smoking and occupational exposure to arsenic in relation to lung cancer. A multiplicative interaction has been reported (143), but also less pronounced interaction (151). A recent study indicates that the interaction lies between an additive and a multiplicative model and that it is less pronounced among heavy smokers (79). Among smokers the histological picture seems to be similar in arsenic exposed smelter workers and controls with lung cancer (148). Nonsmoking smelter workers showed an excess of squamous and small carcinoma in comparison with nonsmoking lung cancer controls.

Many studies demonstrate that ingestion of arsenic increases the risk of skin cancer, primarily

via intake of drinking water (valency state unknown) or drugs (mainly trivalent arsenic). The total doses correspond to several grams of arsenic. Skin tumours caused by arsenic can be of different types – Bowen's disease, invasive squamous cell carcinoma and basal cell carcinoma (163). Some hyperkeratotic lesions and benign tumors (Bowen's disease) may develop to invasive tumours. The tumours are often multiple and localized to the trunk (46, 199). It should be noted that skin cancer has been reported only in one study of workers occupationally exposed to inorganic arsenic although high urinary arsenic excretion is often found, indicating a substantial absorption (63).

A few studies suggest that inorganic arsenic may also induce cancer of the liver, stomach, urinary tract and hematopoietic system, but it is not possible to draw any definite conclusions regarding causality (31, 32, 191).

The International Agency for Research on Cancer has classified inorganic arsenic as a group 1 carcinogen for cancer of the lung and skin (71, 72).

8.2 Animal studies

Experimental studies on the carcinogenicity of inorganic arsenic have produced somewhat contradictory results (143). Following peroral administration most studies have given negative results, both for tests of initiation and promotion. In one study there was, however, an increased incidence of kidney tumors initiated by diethylnitrosamine in male rats exposed to sodium arsenite in drinking water (164).

Exposure to different types of inorganic arsenic compounds via intratracheal installation has resulted in lung tumors in hamsters and rats (74–77, 146, 147, 196). The excess risk was not substantial, except in one study where calcium arsenate was given in combination with calcium hydroxide and copper sulphate. Lung tumors have also been induced in mice following perinatal administration of arsenic trioxide (161).

9. REPRODUCTION EFFECTS

Studies in mice exposed to tri- or pentavalent inorganic arsenic once during day 7 to 18 of pregnancy indicate that both forms of arsenic easily pass to the fetus (102). Following exposure during early pregnancy arsenic primarily accumulated in neuroepithelial tissue; exposure during later pregnancy resulted in a similar distribution as in the mother. It thus appears plausible that both unmethylated arsenic and methylated metabolites (68) pass to the fetus. The fetus of mice exposed to 7 or 14 mg As(V)/kg at day 18 had about 75% of the arsenic in the form of DMA (69).

There are several studies showing malformations and embryotoxicity following relatively high single doses (about 67–160 μmol or 5–12 mg As/kg body weight) of As(V) to rats and mice during organogenesis (11, 67, 121). As(III) has been reported to give similar teratogenic and embryotoxic effects as As(V), but at lower doses (10). Inhalation of arsenous oxide at a concentration of nearly 30 mg/m^3 has produced malformations in mice (122).

Ferm et al. (45) did not find any toxic effect on the fetus following administration of 27 μmol (2 mg) As(V)/kg body weight as a single dose to hamsters during day 8 of pregnancy, while 40 μmol (3 mg)/kg produced an increased incidence of resorption and malformations, primarily CNS effects. Ferm and Hanlon (44) used a subcutaneous osmotic minipump in hamsters to get a continuous exposure to sodium arsenate during day 4 to 7 of pregnancy. At day 13 (normal length of pregnancy 16 days) there was an increased frequency of resorption and malformations (primarily in the central nervous system and skeleton) at the lowest dose (70 μmol/kg/24 h) during organogenesis.

It is difficult to assess the teratogenicity in man of inorganic arsenic from the published animal data. Humans are probably more sensitive as the rodents used have a more effective methylation of inorganic arsenic (183), and DMA has a lower teratogenic potential than inorganic arsenic (68). There is only one report on fetal death following ingestion of arsenic in man. This resulted from ingestion of about 400 mg of arsenic as arsenous oxide during the later part of the pregnancy (105). It seems likely, however, that effects on the fetus may occur at considerable lower exposures. Arsenic easily passes the placenta, and the arsenic concentration in cord blood has been shown to be similar to that in maternal blood (81).

