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CRITERIA DOCUMENTS

FROM

THE NORDIC EXPERT GROUP

1987

G. Heimbürger and P. Lundberg (Eds)

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PREFACE

The Nordic Council is an corporative international body for the governments in the five countries Denmark, Finland, Iceland, Norway and Sweden. Within the Nordic Council one committee, the Nordic Senior Executive Committee for the Occupational Environment Matter initiated a project with a view to compile and evaluate scientific information on chemical agents relevant to health and safety at work and produce 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
Gunnar Mowé	National Institute of Occupational Health, Norway
Vesa Riihimäki	Institute of Occupational Health, Finland
Adolf Schaich Fries	Danish National Institute of Occupational Health, Denmark

Before 1987 the Expert Group consisted of the following members:

Børge Fallentin	Danish National Institute of Occupational Health, Denmark
Bjørn Gylseth	National Institute of Occupational Health, Norway
Torkell Johannesson	Department of Pharmacology, University of Iceland, Iceland
Vesa Riihimäki	Institute of Occupational Health, Finland
Ole Svane	Directorate of National Labour Inspection, Denmark
Ake Swensson (Chairman)	National Institute of Occupational Health, Sweden
Hans Tjønn	Directorate of Labour Inspection, Norway
Ulf Ulfvarson	Department of Work Science, Royal Institute of Technology, Sweden
Vesa Vaaranen	Institute of Occupational Health, Finland

The documents accepted by the "old" Expert Group but published in 1987 are included in this volume.

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 of a numerical exposure limit value.

Search and collection of literature is executed by a secretariat headed by G. Heimbürger and located in the Institute of Occupational Health, Solna, Sweden. 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 of significance 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 1987. The names of those scientists who have written the separate documents are given in the list of contents, where also the dates of acceptance by the Expert Group are given.

Gunilla Heimbürger — Secretary

Per Lundberg — Chairman

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METHYL BROMIDE

by

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BACKGROUND

Methyl bromide is manufactured by bromination of methane or by hydrobromination of methanol.

Methyl bromide is used as a methylating agent in the chemical industry. Earlier it was widely used as a fire extinguisher, but as many accidents - often with severe consequences - occurred, it has been replaced by less toxic haloalkanes for this purpose. It has also been used extensively as a pesticide, and is very effective against various vermin. Above all it has been used for disinfection of food in storehouses and for soil disinfection, but as many severe poisonings have occurred in this connection the use of methyl bromide as pest control in food warehouses and insect control in soils has also decreased. Due to the great toxicity of methyl bromide its use is safeguarded by special regulations.

Denmark: Methyl bromide may be handled only by persons with a special license. Every occasion of soil disinfection must be reported to the authorities in advance.

Finland: Handling of methyl bromide is permitted only for authorized companies. Every occasion must be reported to the medical board.

Iceland: Methyl bromide is not used as a pesticide.

Norway: Methyl bromide may be used only by authorized exterminators. It is not used as a soil disinfectant.

Sweden: Methyl bromide may be bought and handled only by persons with special authorization.

PHYSICAL AND CHEMICAL PROPERTIES

Chemical name	methyl bromide
CAS number	74-83-9
Synonym	bromomethane
Formula	CH_3Br
General description	At room temperature a colourless gas with a very faint odor, in high concentrations resembling chloroform. Solubility in water: 0.9 g/l at 20°C. Soluble in diethyl ether, ethanol and chloroform. Stable in air. Burns in oxygen. Pyrolyses at 800°C.
Molecular weight	94.95
Boiling point (at 101.3 kPa)	4.5°C
Vapor pressure (at 23.3°C)	203 kPa (2atm)
Density (at -10.0°C)	1.736
Conversion factors (25°C, 101.3 kPa)	1 mg/m^3 = 0.257 ppm 1 ppm = 3.89 mg/m^3

In contact with open fire or hot metal, methyl bromide is decomposed and CO_2 and HBr are formed. The latter is very corrosive and is a strong irritant to skin and mucous membranes.
(38,46,58,62,80,82,84).

TOXICOLOGY

1. Toxicological model

1.1. Uptake

In the industrial environment, uptake via the lungs is by far the most important. Significant uptake through the skin may occur on direct contact with the liquid or with exposure to very high concentrations in ambient air.

1.1.1. Respiratory organs

Many cases of intoxication after inhalation of methyl bromide have been reported (cf section 4).

In an experiment rats were exposed for six hours to concentrations of 6 - 35 - 660 - 1170 mg $^{14}CH_3Br/m^3$ (1.6 - 9.0 - 170 - 310 ppm = 50 - 300 - 5.700 - 10.400 nmol/l). Uptake of methyl bromide was determined from excretion of ^{14}C in the feces and urine during the next 66 hours and the content of ^{14}C in the animals at the end of this period. The uptake was determined to be 8.7 - 40 - 660- 650 µmol /kg of body weight. The amount of methyl bromide inhaled was calculated from the air concentration, respiration frequency and tidal volume. The relative uptake in the lungs was calculated to be 48 - 48 - 38 - 27 %. Thus there is a ceiling for resorption (56). Other studies have shown that the pulmonary uptake in rats follows first-order kinetics up to lethal concentrations. The concentration in the body depends on a very fast nonenzymatic metabolization (3).

Exposure of rats to $^{14}CH_3Br$ at a concentration of 35 mg/m^3 (9.0 ppm = 337 nmol/l) caused a slow increase of the ^{14}C-activity of the blood during the first hour; thereafter the increase was faster and linear but towards the end of the six-hour exposure period the curve leveled off slightly (12).

1.1.2. Skin

In a review it is stated that it has not been demonstrated that methyl bromide can be resorbed through intact skin. Skin uptake, if any is assumed to be negligible compared to the uptake via the lungs, and a good respiratory protection is considered to give satisfactory protection (84). However, in an in vitro experiment with excised human skin it was shown that methyl bromide penetrates the skin (85). One case study also indicates of an uptake through the skin at exposure to high concentrations or direct contact with liquid methyl bromide: Three men were working for 1.5 h injecting methyl bromide gas into a six-story mill. They used oxygen masks. One man was working with a naked upper body because of the heat. It was difficult to inject the gas into all the pipes between the different stories of the mill, and drops of methyl bromide splashed back and hit the workers, mostly on the breast, abdomen and thighs. Nobody was uncomfortable

during the work, but one hour after they had finished they all developed the typical symptoms of methyl bromide poisoning. The man who had worked with a naked upper body died. The other two recovered (42). Thus direct skin contact with liquid methyl bromide and possibly also exposure to very high concentrations in the air may constitute a risk of skin uptake of dangerous amounts. Further, it is to be noted that methyl bromide very easily penetrates the clothes; this is apparent from the fact that skin lesions are very often observed on areas covered by the clothes (42).

1.1.3. Gastrointestinal tract

No data are available concerning humans.
The resorption was complete when methyl bromide was given orally to rats (55).

1.2. Distribution

In earlier studies methyl bromide was not found in tissues from persons poisoned or in animals experimentally exposed to methyl bromide. This is thought to be due to the very rapid decomposition of methyl bromide in the organism. These results have been confirmed in later studies of material from poisoned persons who died 6.5 to 36 h after an acute short-term exposure. Analysis with mass spectrography showed methyl bromide in the brain in a concentration around the detection limit (<1 ppm), but not in the blood or other organs. In another case, in which death occurred 36 h after exposure, methyl bromide could be detected neither in the blood nor in any other organ. In a third case, amounts around the detection limit were found in the eye chamber fluid but not in blood, fat or brain tissue (53).

When rats were exposed to 970 mg methyl bromide/m^3 (250 ppm) for 8 hours the concentrations of methyl bromide immediately after the exposure were: 0.1 mg/kg in blood, 10 mg/kg in fat, 0.01 mg/kg in brain and 0.006 mg/kg in liver. In all organs the concentration diminished linearly with time, and after 24 h the concentration was about 0.001 mg/kg in all organs. The amount of methyl bromide in blood and fat was halved in 0.5 h (34).

As methyl bromide is biotransformed very rapidly, the problem of distribution has been studied mostly for the biotransformation products. In some cases of

poisoning increased amounts of bromine were found in the blood, 28 - 400 mg/l (16,66). In other studies on poisonings the serum content of bromine was 20 - 500 mg/l (21,51,53,87). After administration of sodium bromide 30 - 40% of the total bromine content of the blood was found in the erythrocytes (79). After exposure to methyl bromide the bromine content of whole blood was found to be 28 mg/kg and of serum 66 mg/kg (65).

The bromine content in the cerebrospinal fluid has also been determined in cases of poisoning. When the serum bromine was 187 mg/l the content in the cerebrospinal fluid was 46 mg/l (21). At autopsy of one case of poisoning the bromine content was found to be 200 mg/kg in the brain, 130 mg/kg in the lungs and 180 mg/kg in the liver (31).

In an experiment rats were exposed to 35 mg/m^3 (337 nmol/l) of ^{14}C-methyl bromide for 6 h. Immediately after the exposure the activity was highest in the lungs, adrenals, liver and kidneys, corresponding to 250-180 µmol methyl bromide equivalents/kg. The content in the brain was 54 µmol/kg and in the blood 30 µmol/kg. In the following hours the activity rapidly decreased in all organs except the liver, in which the activity decreased more slowly during the entire observation period (60 h). Fat tissue was analyzed but no results were reported (12).

1.3. Metabolism

The metabolism of methyl bromide has not been completely elucidated. Earlier the methyl bromide was thought to be hydrolyzed in the organism primarily into methanol and carbon dioxide (24,26). This was confirmed in later studies (12). Duvoir et al (24) found small amounts of methanol and formaldehyde in the brain after lethal poisonings. Viner (81) demonstrated the presence of methanol in urine from persons poisoned by methyl bromide.

When rats were exposed to 970 mg methyl bromide/m^3 (250 ppm) for 8 hours, 22 mg methanol/kg was found in the blood and <10 mg/kg in the brain, liver and kidneys immediately after the exposure. One hour later the content was <10 mg/kg in all the organs (34).

In water solution methyl bromide is slowly hydrolyzed to methanol and hydrogen bromide. The reaction occurs faster in alkaline solutions (46,82). It has been

shown that methyl bromide in vitro reacts with SH-and NH_2-groups of several amino acids (23,50). In vitro reaction with free SH-groups of e.g. reduced glutathione has been demonstrated (50). It has also been shown in animal experiments that the amount of reduced glutathione in the liver decreases on exposure to methyl bromide: the higher the exposure the greater the decrease (2). It has been supposed that conjugation of methyl bromide with glutathione is an important path of methyl bromide biotransformation. Roycroft (69a) found that methyl bromide increased the activity of S-alkyl transferase at the same time as the amount of non-protein-SH decreased, which was supposed to prove conjugation of methyl bromide to the sulfhydryl group of glutathione.

Furthermore, methyl bromide can undergo nucleophilic substitution with other substances, and N-methyl substitution with many amino acids including glycine, alanine, leucine and glutamic acid, which has been demonstrated in vitro (1).

Bond et al (12) suggest that methyl bromide is converted in the body much like other methyl halides. On the basis of their studies Kornbrust and Bus (47) suggest that methyl chloride in the body reacts with glutathione to form S-methyl glutathione, which is further metabolized and excreted in the urine. Barnsley and Young (8) found methyl iodide to be metabolized in several different ways, yielding primarily S-methyl cysteine and methyl mercaptic acid, which are excreted in the urine. Injection of cysteine to rats 5 min after exposure to a deadly dose of methyl bromide resulted in a substantial reduction of intoxication symptoms and survival of the animals (60). Methyl bromide is metabolized very rapidly in all organs. When rats were exposed to 970 mg/m^3 (250 ppm) for 8 h the amount of methyl bromide in the organs rapidly increased during the first hour but was practically constant thereafter. The amount of bromine increased all the time. After exposure was terminated the amount of methyl bromide in blood and fat was halved in 30 min (34).