And increased frequency of malformations in children of female employees at a smeltery has been reported as well as other effects on pregnancy outcome (131, 132). The role of arsenic exposure for these findings is not clear.

10. RELATIONSHIP BETWEEN EXPOSURE, EFFECT AND RESPONSE

Short term exposure to high doses of inorganic arsenic can induce a number of effects in different organs. As the effects of long term exposure are more important for the discussion of occupational exposure limits only such relationships will be taken up here.

Increased lung cancer risks have generally been observed following occupational exposure to inorganic arsenic. Figure 1 shows the relation between cumulative arsenic exposure (air concentration x duration) and lung cancer risk in the three major smelter cohorts under study: Anaconda and Tacoma in the United States and Rönnskärsverken in Sweden (41, 80, 96). Only the lower exposure groups in the Anaconda and Rönnskär cohorts have been included because they have the greatest interest for the discussion of occupational exposure limits.

It is clear from the figure that the lung cancer risk increases with increasing cumulative arsenic exposure. This increase continues among the more heavily exposed in the Anaconda and Rönnskär cohorts with SMRs in the range of 1000 for the groups with highest exposure (not included in the figure). There appears to be an excess risk also among the lowest exposure groups with SMRs of about 150–250 (corresponding to an excess risk of 50–150%). This can partly be explained by a higher percentage of smokers among the smelter workers than in the general population, which constituted the comparison (144), and a more pronounced exposure to other agents which increase the lung cancer risk. The lung cancer risk appears to increase almost linearly with increasing dose, corresponding to an excess risk of about 3–10% per mg/m^3 and year as cumulative arsenic exposure. In these estimates it is assumed that the excess risk is related to the background risk according to a relative risk model. The cohort in Tacoma and partly also the other cohorts suggest that the excess risk per unit exposure may be higher at low doses.

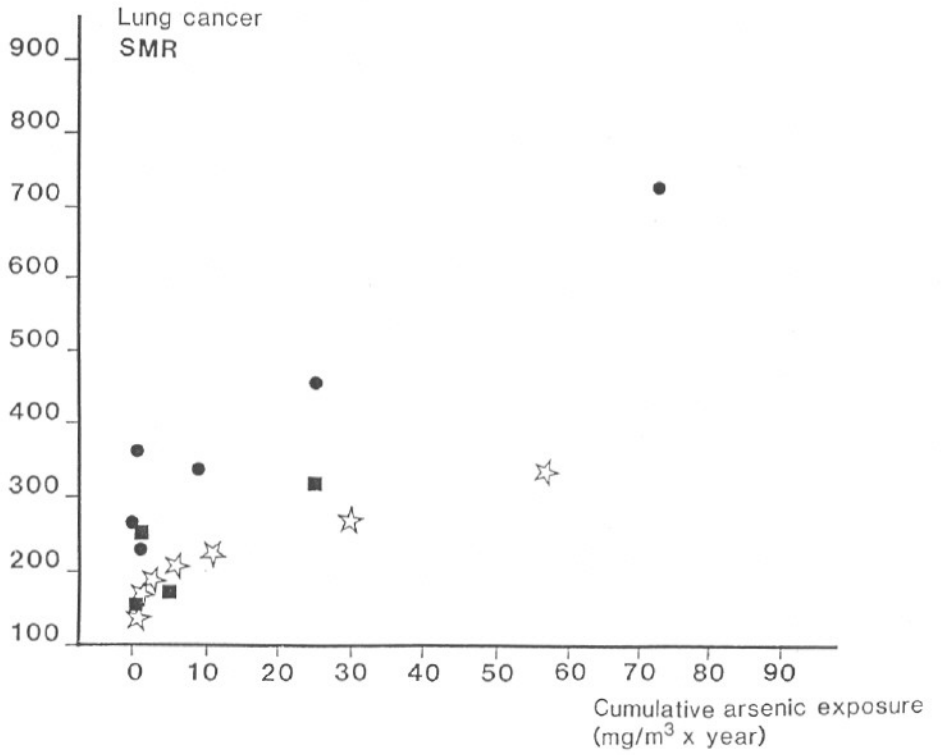

Figure 1. Relation between cumulative arsenic exposure and standardized mortality ratio (SMR) in three smelter worker cohorts: ■ Anaconda (97), ☆ Tacoma (41) and ● Rönnskärsverken (80). The exposure in the Anaconda cohort has been calculated from the midpoint in the intervals and the SMRs have been estimated from cohorts with different years of hiring.