1.4. Elimination

1.4.1. Lungs

There are no human data on the elimination of methyl bromide through the lungs.

When rats were given 23.7 mg/kg (250 µmol/kg) of ^{14}C-methyl bromide intraperitoneally or orally 20 and 4 % respectively of the activity were exhaled within

72 h as unmodified methyl bromide; 45 and 32 % respectively were exhaled as CO_2. Half the amount of exhaled activity was exhaled within 3-4 h and practically the whole amount within 24 h. No unmodified methyl bromide was exhaled later than 7 h after the intraperitoneal injection. When methyl bromide was administered orally the exhalation of activity was initially somewhat slower than after the intraperitoneal injection (55).

After inhalation of ^{14}C-methyl bromide in concentrations from 6 to 1200 mg/m^3 (1.6 - 310 ppm) for 6 h, rats exhaled 47-50 % of the activity as CO_2 during the next 65 h. The exhalation of activity was almost completed after the first 10 h. Only 0.4-4 % of the activity was exhaled as methyl bromide, and the amount exhaled increased with increasing exposure (12,56).

1.4.2. Kidneys

There are no reports on elimination of unchanged methyl bromide in the urine.

Increased elimination of bromine via the urine has been demonstrated in cases of poisoning (66). Persons who handled methyl bromide as a pesticide excreted more bromine in the urine than did unexposed persons, 9 mg/l and 6.3 mg/l respectively. The exposed persons had no symptoms of poisoning (36). Alkali bromide is eliminated mainly via the kidneys (78,79).

When rats were given 23.7 mg/kg (250 µmol/kg) of ^{14}C-methyl bromide intraperitoneally and orally, 16 and 43 % respectively of the activity was eliminated in the urine. The elimination was initially slower when the methyl bromide was given orally (55).

In an experiment in which rats inhaled 35 mg ^{14}C-methyl bromide/m^3 (337 nmol/l) for 6 h, 23% of the activity was excreted in the urine within 65 h (12). In another experiment with inhalation of 6 - 1170 mg/m^3 for 6 hours, 18-23 % of the resorbed activity was eliminated within 20 hours (56). No unchanged methyl bromide was found in the urine (12).

The renal excretion of bromine is highly dependent on the supply of chloride. In experiments with rats the bromine excretion increased 10 times when the chloride supply was increased from 10 to 150 mg/day and animal (12).

1.4.3. Gastrointestinal tract

There is no information on elimination of methyl bromide or its metabolites via the feces in humans exposed to methyl bromide.

Bromine is excreted via the gastric mucous membrane but is reabsorbed rapidly and effectively in the intestine (77,78).

In rat experiments unchanged methyl bromide has not been found in the feces (12). When rats were given 250 mg/kg of ^{14}C-methyl bromide intraperitoneally or orally, 26 % of the activity was excreted via the bile within 4 h and was rapidly reabsorbed in the intestine. The activity of the feces was only 1-2 % of the dose administered (55). When rats were exposed to 6 - 1170 mg ^{14}C-methyl bromide/m^3 for 6 h only 2 % of the resorbed activity was found in the feces within 65 h (12,56).

1.4.4. Other routes

In a group of workers exposed to methyl bromide but without any symptoms of poisoning the amount of bromine in the hair was about three times as high as in unexposed workers (11.2 and 4.2 µg/g). The amount of bromine in the hair from a worker with suspected methyl bromide poisoning was 400 µg/g. When he returned to work after 1.5 years the amount was 11.5 µg/g (65).

1.5 Biological half times

There is no information regarding humans.

In a test rats inhaled 970 mg methyl bromide/m^3 (250 ppm) for 8 h. Immediately after the exposure methyl bromide could be demonstrated in blood, fatty tissues, brain, liver, kidneys and muscles (cf 1.2). In blood and fatty tissues the concentration was halved within 30 min. The very low concentrations in brain and liver decreased more slowly (34). After administration of alkali bromide to humans, the half-time of the bromine concentration in blood was about 12 days (79). In severe cases of methyl bromide poisoning the half-time of bromine concentration in serum has been reported to be about 16 days (65). The urine bromine excretion is closely dependent on the chloride supply and is increased with raised chloride supply (67).

After inhalation of ^{14}C-methyl bromide by rats the exhalation of activity in the form of CO_2 occurred in two phases with half times of 4.1 and 17 h. The half-time of the activity concentration in the urine was 9.9 h. The half-times of the activity content were 8 h in blood, 33 h in liver and 5 h in brain (56).

1.6. Factors influencing the metabolic model

In cell cultures the addition of GSH reduces the toxicity of methyl bromide (64).

For rats it has been demonstrated that reduction of body GSH reduces the biotransformation of methyl bromide measured as elimination of activity in the urine and exhalation of $^{14}CO_2$ (47).

The chloride supply affects bromine excretion in the urine: increased chloride supply causes increased bromine excretion (67).

2. TOXICOLOGICAL MECHANISMS

The toxicological mechanisms of the action of methyl bromide are not completely clear (34).

Miller and Haggard (59) assumed that methyl bromide could pass through the cell membrane, whereas bromide ions could not. The methyl bromide was then thought to be hydrolyzed within the cell to cause an intracellular bromism. This theory has been discussed and judged improbable in later publications (16,17). Methanol has been detected in blood and urine in cases of methyl bromide poisoning (24,81). In animal experiments methanol has been detected in the blood after administration of methyl bromide, but the amounts were very low and decreased very rapidly when exposure was terminated. Earlier, methanol was thought to be of importance for the development of the poisoning. However, the amount of methanol formed is too small to be significant and furthermore the clinical picture is not at all that of methanol poisoning (1). Today the prevalent view is that the damage is caused by the methyl bromide molecule and not by any of its metabolites (34,39a). Results from in vitro experiments have shown that methyl bromide reacts with NH_2- and SH-groups of many amino acids in water solution (23,50). It has been demonstrated in tissue cultures that methyl bromide can react with proteins and other cell components. The metabolites do not have the same effect (64). In animal experiments, administration of methyl

bromide causes a decrease of the concentration of noradrenaline in the hypothalamus and of cAMP in the cerebellum but not in the striatum (34).

3. TOXICITY

Systematic tests on the toxicity of methyl bromide are scarce.

Table 1. Methyl bromide: LD_{50} and LLD*) for different animal species

Species	Way of administration	mg/kg	Ref
Rat	orally in peanut oil	LD_{50} = 214	(18)
Rat	orally in olive oil (observation time 7 h)	LD_{50} = <100	(59)
Rabbit	subcutaneously in olive oil	LLD = 130	(43)
Rabbit	orally in olive oil	LLD = 60-65	(22)

*) LLD = lowest lethal dose

Table 2. Methyl bromide in air: LC_{50} for different animal species

Species	Exposure time	mg/m^3	ppm	Ref
Mouse	1 h	4680	1200	(2)
Mouse	0.5 h	6600	1700	(7)
Rat	8 h	1200	302	(34)
Rat	0.5 h	11000	2900	(7)

The dose-response curve is very steep when determining the LC_{50} for mice. The difference between LC_{10} and LC_{90} is equivalent to a doubling of the dose (2). About the toxicity of repeated doses cf 9 and table 5.

4. EFFECTS ON ORGANS

4.1. Skin and mucous membranes

Accidental exposures to high concentrations of methyl bromide in the air very often but not always lead to skin injuries. Usually these appear with several hours latency. The initial symptoms are tingling and burning sensations and itching in the exposed area. Later on erythema and vesiculation appear (9,10,13,14, 42,68). Generally, but not always, the skin reactions appear after general symptoms have been experienced. However, severe skin reactions with vesiculation may be associated with only insignificant general symptoms (32). Even a severe acute exposure with splash directly onto the skin does not necessarily cause a skin reaction (15,51). On the other hand, there are observations indicating that even relatively small repeated exposures can cause skin irritation even if the single exposure has no effect (13). In an inquiry study among employees in a factory packing methyl bromide, 25 % of the workers reported that they had experienced skin injuries in the form of irritation, erythema and vesiculation. They had not had any other symptoms (83). From a Japanese survey it appears that skin injuries are rare in systemic intoxication (6). This is well in accordance with the general impression from descriptions of acute systemic intoxication.

Conjunctival irritation and pain have been described in connection with exposure to high concentrations, but only in some cases (15,16,27,28,51,53). In reports on acute poisonings symptoms involving the conjunctiva are not particularly common, and when present are usually not severe. Even in cases of splash against the face the conjunctival symptoms have been slight (15,51).

4.2. Respiratory organs

In lethal cases of acute poisoning lung edema has often, but not always, developed (6,16,27,51,53,65a,69,73,74). Irritation of the nose, trachea and bronchi have been mentioned in only a few reports of this kind. In less severe cases, though still with very pronounced neurological signs, there are no observations of symptoms involving the respiratory organs (6,16,53,65a). Nor is there any information on irritation of the nose, throat or trachea-bronchi.

In animal experiments pulmonary edema has been reported at exposure to high concentrations, 3100 mg/m^3 (800 ppm) or more (2,39). In one experiment with continuous exposure of rats to 40 mg/m^3 (10 ppm) for three weeks, increased relative weight of the lungs and some petechiae were observed. The weight difference was, however, mainly due to on the decrease of body weight during the experiment. Exposure in the same way to 3.9 mg/m^3 (1 ppm) or to 20 mg/m^3 (5 ppm) gave no observable effect on the lungs (71).

4.3. Gastrointestinal tract

In acute poisonings the first symptoms are usually nausea and vomiting, which are thought to be centrally caused (cf 4.7). Stomach pain and watery diarrhea were reported in an isolated case of acute poisoning (53).

In animal experiments with exposure to lethal concentrations of methyl bromide in air, hemorhage in the colon has been observed in mice (2). Rabbits given an oral dose of 56 - 70 mg/kg in olive oil developed mucous membrane injuries in the stomach and duodenum, with hyperemia and hemorrhage (22).

4.4. Liver

The liver is not a prominent target organ in methyl bromide poisoning. Changes in the liver are rarely observed in lethal human poisonings (27,51,53,54,66,69, 74,86).

Exposure of mice to 4700 mg/m^3 (1200 ppm) for 1 h caused liver injuries. LC-50 was reported to be 4700 mg/m^3 (1200 ppm) (2). Continuous exposure of rats to 40 mg/m^3 (10 ppm) for three weeks caused decrease of the absolute and relative liver weight, increase of S-ASAT and S-LDH and decrease of the S-ChE-activity (71).

4.5. Kidneys

Oliguria and anuria have been noted in human cases of lethal poisoning. Tubular necrosis has been found at autopsy (16,19,26,53,65a,69). However, kidney function has been normal in many lethal cases and at autopsy the tubuli were normal (51,53,54,57a).

In severe cases of poisoning with recovery after a longer or shorter time, oliguria, proteinuria and hematuria have sometimes been observed. The symptoms were reversible and kidney function was restored to normal (4,9,16,27,53,65a,74).

In mild cases of methyl bromide poisoning without neurological symptoms no signs of kidney damage have been observed.

In animal experiments kidney damage has been observed only at exposure to very high sublethal or lethal concentrations. Kidney damage has been demonstrated in mice after exposure to an air concentration of 3500 mg/m^3 (900 ppm) for one hour. The LC_{50} was reportedto be 4700 mg/m^3 (1200 ppm) (2).