There is a need for some caution in the comparison of SMRs between different exposure groups and studies. The SMRs are computed based on the morbidity (mortality) in a reference population which is different in the different studies. Within a certain study the age structure may vary between different dose groups, which implies that the SMRs are not directly comparable (unless the underlying relative risk is constant over different age strata). The risk estimation is based on a linear relative risk model, in which it is assumed that the excess relative risk is directly related to the dose, and this is also assumed in the comparison of different ages and populations.

The cumulative exposure measure consists of two components: intensity (air concentration) and duration. A few studies indicate that the intensity of arsenic exposure is of greater importance than the duration (80, 97). The risk estimation may also be affected by errors in the exposure estimation and confounding as well as by interactions with smoking and other agents in the occupational environments. These factors are not likely to explain the observed association but they may be of importance for the risk estimation. The influence of the chemical form of arsenic for the induction of lung cancer has not been clarified.

Exposure-response relationships for other health effects in workers exposed to arsenic have been less well investigated. Airway irritation, effects on the peripheral vascular system or peripheral nervous system, reproduction effects, skin sensitization as well as tumours of other organs than the lung are of interest. It cannot be excluded that some of these effects may occur at exposures near current occupational exposure limits.

11. RESEARCH NEEDS

It is well documented that inorganic arsenic is detoxified in the body through methylation to MMA and DMA. Further studies are needed to assess the variation in methylating capacity in the population, the reasons for such differences between individuals and the influence of the dose on the detoxification. This information is needed to assess the relation between exposure and risk of health effects on an individual level. Some animal studies have shown an initial accumulation of arsenic in some organs/tissues following continuous exposure. After a few weeks the concentrations are reduced in several organs. This has been interpreted as a tolerance to arsenic. The mechanism for this phenomenon, as well as its importance for humans exposed

to different arsenic compounds, should be clarified.

Several studies have shown that the concentration of arsenic metabolites in urine provides a good estimate of the exposure to arsenic trioxide. Further studies are needed to assess the relation between urinary arsenic concentrations and exposure to other arsenic compounds, primarily compounds of low solubility.

The major difficulty in assessing dose-response relationship for different health effects associated with exposure to inorganic arsenic stems from imprecise exposure estimates. It would be of great value to describe in detail the arsenic compounds occurring in different occupational environments, and not only the total arsenic concentration.

The results from epidemiologic studies and animal experiments on arsenic carcinogenicity are partly inconsistent. In view of the fact that some cytogenetic studies indicate that arsenic can influence repair mechanisms for DNA, it is desirable with more studies on interaction effects between arsenic and other agents, particularly tobacco smoke, in both epidemiologic and experimental systems.

12. DISCUSSION AND EVALUATION

Inorganic arsenic compounds can give rise to acute and chronic health effects in the respiratory tract, gastrointestinal tract, skin, cardiovascular system, nervous system and the hematopoietic organs. There is, however, still a great uncertainty regarding dose-response relationships.

In general, trivalent inorganic arsenic has a higher acute toxicity than pentavalent. Trivalent arsenic is very reactive and inhibits the activity of a number of enzyme systems, while the pentavalent arsenate can replace phosphate in different reactions. Following reduction of pentavalent arsenic to trivalent in the body the effects of exposure to pentavalent arsenic are largely similar to those of trivalent arsenic.

Following inhalation of soluble arsenic compounds, such as arsenous oxide, there is rapid absorption of the major part, either in the respiratory tract or in the gastrointestinal tract

following clearance. Particles containing less soluble arsenic compounds, such as arsenic trisulphate, calcium arsenate, lead arsenate and gallium arsenide have considerably longer half-lives in the lung. How this affects the risk of lung cancer is not known.