4.6. Blood and blood-forming organs

In severe cases of methyl bromide poisoning leucocytosis has often been observed in the intital phase (43). This may well be a secondary effect of the pulmonary damage occurring in severe cases.

4.7. Central nervous system

In acute poisoning the initial symptoms are dizziness, nausea and vomiting which in peracute poisoning may appear after only a few minutes of exposure. In less acute cases the symptoms begin after a few hours at work or up to ten hours after exposure. Neurological symptoms and signs appear after longer or shorter intervals or, in peracute cases, almost simultaneously. The initial symptoms are a feeling of stiffness and uncontrolled twitching of some muscles or limbs. The symptoms gradually increase to continuous myoclonies and general epileptic fits, and can progress to status epilepticus and coma. The death rate is high and the immediate cause of death is often pulmonary edema and respiratory insufficiency. In surviving cases a more or less complete recovery from the symptoms may occur over months or years. After severe poisonings more or less disabling sequelae may remain, often in the form of severe intention tremor, coordination disturbances, and myoclonic and epileptic fits (4,16,19,26,27,51,53,54, 57,65a, 66,86).

The neurological picture may vary considerably, but is usually dominated by motor symptoms. Sensibility disturbances are seldom mentioned in reports on

these severe acute intoxications. Signs of lesions in the cerebellum and in the central ganglia are especially prominent. In isolated cases brain atrophy has been reported as a result of the poisoning (6). Andersen and his coworkers (4) describe a very severe intoxication case in which a progressive brain atrophy was demonstrated by CT-scanning, and this atrophy became stationary 35 days after the acute poisoning. Thorough examination of the central nervous system at autopsy of acute intoxication cases has shown diffuse changes in the cerebrum with loss of neurons. Furthermore, very severe lesions with massive loss of neurons were found especially in the cerebellum and in some central ganglia (26, 27, 69).

Exposure to lower concentrations over a period of weeks or months may lead to insidious development of a somewhat different picture with fatigue, headache, focusing and accommodation problems, numbness mostly in the feet and hands, hypesthesia, memory troubles and, slurred speech (14,16,17,40,41,45,87). Auditory hallucinations, confusion and psychic agitation to distinct manic states have been reported in some cases (6,14,37,40,54,74,87). If the exposure is stopped the symptoms usually develop no further and regression is seen over a few months. If the exposure continues the clinical picture develops to that of acute poisoning.

Watrous (83) examined 90 persons who had been exposed for two weeks to less than 140 mg/m^3 (35 ppm) of methyl bromide in a factory. Mild and vague symptoms such as headache, dizziness, focusing difficulties with blurred vision and muscular pains were reported by 31 persons. A neurological examination revealed nothing abnormal. All the troubles disappeared within 2 weeks after termination of the exposure. Clarke and coworkers (16) report that the picture was dominated by nausea, loss of appetite, headache and muscle pains after long-term exposure to concentrations up to 140 mg/m^3 (35 ppm). All the symptoms disappeared rapidly on cessation of the exposure. Similar observations have been reported by other authors (6,45).

In animal experiments the clinical picture is dominated by motor symptoms, paralysis and convulsions (1,2,5,39).

4.8. Peripheral nervous system

After long term exposure to low concentrations of methyl bromide in the air, symptoms from the peripheral nervous system have been described: numbness,

paresthesias and paresis mostly in the limbs (14,15,25,45,72). In some cases impairment of various types of skin sensibility of the type glove-stocking anesthesia has been reported (14,72). Impairment of the vibration sense in the feet and legs up to the knee has been described (6,25,45). In all the reports on severe acute poisonings these symptoms are lacking. The conduction velocity of peripheral nerves has been examined in a few cases of poisoning. Schaumberg and Spencer (72) reported reduced conduction velocity. Chavez and coworkers (15) reported normal conduction velocity with low amplitudes in n. peroneus.

Rabbits exposed to 250 mg methyl bromide/m^3 (65 ppm) 7.5 h/day, 4 days /week for 4 weeks, altogether 100 h, began to lose weight after the second week and at the end of the experiment they had pareses (5). A group of rabbits were exposed to 105 mg/m^3 (27 ppm) 7.5 h/day, 4 days/week, altogether 900 h over a period of 8 months. They showed somewhat less weight increase than control animals but displayed no behavioral disturbances. Peripheral nerve conduction velocity was not markedly changed (70). In earlier experiments Irish and coworkers (39) reported pareses in rabbits after long-term exposure to 130 mg/m^3 (33 ppm). It is unclear whether the difference in results is due to small differences in analytic methods or if the limit for damage to the peripheral nerves lies in the range 105 - 130 mg/m^3.

Rats exposed to 250 mg/m^3 (65 ppm) 7.5 h/day, 4 days/week for 4 weeks showed no general reactions and no effects on the nerve conducting velocity (5). Exposure of rats to 215 mg/m^3 (55 ppm) 6 h/day, 5 days/week for 36 weeks distributed over one year, altogether 1080 h, resulted in no change in weight increase, behavior or conduction velocity in the peripheral nerves compared with controls (70).

4.9. Eyes

Focusing and accommodation difficulties are not uncommon, especially after long-term low-grade exposure. The symptoms are reversible. Impairment of visual acuity is more uncommon. It may develop slowly to blindness that may regress in some weeks. Abnormalities in the retinogram, narrowing of the visual fields and central scotomas, and impairment of color vision have been described. (14,15,28,44).

4.10. Gonads

There is no information on damage to the gonads by methyl bromide.

4.11. Fetus

Exposure of rats to 80 mg/m^3 (20 ppm) or 270 mg/m^3 (70 ppm) 6 h/day during days 1 - 19 of gestation had no negative effects on the dams, no toxic effects on the fetuses and no teratogenetic effect. Rabbits exposed to 80 mg/m^3 (20 ppm) during days 1 - 15 of gestation showed some signs of poisoning. This exposure had no fetotoxic or teratogenic effect (29,75).

5. ALLERGY

There is no information about allergy to methyl bromide.

6. GENOTOXICITY

In Ames tests with Salmonella typhimurium TA 100 and TA 1535 an increased mutation frequency was found. Tests with the strains TA 1537, TA 1538 and TA 98 showed no such increase. In a test with TA 100 the mutation frequency was dose dependent and increased with the air concentration of methyl bromide up to 5000 mg/m^3, whereafter it decreased abruptly as the dose led to cell death (61). In other studies increased mutation frequency was found for TA 100 at air concentrations of 1.90 x 10^3 mg/m^3 and higher. Addition of S 9 mix (metabolic activation) from rats induced by Aroclor 1254 did not change the result. In a similar test with TA 98 no effect on the mutation frequency was observed (48).

In a forward mutation system with Escherichia coli the toxicity of methyl bromide was high and the mutation frequency low (20). In another study increased mutation frequency was established (61).

In a fluctuation test with Klebsiella pneumoniae a positive outcome was found at concentrations of 4.75 mg methyl bromide/m^3 air and higher. The mutation frequency increased with increasing air concentrations (48).

In a mouse lymphoma test mutations were induced in L51784 mouse lymphoma

cells at both TK and HPRT locus. The mutation frequency was dose related and was detected even in the non-toxic range, 0.03 - 0.3 mg/l medium (48).

In studies with Drosophila on methyl bromide induction of sex-related recessive lethals, the exposure of the male flies started when they were one day old. After exposure they were mated with unexposed females. From then on they were mated with new females at 2 - 3 day intervals. The results are summarized in table 3.

Table 3. Mutation frequency induced by methyl bromide exposure of Drosophila

Air concentration (mg/m^3)	Exposure	Result
600	Continuously	The flies died on day 4.
600	6 h	No increase of mutations.
600	6 h x 5	The flies died.
478	6 h x 5	Increased mutation frequency.
375	6 h x 5	- " -
287	6 h x 5	No increase of mutation frequency.
200	6 h x 15	Increased mutation frequency.
70	6 h x 15	No increase of mutation frequency.

The authors point out that a significant increase of mutation frequency was found at high but not lethal exposure levels (48).

7. CANCEROGENICITY

There is no information concerning humans.

In an experiment rats were given 0 - 0.4 - 2 - 10 - 50 mg methyl bromide/kg body weight orally 5 days/week for 13 weeks. The dose was dissolved in peanut oil.

The animals were killed after 90 days. Thirteen out of 20 animals receiving 50 mg/kg had squamous cell carcinomas in the forestomach; most of them had not infiltrated. All animals had diffuse cell hyperplasia. At the lower doses no cancers had developed. In the two middle groups dose-related slight cell hyperplasia was found. There were no metastases in the liver or lungs. Animals given the highest dose had slight anemia and slight leucocytosis (18).

In an experiment with virus transformation of Syrian hamster embryo cells methyl bromide was without effect (30).

8. INDICATORS OF EXPOSURE

8.1. Determination of methyl bromide in air

Methyl bromide in the air can be determined by sampling in charcoal tubes. The sample is desorbed in carbon disulfide and analyzed by gas chromatography on a stainless steel column packed with 10% free fatty acid polymer (FFAP) on Chromosorb WHP (63). The sampling method lacked significant bias in the range of 71-160 mg /m^3 and had a total precision (mean deviation) of 0.103. At 30 mg/m^3 the bias was -13%. The precision of the analysis (mean deviation) was 0.053. With a sampling volume of 5 l of air the applicable concentration range of the method was 40 - 400 mg/m^3 (10-100 ppm). As far as it is known there are no interfering compounds.

8.2. Biological indicators

Methyl bromide in blood can be determined by combined gas chromatography and mass spectrometry (34). Little is known about the relation between exposure to methyl bromide and the concentration in blood. In animals the blood concentration has been found to be related to the concentration in inhaled air. However, the half time is very short and it is doubtful that the methyl bromide content of the blood can be used as a biological indicator for practical purposes.

Some of the resorbed methyl bromide is exhaled again. There are, however, no studies permitting evaluation of this exhalation as a biological indicator of exposure. The half time is very short.

A considerable part of the absorbed methyl bromide is biotransformed in the organism, and the amount of body bromine increases during exposure. The half time of bromine in humans has been found to be 12 days (49,79). Therefore, attempts have been made to use serum bromine and urinary bromine as indicators of exposure. In humans the individual variation in bromine content of the blood is considerable, 3.8 - 7.5 mg/l with a central value of 6.1 mg/l (65). According to a literature survey the serum bromine content in unexposed human subjects normally varies between 1.0 and 7.5 mg/l (65). In the literature the bromine content is sometimes related to whole blood and sometimes to serum. After administration of alkali bromides to volunteers the relation between the bromine contents of whole blood and serum was 0.45 (79). This difference is of some importance, as bromine ions pass the erythrocyte membrane only to a small extent while methyl bromide can pass the membrane easily.

In persons occupationally exposed to methyl bromide without experiencing any symptoms of poisoning the serum bromine content was 44 - 85 mg/l. One worker with non-lethal methyl bromide poisoning was shown to have a serum bromine content of 400 mg/l (65). In a survey of data from the literature on cases of poisoning of slight to medium severity the blood content of bromine was found to be on an average 69 µg/g and in severe cases of poisoning 157 µg/g, compared to 6.7 µg/g in unexposed controls. The individual variations were considerable (65).

Determination of bromine content in the blood seems to give reasonable information on the uptake of methyl bromide. However, This is true only if non-occupational sources can be excluded. Any method used by the clinical chemical laboratories can be used for this determination .

Ohmori et al (65) have studied the amount of bromine in the hair of workers exposed to methyl bromide and found a distinct increase compared with controls. This may have potential for long-term control but the method cannot be used for daily routine control. Due attention must of course be paid to the possibility of external contamination.

9. EXPOSURE, EFFECT AND RESPONSE RELATIONSHIPS

It is difficult to get a distinct conception of the dose-effect and dose-response relationships in humans, as in most reports the effects are incompletely described. Most authors report only symptoms from the nervous system.

After long-term exposure to low concentrations in the air, rather vague and non-specific symptoms appear: headache, dizziness, lack of appetite, nausea, vomiting, visual disturbances in the form of convergence and accommodation problems, muscular weakness and sensitivity impairment, numbness and tingling in the extremities. After exposure to higher concentrations leading to acute poisoning, nausea and vomiting occur after a shorter or longer time; thereafter, with varying latency time, symptoms from the motor system with muscle twitchings, myoclonias and epileptic fits rapidly develop. Symptoms from other organs such as the liver and kidneys are mentioned only sporadically. However, in many lethal cases of poisoning injuries to the kidneys and liver have been established at autopsy and so has pulmonary edema.

The information about exposure is usually very approximate. For cases of acute poisoning, reported estimates of exposure level vary from 230,000 mg/m^3 (60,000 ppm) for one minute (86) to 31,000 mg/m^3 (8000ppm) for a few minutes (57a) to more than 4000 mg/m^3 (1000 ppm), which causes very severe intoxications that only very rarely are survived (52). Several authors have tried to relate the effects on the central nervous system to different degrees of exposure given for cases of acute poisoning. Exposure to 4000 mg/m^3 (1000 ppm) or more causes the usual initial symptoms followed by symptoms of severe damage to the central nervous system. Survival is rare. Exposure to 800 - 1600 mg/m^3 (200 - 400 ppm) causes milder illness with headache, nausea, vomiting, muscle pains, visual disturbances. These intoxications are seldom lethal (16,19, 58,62).

Watrous (83) examined 90 persons who for two weeks had worked in a plant where exposure to methyl bromide often rose to 140 mg/m^3 (35 ppm). During this time 31 persons in the group reported mild symptoms such as headache, vertigo and blurred vision. Neurological examination revealed nothing abnormal. All the symptoms disappeared within two months after the exposure was stopped.

In an extensive study on mice the discussed relations were studied, table 4. In mice no pulmonary edema develops, not even in lethal poisonings. The dose-response curve for mortality was so steep that the difference between LC_{10} and LC_{90} was covered by doubling of the concentration.

The dose-response curve for kidney damage is closely parallel to that of mortality with only a slight shift to the left. It was quite clear that injuries to the

central nervous system, liver and kidneys only appeared at near-lethal exposures (2). The effect of repeated inhalations of methyl bromide has been studied in different animal species, table 5. There are considerable differences in sensitivity between different animal species. Also in these experiments damage to the central nervous system is predominant. Liver and kidney injuries were detected only at exposures within the area of lethal concentrations.

Mutagenic effects of methyl bromide have been found when high doses were used. High doses given orally to rats gave a high incidence of ventricular cancer. The dosage was 0.23 x LD_{50} and 0.5 x LLD. A dose of 0.05 x LD_{50} or lower caused no cancers but resulted in local epithelial hyperplasia.

Table 4. Effect of 1 h exposure of mice to different air concentrations of methyl bromide (2).

mg/m^3	ppm	Effect
2200	570	Seemed quite normal.
2720	700	Hyperactive the first 20 h after exposure.
3500	900	Significantly less weight gain; walking disturbance; inert, stopped cleaning themselves 20 h after exposure; kidneys enlarged.
3820	980	Increased depth of breath and decreased breathing frequency, tremor 20 h after the end of exposure; hemorrhages in cerebrum.
3870	990	Half of the animals had kidney injuries.
4680	1200	LC_{50}
4700	1200	Before death fasciculations, can not keep themselves upright; incoordination; reduced liver GSH-content; hemorrhages in liver and brain.
5770	1480	Symptoms appear faster, within 3 h after exposure. Hemorrhages in colon.

Table 5. Long term animal studies with methyl bromide inhalation

mg/m^3	ppm	Manner of exp	Time	Rat	Guinea pig	Rabbit	Monkey	Ref
1170	300	4h/d, 5d/w	3 weeks	3/12 died, 1 had convulsions.	-	-	-	35
850	220	7.5h/d, 5d/w		Died after 3-4 exp, nothing at section.	Died after 1-3 exp, lung injuries at section.	-		39
780	200	4h/d, 5d/w	3 weeks	All survived, poorer weight gain.	-	-	-	35
420	110	7.5h/d, 5d/w	6 months	Poor general appearance, convulsions, some after 6-14 exp, others after 16-58. 5 survived, growth redardation, lung sections showed histopathologic changes.	4/11 died after 64-91 exp. No positive pathological findings at section.	-	1 monkey died after 11 exp.	39
270	70	6-7h/d	15 days	No obvious effect, fetus without defect.	-	24/25 died before day 27, the first ones died on day 9.	-	29
250	62	7.5h/d, 5d/w	6 months	No obvious effect	No obvious effect	4 died, 38/42 had paralysis, restitution when exp was terminated.	3/6 paresis after 19-45 exp.	39
250	65	7.5h/d, 4d/w	a total of 100 h in 1 month	No effect on conduction velocity in peripheral nerves. No coordination disturbance. No influence of general motility. No effect on open field test.	-	Weight loss after week 2, weight was less at the end of exp than in the beginning. Lowered conduction velocity in nn ulnaris and ischiadicus at the end.	-	5

Table 5. cont.

mg/m^3	ppm	Manner of exp	Time	Rat	Guinea pig	Rabbit	Monkey	Ref
210	55	6h/d, 5d/w	1 year 1080 h	No effect in any test.	-	-	-	5
130	33	7.5h/d, 5d/w	6 months	-	-	34/58 paralysis in hind legs	-	39
105	27	7.5h/d, 4d/w	900 h in 8 months	-	-	Poorer weight gain than controls. All tests negative.	-	70
78	20	6-7h/d	15 d	-	-	No effect. Fetus normal.		29
65	17	7.5h/d	6 months	-	-	No effect	No effect	39
40	10	cont.	3 weeks	2/5 died. Lower absolute liver weight. Body weight decrease, increase of S-ASAT, S-LDH, S-GSH. Decrease of S-ChE.	-	-	-	71
20	5	cont.	3 weeks	No general reaction. Insignificant changes in blood chemistry.	-	-	-	71
4	1	cont.	3 weeks	No effects	-	-	-	71

10. NEEED OF FURTHER RESEARCH

A reliable biological exposure indicator would have great practical value. It seems that the concentration of bromine in the blood and urine might be useful for this purpose. However, the relation between exposure to methyl bromide and the concentrations of bromine, and the kinetics of biotransformation, need to be thoroughly studied..

The relation between the bromine concentration in serum and that in whole blood may be relevant for diagnosis and monitoring purposes and ought to be studied.

Further study of carcinogenic action of prolonged low grade exposure is desirable. It has been reported that long term inhalation experiments with rats are in progress at the Netherlands National Institute of Public Health (18) and at the U.S. National Toxicology Program (62).

11. DISCUSSION AND EVALUATION

Methyl bromide is a very dangerous gas, as it is practically odorless and non-irritating - at least in concentrations of interest in the context of industrial hygiene. Very severe acute poisonings may occur suddenly without the person in question having noticed any smell or irritation.

The discussion of occupational exposure limits must be based on the early symptoms from the central nervous system, symptoms that appear insidiously.

The experimental results showing local cancerogenic effects in rats when methyl bromide was given orally must be taken into consideration. Cancerogenicity cannot be accurately assessed until further experiments have been completed. The mutagenic power is low. No teratogenic effects have been found.

The poisoning may develop insidiously, beginning with vague, nonspecific symptoms and developing to a severe neurological condition. Recovery is usually very slow and severe sequelae are common. It is therefore important to consider the very first symptoms that may appear and the discussion of exposure limits must be based on them. The insidious development of the poisoning, the gravity of the damage to the central nervous system and the risk of serious sequelae must be considered very seriously.

SUMMARY

A. Swensson: Methyl bromide. 74. Nordic expert group for documentation of occupational exposure limits.

A critical survey of the literature relevant for a discussion of an occupational exposure limit is given.

The discussion should be based on the vague symptoms that develop insidiously after prolonged exposure to low concentrations in the air.

The severe sequelae after even moderate poisonings must be taken into consideration as well as the possibility of carcinogenic effects.

Key words: Methyl bromide, occupational exposure limit, intoxication picture, metabolism, brain damage, polyneuritis, cancer.

A Swedish version is available; 89 references.

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APPENDIX I. Occupational exposure limits for airborne methyl bromide.

Country	mg/m^3	ppm	Year	Note	Ref.
BRD	20	5	1985	S	7
Denmark	20	5	1985	S	3
Finland	60 90	15 25	1981	S (15 min)	14
France	20	5	1986	8 h	16
Great Britain	60	15	1986	S	6
Iceland	60	15	1978	S	12
Netherlands	60	15	1985	S	10
Norway	20	5	1984	S	1
Soviet Union	1		1978	g	8
Sweden	60 80	15 20	1985	S STV	4
USA (ACGIH) (NIOSH/OSHA)	20 80	5 20	1986-87 1978	S S	13 11

g = gas, S = skin, STV = short term value, M = maximum.

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n-DECANE and n-UNDECANE

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Appendix I: The list of allowed or recommended thresholds for exposures to vapours of n-decane, n-undecane and similar alkanes.

Appendix II: Methods for measurements and analysis of n-decane and n-undecane in air.

BACKGROUND

n-Decane is used for organic synthesis, as a solvent, and in laboratories (38).

n-Undecane originates like decane from crude oil distillates (38) and is probably used in the same processes.

n-Decane is an abundant substance occurring in different types of fuels and solvents, e.g. kerosene or white spirits (38). The traditional white spirit (Varnolene(R)) contains about 4% n-undecane and 10.6% n-decane (15). The production in USA in 1975 was about 910 kg of pure n-decane.

PHYSICAL-CHEMICAL DATA

Both compounds are colorless liquids at room temperature and are almost insoluble in water. They are easily dissolved in organic solvents. The physical and chemical properties are shown in Table 1.

Table 1. Physico-chemical properties (38, 45).

Chemical name	n-decane	n-undecane
Synonyms:	Decylhydride	Hendecan
CAS-number:	124-18-5	1120-21-4
Molecular formula:	$CH_3(CH_2)_8CH_3$	$CH_3(CH_2)_9CH_3$
Molecular weight:	142.3	156.3
Boiling point (101.3 kPa):	174.1 °C	195 °C
Specific gravity (20 °C):	0.7301	0.7402
Vapour pressure (25 °C):	ca. 0.40 kPa	0.052 kPa
Saturation concentration in air (25 °C):	ca. 7900 mg/m^3 (1359 ppm)	3280 mg/m^3 (521 ppm)
Conversion factors (25 °C, 101.3 kPa):	1 ppm = 5.82 mg/m^3 1 mg/m^3 = 0.172 ppm	1 ppm = 6.39 mg/m^3 1 mg/m^3 = 0.156 ppm

1. METABOLIC MODEL

1.1 Uptake

No information was available about n-decane and n-undecane absorption through skin, lungs or mucous membranes. Indirect evidence for skin absorption was found in an investigation (30) showing damage to spleen, kidneys and lungs of mice after application of 100-150 mg n-decane on skin 3 times a week for 50 weeks.

The exposure would normally take place in the form of vapours. Therefore the compounds, like most other n-alkanes (1, 25, 38) under normal occupational environmental conditions, would be absorbed through the lungs. Lung absorption is therefore the main absorption route.