The concentration of arsenic metabolites in urine may be used as a marker of exposure to inorganic arsenic. A correlation between urinary arsenic concentration and arsenic in air has been observed in smelter workers exposed to arsenous oxide. Corresponding associations for other arsenic compounds have not been reported.

Lung cancer should be regarded as the critical effect following long term exposure to inorganic arsenic via the respiratory tract. A linear dose-response relationship assuming no threshold is often used to estimate cancer risks at low exposures. This model has been used in several evaluations of inorganic arsenic and lung cancer (42, 191, 193). It should be noted, however, that this model only gives a rough estimate of the cancer risk, particularly when extrapolations are done to low doses.

From the major cohort studies of arsenic exposed smelter workers described in chapter 10 it was estimated that the relative risk of lung cancer increases by about 3-10 % per mg arsenic/m^3 and year as cumulative arsenic exposure. This risk is lower than estimates by OSHA (139) and EPA (42) by a factor of about 3. The difference is partly due to the fact that their risk estimations did not account for an increased background risk among the smelter workers with the lowest arsenic exposure and because a use of respirators was assumed. If the interaction between smoking and arsenic exposure follows a multiplicative model, which is supported by some data, the risk estimation for arsenic is independent of smoking habits.

This risk estimation implies that an exposure to arsenic during 50 years at a level of 0.03 mg/m^3 would correspond to an excess risk of lung cancer of about 10% in comparison with the background risk. Assuming a life time risk of 4% for lung cancer this corresponds to an absolute risk increase of about 0.4%. Interactions between arsenic and other agents in the occupational environment or with tobacco smoking have not been considered in this estimation.

No conclusions can be drawn on differences in carcinogenic activity between different inorganic arsenic compounds. Consequently the discussion regarding occupational exposure limits must

relate to inorganic arsenic without differentiation between different forms.

13. SUMMARY

Göran Pershagen and Marie Vahter. Inorganic arsenic. Nordic Expert Group for Documentation of Occupational Exposure Limits. Arbete och Hälsa 1991.

The document constitutes a survey of the literature on inorganic arsenic (except arsine), to be used as background for discussion on occupational exposure limits. Lung cancer is considered the critical effect following long-term exposure to airborne arsenic. It can be estimated that the risk for lung cancer increases 3-10% per mg As/m^3 and year of exposure. Thus, 50 years of exposure to 0.03 mg As/m^3 would correspond to an excess lung cancer risk of about 10% compared with the background rate. Interactions between arsenic exposure and other environmental pollutants or smoking have not been considered in the risk estimation.

Number of references: 200

Keywords: inorganic arsenic, arsenic trioxide, arsenic trisulphide, gallium arsenide, occupational exposure, occupational exposure limits, air, urine, indicator of exposure, metabolism, methylation, detoxication, lung cancer, skin cancer, vascular disease, neuropathy, DNA-repair, chromosomal aberrations.

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Appendix 1. List of allowed or recommended maximum exposure limits of arsenic and inorganic compounds except arsenic hydrogen (as As).

Country	mg/m^3	ppm	Year	Ref.
Denmark	0.05	-	1988	1
Finland	0.01	-	1987	2
Iceland	0.05	-	1978	3
Netherlands	0.025	-	1989	4
	0.05 (15 min)	-		
Norway[1)]	0.01	-	1989	5
Sweden[1)]	0.03	-	1990	6
USA (ACGIH)[2)]	0.02	-	1990-91	7
(OSHA)	0.01	-	1990-91	8

1) Regarded as carcinogen
2) Suspected carcinogen

Appendix 2. List of allowed or recommended maximum exposure limits of arsine.

Country	mg/m^3	ppm	Year	Ref.
Denmark	0.05	0.01	1988	1
Finland	-	-	1987	2
Iceland	0.05	0.01	1978	3
Netherlands	0.025	-	1989	4
Norway[1)]	0.01	0.003	1989	5
Sweden[1)]	-	-	1990	6
USA (ACGIH)	0.16	0.05	1990-91	7
(OSHA)	0.2	0.05	1990-91	8

1) Regarded as carcinogen

Appendix 3. List of allowed or recommended maximum exposure limits of arsenic trioxide.