1.2 Distribution

The absorption and distribution of n-decane from the blood phase and to the tissue of rabbits were investigated after intravenous injection of radioactive labelled n-decane in a paraffin emulsion (4). During a period of one hour a very rapid absorption in the liver took place followed by a re-distribution into spleen and adrenal glands. The radioactivity of the organs was subsequently reduced over a period of one month. It was not investigated if the activity was due to the originally injected n-decane or metabolites of this compound. The exposure through injection will most likely lead to another distribution of the compound than with inhalation, as the distribution of the paraffin emulsion particles to the organs and the subsequent absorption in these organs may differ significantly.

1.3 Biotransformation

n-Decane is oxidized to n-decanol through the microsomal cytochrome P-450 enzyme system (17, 26).

The metabolites found after oxidation (in vitro) in mice liver microsomes were mainly 1-decanol, n-decanoic acid, 1.10 decandiole and an unidentified polar compound (17). The carboxylic acid may be oxidized by β-oxidation to produce CO_2.

An alternative degradation was seen in rat liver where decanoic acid is further oxidized by the microsomale P-450-system into 10-hydroxydecanoic acid. Through a combined cytosol and microsomal function this compound may be oxidized, to produce dicarboxylic acid. This acid can be expected to oxidize through β-oxidization into CO_2 (16).

The oxidation of n-decane by the microsomal cytochrome P-450 takes place primarily in the liver. In mice, rabbits and oxen a relatively high enzyme activity has been found in the lung tissues (16,17).

1.4 Excretion

No relevant information is available about excretion of these compounds, but the excretion of alkanes and their metabolites is expected primarily to occur through the exhalation of the unchanged compound CO_2 and other volatile metabolites.

2. TOXICOLOGICAL MECHANISMS

Investigations of alkanes and their toxic action has been focused on their effect on the cell membranes. This is due to the strong lipophilic action of alkane combined with a minimal solubility in water for the heavier alkanes.

The different toxicological mechanisms have been investigated mainly in relation to the effect on the nervous system.

In vitro it has been shown that the anaesthetic alkanes affect the nerves by blocking the potassium and sodium ionic currents

across the cell membrane, upon which the nerve impulses depend (13). The mechanism is, that the proteins regulating the potassium/sodium currents through the cell membrane are changed in such a way that conductivity is reduced.

Model experiments have shown that when n-decane is dissolved in the cell membrane it increases membrane thickness and at the same time reduces activity of the ATP-ase in the membrane (19). This and a similar observation for n-pentane on the nerve cells, has led to the hypothesis that these alkanes act through a thickening of the membrane, thereby disturbing normal membrane enzyme function (12, 9). It is still under discussion whether something similar happens during in vivo exposure (9, 20, 28, 37, 39). The Ca^{++} and Mg^{++} dependent ATP-ase in the sarcoplasmatic reticulum is dependent on the length of the molecules in the membrane, and thereby on membrane thickness (19). It is expected that if the sarcoplasmatic reticulum has an optimal structure for the function of ATP-ase then n-decane will have a negative effect on the activity on this ATP-ase, and subsequently on muscle action.

The exposure of cultivated human lung fibroblasts to n-decane emulsions with a high concentration of 25 mmol/l induced a 6% increase in the membrane penetration measured by loss of radioactive labelled nucleotides. The metabolites n-decanol, n-decanal, n-undeanal and n-decanoic acid and n-undecanoic acid were very active in an aqueous emulsion at a concentration of 25 mmol/l (42).

3. EFFECTS ON THE ORGANS

3.1 Skin and mucous membranes

A pilot study on carcinogenicity running for 2-4 weeks showed that 5 mg of n-decane or n-undecane dissolved in acetone and applied 3 times a week was the highest dose causing only minimal skin damage to mice. The concentration of the solutions is not given (43). This pilot study does not include information of the

effect of acetone alone. Acetone would, however, evaporate relatively quickly. Furthermore no information is given about the size of the exposed skin area. It may be concluded, that this pilot study does not fully describe the effects, nor the dose-effect relationships.

65 mice were exposed to between 0.10-0.15 g of n-decane three times a week for 50 weeks, or a total of 16.53 g per mouse. The exposure caused skin damage, as well as damage to organs. The effects on the skin were macroscopic thickening, dryness and desquamation. The microscopic changes were ulceration, dermal fibrosis, pigmentation, and hyper- and perikeratosis corresponding to a keratinization in the areas of application. No information is given about the size of the exposed area and the experimental conditions as such (30).

The skin-irritating effect of n-decane on rabbits was examined (21). 0.2 ml n-decane was applied to an 8 x 10 cm^2 skin area and covered with Japan paper for one hour. After removal the effect was observed for fourteen days. On the first and second day a hemorrhage and strong erythema was observed macroscopically and on the third to seventh day, encrustation, reduced elasticity and cracks were seen. After 14 days the skin again appeared normal. Histological examination showed a strong cell growth, parakeratosis and encrustation. The increased cell growth continued, although to a lesser extent until 14 days after the exposure. A reduction of the skin content of DNA and RNA to about 50 and 25% of the initial value in a control group was observed on the third day. At the same time the biosynthesis of DNA, RNA and phospholipids were reduced to about 0. Syntheses and the concentrations increase to above the normal value, gradually reducing towards normal after some days.

3.2 The respiratory organs

Only few investigations of the effects of n-decane and n-undecane on the respiratory organs exist.

Application of 100-150 mg of n-decane on the skin of mice 3 times a week for 50 weeks caused pathological effects on the lung tissue in the form of hemorrhages, inflammation and necrosis (30).

Inhalation exposure of rats to 3140 mg/m^3 (540 ppm) n-decane for up to 91 days, 18 hours a day, 7 days a week, showed no micro-or macroscopic effects on the respiratory organs (30).

Aspiration of 0.2 ml n-decane caused lung oedemas, hemorrhages and increased weight of the lung for 5 of a total of 5 exposed rats. This dose was fatal within 24 hours (11).

n-Decanal, n-undecanal and n-decanoic acid, which are expected to be metabolites of n-decane and n-undecane, caused a blocking of the ciliary movements in cultures of embryo chicken trachea at a conentration of 5 mmol/l. n-Decanol had no effect, and n-decane was not tested (35). These effects indicate an increased risk of impairment of the respiratory defense system.

Lung irritation caused by n-alkanes increases in intensity as the number of carbon atoms in the compound increase (41). In mice n-pentane and compounds up to n-heptane cause an increase in airway irritation as measured by the respiratory rate and the depth and on figuration of the respiration. This is, however, only seen at very high concentrations close to the condensation concentration (41). By analogy, it can be expected that n-decane and n-undecane will change lung function in mice.

3.3 The liver

No information is available about liver damage.

3.4 The kidneys

A subchronic inhalation study with rats which were exposed 6 hours day, 5 days a week for 3 weeks to different types of crude oil distillates indicated that the medium and long chained alkanes are among the most potent kidney-toxic solvents (14). The investigation showed the largest effect for the high boiling distillates and an exceptionally high effect among those distillates containing a high proportion of alkanes and alkenes. The toxicity was according to the authors probably highest for the branched alkanes, but a sharp separation of the effect of different compounds was impossible. The boiling point intervals mentioned in the publication indicates that the concentration of n-decane and n-undecane may not have been large in the distillates used. The results, however, show that alkanes have a stronger effect as the size of the molecule increases. A consistent dose/effect curve was found for concentrations between 0 and 4000 ppm. Tubular degeneration was seen after exposure to concentrations down to 35 ppm of the distillate containing the highest proportion of high boiling alkanes. The effects seen on the rats were formation of hyalin droplets, degeneration of the proximal tubules in the cortex, tubular dilatation and necrosis in the corticomedullary part.

In an experiment, rats were exposed to dearomatized white spirit or to a mixture containing 100% isoalkanes, which is a mixture of branched and normal decanes and undecanes. A decreased growth rate and changes in the kidneys were shown among the male rats exposed to 1900 and 5610 mg/m^3 for 6 hours a day up to 12 weeks. The effects on the kidneys were tubular cell degeneration in the cortex, tubular dilatation with precipitation of proteins, probably a disintegration of cell material in the corticomedullare part (36). Many authors seem to be convinced that male rats may be more sensitive to carbon hydrates and that such effects are not expected among other animals. These effects, therefore, may not be relevant for humans.

Fuel for jet engines contains mainly C_{10}-C_{15} alkanes. A single, orally administered dose of fuel corresponding to 24 ml/kg body weight caused kidney damage in rats in the form of hyalin droplets and an increased creatinine concentration in the serum. Furthermore, an increased lactate dehydrogenase activity and an increased glutamic pyruvic transaminase activity was observed (33).

A long-term exposure experiment, running for 50 weeks, in which 100-150 mg n-decane were applied to the skin of mice 3 times a week showed pathological effects on the kidneys in the form of hemorrhages, inflammation and pigmentation (30). For a mouse weighing 30 g, this corresponds to a dose of 5 g/kg per application.

3.5 The blood and blood forming organs

One large dose of C_{10}-C_{15} jet fuel corresponding to 25 mg/kg body weight administered per os caused a reversible decrease in the number of leucocytes and an increase in number of erythrocytes among rats. The increase in erythrocyte number was assigned to an induced dehydration (33).

3.6 Gastrointestinal and cardiovascular system

No information is available about effects on these organs.

3.7 The central and peripheral nervous system

Table 2 shows the odour threshold for n-decane and n-undecane

Table 2. Odour threshold for n-decane and n-undecane

n-Decane	detection threshold	11.3 mg/m^3	(44)
n-Undecane	detection threshold	23.0 mg/m^3	(44)
n-Undecane	recognition threshold	376.0 mg/m^3	(44)

The detection limit of the pollutant is the concentration at which 50% of the panel persons detect an odour. The recognition limit is the concentration at which 50% of the subjects can identify the odour.

n-Decane is probably not anaesthetic when inhaled as vapours (29). This suggestion is supported by a comparison to the a-

naesthetic potential of other liquid alkanes. For these compounds, the thermodynamic activity expressed by p_t/p_s decreases with increasing number of carbon atoms in the molecule (8, 29, 39), where p_t is the partial pressure of the compound at anaesthetic concentration, and p_s is the partial pressure of saturated vapour. The ratio between the two pressures is the anaesthetic potential of the compound. The anaesthetic effect increases, however, with increasing number of carbon atoms when the dose is expressed by the absolute concentration (10). This is due to a simultaneously decrease of p_s.

When an emulsion containing n-decane or n-undecane is administered by intravenous infusion 30 mg/min (0.04 ml/min), these compounds are found to be among the most effective anaesthetic alkanes. For 10 mice exposed to n-decane or n-undecane the average anaesthetic doses were 582 mg/kg and 100 mg/kg, respectively (18).

Small effects on isolated nerves or axones (in vitro) have been demonstrated in experiments with n-decane. The nerve impulse of isolated frog nerves decreased 10% after about 4 hours of exposure to a Ringer solution of n-decane containing 0.30 mol/l (0.042 mg/l) (12). The nerve impulse was blocked after 20 hours of exposure (32). The speed with which nerve preparations decay increases when they are exposed to alkanes. There was a tendency for n-decane emulsions to produce irreversible decreases in the nerve impulse (12). Changes in the structure of the myeline sheaths as measured through x-ray diffraction increased as time progressed for all the examined alkanes. After prolonged exposure the effect on the myelin structure was irreversible (32).

The two primary metabolites expected from n-decane and n-undecane are n-decanol and n-undecanol, respectively. These two compounds have in in vitro experiments been shown to block nerve impulses (37). In a preparation of the lateral olfactory nerve from guinea pigs one mmol/l n-decanol blocks the nerve impulses after 100 minutes of exposure. A similar effect is seen for 0.5 mmol/l n-undecanol after 300 minutes. These nerve blockings are in both cases reversible.

3.8 Reproduction and foetus

There is no known effect on the reproductive organs or foetus.

3.9 Other organs

Some investigations with alkane metabolites, e.g. n-decanoic acid have shown that these compounds may affect the muscle function of frogs and rats in in vitro experiments (5, 22, 23). These investigations show that there is an increasing effect with increasing number of carbon atoms in compounds, and that the contraction is reduced, the membrane potential and action potential are changed.

Prolonged (50 weeks) skin application of 100-150 mg n-decane, 3 times a week, on mice caused pathological changes of the spleen in the form of amyloidosis (30).

In vitro experiments with n-decane have shown comitogenic effect on the spleen lymphocytes (2). n-Decane alone shows no mitogenic effect, but the addition of phytohemaglutinine caused a synergistic effect. n-Tetradecane produced an even stronger response, indicating that n-undecane, which has not yet been examined, may have a stronger effect than n-decane. The mitogenic effect of lymphocytes is considered to be an immune reaction.

4. ALLERGY

No information is available about allergy after exposure to n-decane and n-undecane.

5. GENOTOXIC EFFECTS

5.1 Mutation measured in vitro

n-Decane was not mutagenic in in vitro experiments using both V79 Chinese Hamster cells and Salmonella typhimurium TA-100 (7,

24). Application of n-decane to Chinese hamster cells after treatment with a known muta- and carcinogen, methylazoxymethanol acetate (MAM), produced a significant 24% increase of the ouabain resistant mutants when compared to the effect of MAM alone (24). n-Decane may therefore act as a promotor of mutagenicity due to MAM.

n-Decane has also been shown to increase the mutagenic effect of the known carcinogen N-methyl-N1-nitro-N-nitrosoguanidine (MNNG) if applied at the same time in a modified 8-azaguanin resistance experiment with S. typhimurium TA-100 (7).

n-Undecane has not been examined for mutagenic effects, but both dodecane and tetradecane, like n-decane increase, the mutagenic effect of MAM on V79 Chinese Hamster cells. n-Undecane is therefore expected to have a similar effect.

5.2 Chromosome damage

No information is available in the literature of possible chromosome damage due to n-decane and n-undecane.

6. CARCINOGENIC EFFECT

The tumour promoting activity of alkanes and alkanoles has been examined in mice (40). One week after the skin application of 7.12 dimethylbenz(a)antracene, 4 mg of test compound was applied 3 times a week for 60 weeks to a total of 720 mg. n-Decane was shown to cause tumours in two mice out of 30 exposed mice after week 52 and week 53, while the expected metabolite n-decanol caused six tumours in 30 exposed mice after 25-36 weeks. No tumours were observed in the two control groups. Though effect of n-decane was not significant, n-decanol is expected to be a primary metabolite of n-decane.

In another major experiment of the cocarcinogens and tumour promoters in tobacco smoke, the cocarcinogenic effect of n-decane

and n-undecane was examined together with benzo(a)pyrene (B(a)P). 50 mice were applied 25 mg of test compound + 5 µg of B(a)P in 0.1 ml of acetone 3 times a week for 440 days (a total of 4.7 g test compound and 0.9 mg of B(a)P). Neither n-decane nor n-undecane caused papillomas on the skin. But both compounds were potent cocarcinogens causing 44 and 38 of the 50 mice to develop papillomas, respectively. 41 and 34 of these were squamous carcinomas, respectively. In the control group which had been treated only with B(a)P 16 mice were observed to have papillomas and out of these 12 were squamous carcinomas (43).

The cocarcinogene effect on n-decane has further been demonstrated by a simultaneous application of n-decane and ultraviolet light (3).

7. INDICATORS OF EXPOSURE

7.1 Air concentrations

The concentration in the inhaled air is normally used as a measure for exposure. The methods and analytical procedures for measurements of the concentration are shown in appendix II.

7.2 Biological indicators

No information is available about biological indicators of exposure to n-decane and n-undecane.

8. THE RELATION BETWEEN EXPOSURE, EFFECT AND RESPONSE

8.1 The effects of short-term exposure

8.1.1 Acute reversible effects

Exposure (skin application) to 100% n-decane for one hour causes reversible acute skin damage on rabbits (21).

8.1.2 Irreversible damage

Intravenous bolus injection of an emulsion of n-undecane in mice caused a LD_{50} value of 517 mg/kg body weight.

Continous intraveneous infusion into mice revealed an average highest acceptable dose before stop of respiration at 912 mg/kg for n-decane and 1637 mg/kg for n-undecane (18).

8.2 The effects of-long term exposure

8.2.1 Reversible effects

Damage to the skin, the kidneys, the lungs and the spleen has been observed when 100-150 mg of n-decane was applied to the skin of mice 3 times a week for 50 weeks. The observed effect on the skin were encrustation, fibrosis, increased pigmentation, ulceration, hyper- and perikeratosis. Inflammation, increased pigmentation and hemorrhages were observed in the kidneys and amyloidosis was seen on the spleen. The effects in the lungs were microscopic hemorrhages, pigmentations, inflammation and necrosis of tissue (30).

8.2.2 Chronic or irreversible damage

It has been shown that n-decane and n-undecane are cocarcinogens when applied to the skin of mice. No information is available about the dose/response relation.

9. RESEARCH NEEDS

Very little information is available about the toxic effect of the two compounds on humans.

A number of investigations will be needed if the risk of toxic effects on humans are to be evaluated. This is further underlined by the possible cocarcinogenic and comutagenic effect. Special consideration should be given to the effect on the nervous

system, the lungs, the muscles and the kidneys due to the indications of effects on these organs.

Such investigation will be especially needed for n-undecane, which in general has not been investigated.

10. DISCUSSION AND EVALUATION

No relevant information is available about the effects of n-decane and n-undecane on humans.

Based on the existing literature and on knowledge of other similar organic solvents n-decane and n-undecane can be expected to be absorbed through the lungs and transported via the plasma lipoproteins to the different organs used for measurements, for example the liver and the nervous system.

It can also be anticipated that the two compounds mainly will be degraded into CO_2 in the liver, the kidneys and the lungs. Excretion is expected to happen, mainly through the lungs.

Two effects seem to be well documented. Skin irritation after application of liquid n-decane to the skin of rabbits and mice, and cocarcinogenic effect to mice after application to the skin.

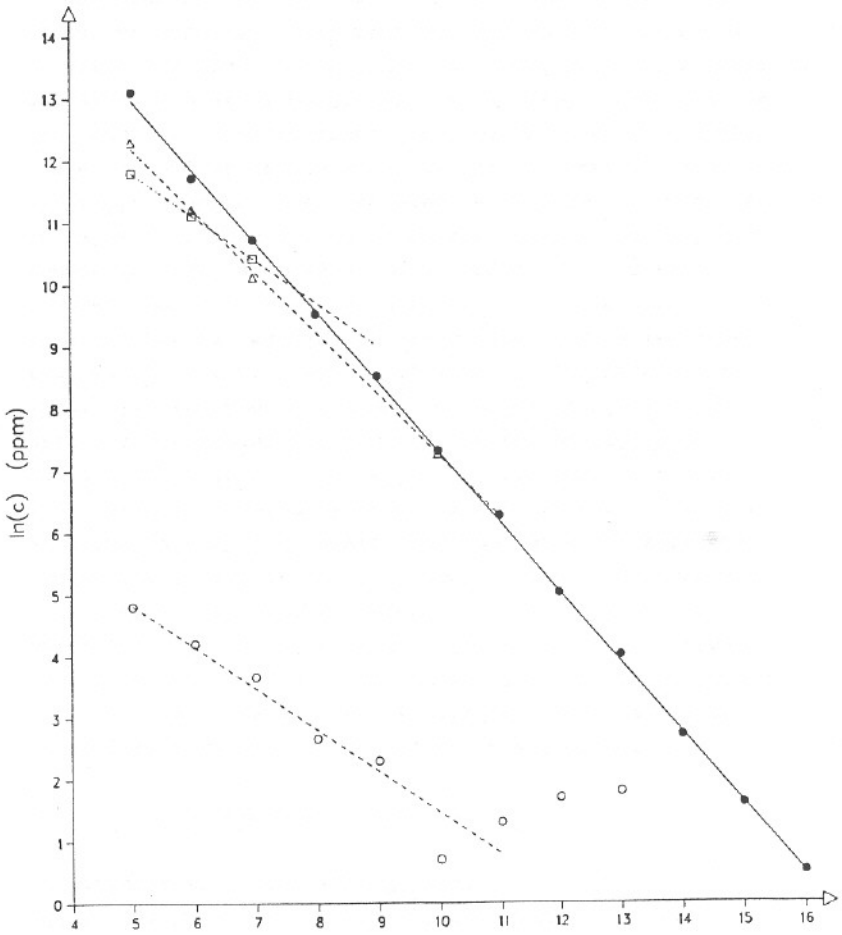
The dose needed to obtain the equal effects is expected to decrease as the number of carbon atoms increase in the alkane molecule. This is illustrated in figure 1, showing a linear relationship between the natural logarithm of doses leading to the same effect and the number of carbon atoms in the alkane molecule. The figure illustrates why n-decane and n-undecane might have no anaesthetic effect when inhaled. The lines on the figure are seen to intersect the line corresponding to saturated vapour concentrations at room air temperature. Therefore, it would not be possible to produce a sufficiently high concentration to cause narcotic effects. Also, it may be concluded, that respiration stop among mice can not be provoked by exposure to vapours of n-nonane, n-decane and higher alkanes. The high concentration needed for that may, however, be created as aerosols.

n-Decane and n-undecane are normally used in mixtures with other and more volatile organic solvents. Such compounds may have a more intensive effect than n-decane and n-undecane. It should, however, not be overlooked that due to the lower vapour pressure exposure to n-decane and n-undecane may be prolonged when compa-

red to the more volatile compounds. The total absorbed dose may therefore be higher for n-decane and n-undecane.

The available information indicates that a possible limiting value for the exposure to n-decane and n-undecane will have to be based on the cocarcigenic effect and the chronic effect on kidneys and nervous system. Very little information is, however, available about the dose/response relationship for these effects. Based on the known effects of organic solvents on the nervous system, this type of effect ought to be considered, too. This is further underlined by the in vitro experiment with nerve preparations. The sparse information about the effects of these compounds on humans makes an estimate for threshold limit values difficult.

Figure 1. The relation between the natural logarithm of concentrations (ppm) leading to the same effect and the number of carbon atoms in different n-alkanes (C_4-C_{16}).



o Odour detection threshold (44)
Δ LC_{50} for the Grain weevil (8)
□ Stop of respiration in mice (41)
• Concentration of saturated vapour at 25°C (38, 27)

11. SUMMARY

S. Kjærgaard and L.Mølhave: n-Decane and n-undecane. 75. Nordic Expert Group for Documentation of Occupational Exposure Limits.

A survey and an evaluation of literature useful as a basis to point out effects on which an occupational threshold limit value should be based.

No relevant human dose/effect or dose/response relations could be established.

Male rats show kidney damage when exposed to solvent mixtures containing n-decane and n-undecane. In animal studies both substances are cocarcinogenic and in very large doses they are lethal. Nerve function is diminished in in vitro experiments.

It is impossible to recommend any threshold limits based on the existing knowledge, and the need for further investigations is pointed out.

A Danish version is available; 45 references.

Keywords: Occupational exposure limits, n-decane, n-undecane, paraffins, carbonhydride mixtures, effects on central nervous system, cocarcinogenecity, kidneys.

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Appendix I: List of acceptable or recommended thresholds for air concentration on n-decane, n-undecane and other similar alkane vapours.