Country	mg/m^3	ppm	Year	Ref.
Denmark	-	-	1988	1
Finland	-	-	1987	2
Iceland	-	-	1978	3
Netherlands	-	-	1989	4
Norway[1)]	-	-	1989	5
Sweden[1)]	-	-	1990	6
USA (ACGIH)[2)]	-	-	1990-91	7
(OSHA)	-	-	1990-91	8

1) Regarded as carcinogen
2) Suspected carcinogen

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SUMMARY

Beije B, Lundberg P (eds). Criteria documents from the Nordic Expert Group 1991. Arbete och Hälsa 1991:50, pp 1–236.

The Nordic Expert Group is a standing committee with the task to produce criteria documents on health effects of occupationally used chemicals. The documents are meant to be used by the regulatory authorities in the five Nordic countries as a scientific basis for the setting of national occupational exposure limits.

The volume consists of English translations of the cirteria documents which have been published in a Scandinavian language during 1991.

Key words: Criteria document, Dimethylethylamine, Dimethylsulfoxide, Inorganic arsenic, Isophorone, Microorganisms, Nordic Expert Group, Occupational exposure limits, Trichloroethene.

SAMMANFATTNING

Beije B, Lundberg P (eds). Kriteriedokument från Nordiska Expertgruppen 1991. Arbete och Hälsa 1991:50, pp 1-236.

Den Nordiska Expertgruppen är en arbetsgrupp med uppgift att producera kriteriedokument om hälsoeffekter av kemiska ämnen i arbetsmiljön. Dokumenten skall användas av tillsynsmyndigheterna i de fem nordiska länderna som ett vetenskapligt underlag vid fastställande av hygieniska gränsvärden.

Volymen omfattar en engelsk översättning av de kriteriedokument som har publicerats på ett skandinaviskt språk under 1991.

Nyckelord: Dimetyletylamine, Dimetylsulfoxid, Hygieniskt gränsvärde, Isoforon, Kriteriedokument, Mikroorganismer, Nordiska Expertgruppen, Oorganisk arsenik, Trikloreten.

APPENDIX

Documents published in English by the Nordic Expert Group.

Acetonitrile	Arbete och Hälsa 1989:37, pp 149-174
Creosote	Arbete och Hälsa 1988:33, pp 7-51
Diacetone alcohol	Arbete och Hälsa 1989:37, pp 59-78
Hydroquinone	Arbete och Hälsa 1989:37, pp 79-114
Methyl bromide	Arbete och Hälsa 1987:40, pp 7-44
Methylene chloride	Arbete och Hälsa 1987:40, pp 74-120
Methyl formate	Arbete och Hälsa 1989:37, pp 175-202
Methyl isobutyl ketone	Arbete och Hälsa 1988:33, pp 53-76
n-Decane and n-Undecane	Arbete och Hälsa 1987:40, pp 45-73
Nitrilotriacetic acid (NTA) and its salts	Arbete och Hälsa 1989:37, pp 115-148
Nitroalkanes	Arbete och Hälsa 1988:33, pp 115-163
N-Nitroso compounds and cancer	Arbete och Hälsa 1991:2, pp 67-128
Organic acid anhydrides	Arbete och Hälsa 1991:2, pp 129-188
Paper dust	Arbete och Hälsa 1989:37, pp 203-246
Thiurames and dimethyldithio-carbamates	Arbete och Hälsa 1991:2, pp 7-66
Styrene	Arbete och Hälsa 1991:2, pp 189-279
Toluene	Arbete och Hälsa 1989:37, pp 7-58
Vinyl acetate	Arbete och Hälsa 1988:33, pp 77-113
Welding gases and fumes	Arbete och Hälsa 1991:2, pp 281-315

Documents published by the Nordic Expert Group (NEG) in collaboration with the Dutch Expert Committee for Occupational Standards (DEC) or the National Institute for Occupational Safety and Health (NIOSH).

7/8-Carbon chain aliphatic monoketones (DEC & NEG)	Arbete och Hälsa 1990:2, pp 1-44
Ethyl acetate (NEG & DEC)	Arbete och Hälsa 1990:35, pp 1-36
Methyl methacrylate (DEC & NEG)	Arbete och Hälsa 1991:36, pp 1-58
Propylen glycol ethers and their acetates (NEG & NIOSH)	Arbete och Hälsa 1990:32, pp 1-47

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