Country	mg/m^3	Year	Compound	Note	Ref.
USSR	300	1978	Saturated aliphatic hydrocarbon C_1-C_{10}	a	2
Sweden	500	1985	Decanes and higher aliphatic hydrocarbons	b	1

a: gas
b: short term value

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APPENDIX II Recommended methods for sampling and analysis of n-decane and n-undecane

Short-term sampling of n-decane and n-undecane may be done in a Douglas bag, Tedlar, Mylar or Saran bags, glass tubes or gaspipettes (1, 2, 4).

Sampling for longer periods may be done through an absorption tube containing active charcoal or silicagel. Hydrocarbons may be desorbed from the silicagel through heat desorption or through liquid desorption using hexane (3, 4) and from a charcoal tube using N,N-dimethylformaid (2) or hexane (3).

The analyses are done by gaschromatography eventually combined with use of a mass spectrometer or by a HPLC chromatographic instrument using ultraviolet or fluorescence detector.

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METHYLENE CHLORIDE

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BACKGROUND

Areas of use

Methylene chloride is used widely, primarily as a paint and varnish remover, but also in vehicle gases for various spray products.

Methylene chloride is used as a solvent for oils, greases, waxes, tars and numerous other organic chemicals. It is particularly valuable as an extractant for substances that do not tolerate high temperatures, e.g. for extraction of oils in the food processing industry, in particular the decaffeination of coffee.

Methylene chloride is also used in degreasing processes, as a coolant in air conditioning equipment, and in the manufacture of photographic films.

Criteria documents on methylene chloride have been published by WHO (52) and by NIOSH (70, 71).

Physical and chemical characteristics.

Chemical name: Dichloromethane
CAS No.: 75-09-2
Synonym: Methylene dichloride
Formula: CH_2Cl_2
At room temperature it is a colorless, non-flammible liquid.
Molecular weight: 84.94
Boiling point: 40°C (101.3 kPa)
Vapor pressure (25°C): 58.1 kPa,
about 2×10^6 mg/m^3 at equilibrium
Odor threshold: 730 – 870 mg/m^3 (210 – 250 ppm) (2, 63)
1 ppm = 3.48 mg/m^3; 1 mg/m^3 = 0.288 ppm

Note: If methylene chloride comes into contact with an open flame, phosgene is formed. The amount of phosgene is dependent on the methylene chloride concentration and on the temperature. Dangerous amounts can be formed (34, 42). It seems that only small amounts of phosgene are formed during welding, and there is probably little risk of lung damage due to phosgene from this source (89).

TOXICOLOGY

1. METABOLIC MODEL

1.1. **Uptake**

Methylene chloride can be absorbed by the skin, the respiratory organs and the digestive tract.

1.1.1. Skin and mucous membranes

Methylene chloride in liquid form can penetrate healthy skin. In a study in which volunteers kept one thumb in various solvents for 30 minutes, it was found that methylene chloride penetrated the skin several times faster than four other solvents. The subjects had a maximum of about 11 mg/m^3 (3 ppm) in exhaled air (99).

In another experiment, the absorption of eight different solvents was studied by application to the skin of mice. Methylene chloride was absorbed several times faster than the other solvents (107).

1.1.2 Respiratory organs

In occupational exposure to methylene chloride, the substance is taken up mainly by the lungs. The concentration in alveolar air rises rapidly during the first few minutes of exposure. The

increase then gradually decelerates, and after an hour or two of exposure further increases in methylene chloride concentration are minimal (9, 29). The methylene chloride concentration in blood rises more slowly, and, if the air concentration is constant, reaches a plateau after about eight hours (30).

Alveolar uptake in human subjects exposed to various concentrations of methylene chloride for from 30 minutes to 7.5 hours was calculated to be between 50 and 70% (9, 30, 31).

The blood/air partition coefficient for methylene chloride is about 10 (37).

1.1.3. Digestive tract

Uptake via the digestive tract is rapid (6, 7). Serious poisoning can result from swallowing methylene chloride in an attempt to commit suicide (15, 90).

1.2. Distribution

Methylene chloride is distributed by the blood to the body's tissues. Animals given methylene chloride by inhalation or intraperitoneal injection accumulate the substance in adipose tissue (14, 21, 27, 93). The concentration in fat is higher than that in other tissues even a relatively long time after exposure (93). Researchers who have used ^{14}C-labeled methylene chloride and also studied metabolite concentrations have found a rapid reduction of concentrations in adipose tissue; the liver, kidneys and lungs are the organs that show the highest radioactivity 48 hours after a single peroral dose or six hours of inhalation (14, 21, 27, 65, 66). Concentrations in the brain are always low in relation to those in other organs (21, 27, 65, 66, 93).

The liver/blood and muscle/blood partition coefficients for methylene chloride are both about 1, while the fat/blood coefficient is about 10 (37).

Methylene chloride passes the placental barrier in rats (4) and man, and can also be found in breast milk of exposed women (113).

1.3. Biotransformation

Two pathways for biotransformation of methylene chloride have been identified. The first is a cytochrome P-450 dependent metabolism to carbon monoxide and the second is a glutathione-S-transferase dependent metabolism to formaldehyde and formic acid, in which the formaldehyde is subsequently metabolized to carbon dioxide (3).

The biotransformation of methylene chloride is dose-dependent (30, 31, 65, 66, 80). At higher doses, a relatively smaller proportion is metabolized and a larger proportion exhaled unchanged. When rats were exposed by inhalation to 175 mg/m^3 (50 ppm) of radioactively labeled methylene chloride for 6 hours, within the next 48 hours they exhaled about 25% of the dose as carbon dioxide and an equal amount as carbon monoxide, while only 5% was exhaled as methylene chloride. With exposure to 1750 mg/m^3 (500 ppm), exhaled carbon dioxide and carbon monoxide each accounted for about 20%, while 30% was exhaled as unchanged methylene chloride (66). The rest of the dose was mainly retained in the body or found in urine as unidentified radioactive metabolites. Human subjects exposed by inhalation to 175, 350, 520 or 700 mg/m^3 (50, 100, 150 or 200 ppm) for 7.5 hours exhaled 25 to 35% as carbon monoxide, while 5% was exhaled unchanged. This indicates that people exposed to low concentrations of methylene chloride probably exhale a large proportion of uptake as carbon dioxide (30).

Data from experiments with human subjects indicate that with eight hours of exposure during rest to more than 870 mg/m^3 (250 ppm), the metabolism of methylene chloride to carbon monoxide can become saturated (30). Metabolism to carbon monoxide seems to be saturated in rats at about the same exposure level (61, 66).

Formaldehyde or formic acid have not been detected in increased amounts in urine of subjects experimentally exposed to methylene chloride (31).

1.4. Elimination

1.4.1. Respiratory organs

Methylene chloride concentrations in exhaled air and blood drop very rapidly as soon as exposure is terminated. Elimination via the lungs gradually slows. Seven hours after 7.5 hours of exposure to 520 mg/m^3 (150 ppm), the concentration in exhaled air was below 0.35 mg/m^3 (0.1 ppm) (9, 30, 101). About 5% of an inhaled dose of methylene chloride is exhaled unchanged as long as biotransformation systems are not saturated (30, 66).

After exposure, the carbon monoxide concentration in exhaled air drops much more slowly than the methylene chloride concentration. Carbon monoxide concentrations in exhaled air seem to be about halved during the first four hours after exposure to concentrations no higher than 700 mg/m^3 (200 ppm) (30).

1.4.2. Kidneys

Rats that inhaled ^{14}C-methylene chloride in concentrations of 175, 1750 or 5220 mg/m^3 (50, 500 or 1500 ppm) for six hours excreted about 8% of the radioactivity in urine in the next 48 hours. None of this was unmetabolized methylene chloride (66). Excretion of methylene chloride in urine is also negligible in man (30, 31).

1.4.3. Digestive tract

Rats given $^{14}CH_2Cl_2$ via inhalation excreted about 2% of the absorbed radioactivity in feces within 48 hours (66).

1.4.4. Other paths of elimination

Nothing is known about other paths of elimination.

1.5. Biological half times

Using computer simulations, the elimination of methylene chloride has been described as a system with several compartments, the first two of which have a halving time of a few minutes or less. After that are three compartments with halving times of 2 to 3 minutes (vessel-rich group), 15 to 30 minutes (the muscle group) and 5 to 15 hours (adipose tissue). The lower time estimates apply to light exercise, the higher ones to resting (37). These data agree relatively well with experimental results and calculations of halving times for methylene chloride in alveolar air and blood (9, 35, 88, 101) and in fat (35, 88).

Test subjects exposed to 175, 350, 520 or 700 mg/m^3 (50, 100, 150 or 200 ppm) for 7.5 hours had a halving time of about 6 hours for COHb in blood (30). The COHb concentration initially drops more slowly at high exposures than at lower exposures. This is interpreted as indicating that the biotransformation of methylene chloride is saturated, leading to an accumulation of methylene chloride in fat; the metabolization of methylene chloride to carbon monoxide thus continues after termination of exposure (66).

1.6. Factors that can affect the metabolic model

Increasing physical exercise with the consequent increase in alveolar ventilation increases the total uptake of methylene chloride. Physical exertion has less effect on uptake of methylene chloride than on uptake of many other solvents, however. With exposure to 1750 mg/m^3 during rest and during work loads of 50, 100 and 150 watts for four consecutive 30-minute periods, the total uptake increased during the 50-watt period to over double the resting intake, but the increase was clearly smaller for the two subsequent work periods (9). These relationships have been confirmed in other experiments (31).

The COHb concentration also seems to increase with increased work load, but heavy exertion (ventilation 45 liters/minute) doesn't seem to yield higher COHb concentrations than medium-heavy exertion (ventilation 30 liters/minute) (31).

The total uptake of methylene chloride is clearly higher for fat people than for thin people or people of normal weight. After exposure, however, fat people have lower concentrations in adipose tissue than thin people have (35).

Rats or monkeys simultaneously exposed to ethanol (12,500 mg/m^3 to 16,500 mg/m^3; 6675 ppm to 8700 ppm) or isopropanol (3300 mg/m^3 to 12,000 mg/m^3; 1350 ppm to 5000 ppm) and methylene chloride (17,500 mg/m^3; 5000 ppm), and rats given intraperitoneal injections of toluene (460 mg/mg) before a one-hour exposure to methylene chloride (17,500 mg/m^3; 5000 ppm) showed much lower concentrations of COHb than animals exposed to methylene chloride alone (23).

Rats that inhaled methylene chloride in concentrations of 1750 to 10,500 mg/m^3 (500 to 3000 ppm) 6 hours/day for three days showed no cytochrome P-450 induction, while 10 days of exposure to 1750 mg/m^3 (500 ppm) resulted in slight induction (74, 106).

The three-day treatment apparently induced specific isoenzymes of cytochrome P-450, however, and the high exposures seemed to increase production of some benz(a)pyrene metabolites (106).

Repeated exposure to methylene chloride does not induce COHb formation (60), and common inducers of "mixed function" oxidases did not increase COHb formation from methylene chloride (102).

2. TOXICOLOGICAL MECHANISMS

As mentioned earlier, methylene chloride is metabolized to carbon monoxide and carbon dioxide. Both of these metabolic pathways give rise to reactive intermediaries. Both glutathione conjugation and microsomal oxidation seem to be associated with the mutagenicity of methylene chloride (55).

The toxic effects of carbon monoxide are considered to be related to its affinity for hemoglobin, about 300 times stronger than that of oxygen. Carbon monoxide also displaces the dissociation curve for the remaining oxyhemoglobin so that the oxygen is less accessible to the tissues. It has been shown that factory workers exposed to methylene chloride have an oxygen dissociation curve displaced to the left. For workers exposed to over 1000 mg/m^3 (300 ppm) (8-hour time-weighted average), the partial oxygen pressure necessary to saturate 50% of the blood's oxygen-binding capacity with oxygen was lowered by about 250 – 500 Pa, or about 10 to 20% lower than for unexposed controls. For workers exposed to 350 mg/m^3 (100 ppm) or less the reduction was only 1 to 4% (80). The small amount of free carbon monoxide in plasma also seems to be relevant to toxicity (73).

3. EFFECTS ON ORGANS

3.1. Skin and mucous membranes

Irritation of the conjunctiva in connection with exposure to methylene chloride has been described by several authors (24, 50, 62, 68) and confirmed in experiments with rabbits (11, 32).

Volunteers who sat with one thumb in a methylene chloride bath experienced an intense burning sensation within two minutes. Within 10 minutes the thumb was numb and cold (99). A man who had fainted from exposure to methylene chloride vapor and lain unconscious for 30 minutes with his leg in a pool of methylene chloride developed second and third degree chemical burns on the skin in contact with the liquid (110). Methylene chloride irritates the skin of rabbits (32).

3.2. Respiratory organs

Methylene chloride can irritate the upper respiratory passages (24, 50, 62, 68). Lung edema has been described after high (not further specified) exposures (18, 50).

Of the 33 English workers reported to have been acutely poisoned by methylene chloride during the period 1961 – 1980, only 4 reported symptoms involving the respiratory passages (10).

Rats exposed to 12,900 mg methylene chloride/m^3 (3700 ppm) 5 hours/day, 5 days/week for four weeks showed signs of cell damage and inflammatory reactions in the lungs, as well as increase of pulmonary secretion (92). Mice exposed to an aerosol of streptococci for 3 hours were more likely to develop infections when simultaneously exposed to 350 mg/m^3 (100 ppm), but not to 175 mg/m^3 (50 ppm) methylene chloride (8).

3.3. Liver

In a cross-sectional study, workers exposed to methylene chloride in concentrations of 210 to 1650 mg/m^3 (60 to 475 ppm) (8-hour time-weighted average) showed greater ALAT (alanine aminotransferase) activity and higher bilirubin levels in serum than controls. The workers had been simultaneously exposed to 260 – 2600 mg/m^3 (110 – 1080 ppm) acetone (time-weighted average). In general, high exposure to methylene chloride was accompanied by low exposure to acetone, and vice versa. The exposed group showed a clear dose-response relationship for bilirubin but not for ALAT. The rise in ALAT was considered not to have been caused by the exposure to methylene chloride. It was considered unclear whether the increase in bilirubin was an expression of effects on the liver (78).

Of the 33 English workers reported to have been acutely poisoned by methylene chloride in the period 1961 – 1980, signs of liver damage were described in only one (10).

Two case reports describe liver damage after acute exposures to high concentrations of methylene chloride. In one case, a reversible rise in transaminase was the only symptom (86). In the other case there were signs of considerable liver damage, but these were obscured by severe kidney damage (67).

In various experiments in which single intraperitoneal injections of methylene chloride were given to mice, rats, guinea pigs and dogs, and liver damage measured by sulfobromophthalein clearance or by determination of liver enzyme activity in serum, little or no effect was noted on the studied parameters even at near-lethal doses (28, 41, 58, 59). Pre-treatment with phenobarbitol did not increase the effect (25). Dogs and monkeys continuously exposed to 3500 mg/m^3 (1000 ppm) show deterioration in liver function, measured by sulfobromophthalein retention, after four weeks; after 6 to 7 weeks of constant exposure, dogs, but not monkeys, develop some cirrhosis (64).

Mice exposed to 17,500 mg/m^3 (5000 ppm) methylene chloride for 12 hours showed increased triglyceride concentrations in the liver. An incipient cirrhosis was noted after 14 weeks of continuous exposure to this concentration (108).

Male and female rats exposed to 1750, 3500 or 12,200 mg/m^3 (500, 1500 or 3500 ppm) 6 hours/day, 5 days/week for two years showed a dose-dependent cirrhosis from the 12th month onward (19).

3.4. Kidneys

Kidney damage was described in none of the 33 English workers reported acutely poisoned by methylene chloride (10). One case report describes acute tubular necrosis in a 19-year-old man after a high (not further specified) acute exposure to methylene chloride (67).

Mice and dogs given single intraperitoneal injections of methylene chloride in nearly lethal doses show no effects on phenol sulfonephthalein elimination or on protein and glycose contents in urine (58, 59, 84). Rats exposed to methylene chloride in concentrations of 1750, 3500 or 12,200 mg/m^3 (500, 1500 or 3500 ppm) 6 hours/day, 5 days/week for two years developed no kidney damage from the exposure (19). Rats continuously exposed to 3500 mg/m^3 (1000 ppm) for more than 2,000 hours showed clear degeneration of tubules in the renal cortex (64).

3.5. Blood and blood-forming organs

Ott et al, in their study of workers exposed to methylene chloride, noted increases of hemoglobin values, hematocrit values and number of red blood cells among women exposed to 1650 mg/m^3 (475 ppm) (8-hour time-weighted average). This should be interpreted as a compensatory response to the COHb increase (78).

Changes in blood parameters were described in none of the 33 English workers reported acutely poisoned by exposure to methylene chloride (10).

Intravasal hemolysis and pronounced hemoglobinurea have been described in cases of attempted suicide by peroral intake of methylene chloride (15, 90). In one experiment, in which dogs were given four 2-hour exposures to 1750 – 17,500 mg methylene chloride/m^3 (500 – 5000 ppm), they developed signs of hemolytic anemia (1).

3.6. Digestive tract

Nausea and vomiting are common after inhalation of methylene chloride in concentrations high enough to cause symptoms of poisoning (10, 36).

3.7. Heart and blood vessels

Two cohort studies have been performed concerning cause of death among workers exposed to methylene chloride.

The first study included men employed in an American company where methylene chloride had been the solvent most used since the mid-1940s (38, 48, 49). In the most recent follow-up (through 1984), the cohort included 1013 men who had worked in the company for at least a year between 1964 and 1970. The cohort's average exposure to methylene chloride, from the mid-1950s until today, is estimated to be about 100 mg/m^3 (30 ppm) (8-hour time-weighted average). No definite changes in exposure levels have occurred during this time. Seven exposure groups were defined; average exposures of the groups ranged from <3 to 400 mg/m^3 (<1 to 114 ppm). The frequency of exposure peaks in these groups was also estimated. No over-frequency of ischemic heart disease was found, either in comparison with a control cohort of unexposed factory workers from the same company or in comparison with the population of the state of

New York. Dose-response analyses (dose calculated as exposure level x exposure time) revealed no dose-response relationships (49).

The other cohort included all 1271 men who had been exposed to methylene chloride during at least 3 months of employment in an American factory between 1954 and 1977. They were followed from 3 months after they started work until 1977. Methylene chloride exposure probably varied from 490 to 1650 mg/m^3 (140 to 475 ppm) (time-weighted average). Mortality due to ischemic heart disease was no higher in the cohort than that expected for the U.S. population as a whole, nor was longer exposure or latency time associated with any clear increase (77).

Among the 33 English workers reported acutely poisoned by methylene chloride, there were no reports of effects on the heart (10).

In the factory in which the second cohort study described above was made, the ECGs of 24 men were monitored for 24 hours. Their average age was 52 years; their exposure to methylene chloride ranged from 210 to 1650 mg/m^3 (60 to 475 ppm) (8-hour time-weighted average). No differences in any ECG variables were found in comparisons with controls (79). Nor were any differences found by other authors, who studied the ordinary ECGs of 29 workers exposed to 260 – 350 mg/m^3 (75 – 100 ppm) methylene chloride and compared them with controls (22).

Stewart and Hake 1976 (100) describe a case of a 66-year-old man without previous heart trouble who was exposed to methylene chloride while removing paint. The man had a heart attack, with severe chest pains. The pattern was repeated when he was exposed again a few weeks later, and on a third exposure six months afterwards he died, after complaining of severe chest pains. The authors suspect that the heart attacks were caused by a rise in COHb. Other authors describe a 50-year-old man who was admitted to the hospital with a COHb level of 11% after

extremely high (not further specified) exposure to methylene chloride. The patient's ECG showed several pathological changes that disappeared when the COHb level dropped to normal. The ECG changes were considered to have been caused by the high COHb level (13).

3.8. The central nervous system

In a laboratory experiment, Putz and colleagues exposed human subjects to 700 mg/m^3 (200 ppm) methylene chloride or 80 mg/m^3 (70 ppm) carbon monoxide for four hours. Immediately after exposure, COHb levels were about 5% in both groups. After 1.5 to two hours of exposure, and during the rest of the exposure, both groups had lower results in two simultaneous psychomotor tests. One of these was a reaction time test for stimuli in the periphery of the field of vision, and the other an eye-hand coordination test. The methylene chloride exposure, however, led to considerably worse results than the carbon monoxide exposure. Results in an attention test for auditory stimuli were significantly worse after 4 hours of exposure, with no difference between the two groups. The authors concluded that carbon monoxide is the cause of the measured effects (85). In an experiment in which women were exposed to about 1050 mg methylene chloride/m^3 (300 ppm) for 4 hours, a significant effect was noted on a "Critical Flicker Fusion" test after two hours, and became more pronounced as exposure continued. Results in an alertness test were also significantly lower. These effects were more pronounced at exposures of about 1750 and 2800 mg/m^3 (500 and 800 ppm). This author maintains that the decreases are probably due to an interaction between hypoxia, induced by carbon monoxide, and the narcotic effects of methylene chloride (112).

Subjects exposed to 350 and 700 mg methylene chloride/m^3 (100 and 200 ppm) for 2 hours showed no effects on ability to perform mathematical operations, nor were results in a coordination test affected (29). Subjects exposed to 870, 1740, 2600

and 3470 mg/m^3 during consecutive 30-minute periods showed no deterioration of results in psychological tests of numerical ability, reaction time or short-term memory (40).

Cherry and colleagues examined workers exposed to 260 – 350 mg methylene chloride/m^3 (75 – 100 ppm) (8-hour time-weighted average). In a preliminary interview survey, these workers reported more symptoms such as bad memory, headache and dizziness than controls. This prompted a further study in which the subjects were compared with another reference group, and here there were no differences in frequency of symptoms. In a test of psychomotor speed, the performance of exposed subjects was significantly worse than that of controls, but at the same time they were significantly better in other tests. The authors concluded that there was no evidence that methylene chloride in the given concentrations caused lasting damage to the central nervous system (22).

Among 33 English workers reported acutely poisoned by methylene chloride, 13 lost consciousness from the exposure and all the others experienced milder narcotic effects (10).

Four painters who had worked for an extended period in high (not further specified) concentrations of methylene chloride complained of weakness, fatigue, dizziness, headache, dullness and loss of interest in things that had interested them previously (24).

A chemist who had been exposed to an estimated 1750 mg methylene chloride/m^3 (500 ppm) or more for 5 years began to have acoustic and visual hallucinations (109). The symptoms disappeared when exposure was terminated.

A 52-year-old man who had been exposed to high concentrations of methylene chloride for 4 years was admitted to the hospital for mental confusion, and while there developed acute auditory hallucinations and delusions. These symptoms had been preceded

by a year of headaches, nausea and periods of shortness of breath that occurred during work and became worse as the working day progressed. He improved rapidly in the hospital but had a left-sided seizure before he was discharged. The EEG made after the attack showed a change in the right brain. When he was examined again after 6 weeks without exposure all the symptoms had disappeared and the EEG was normal (103).

There is one case report of bilateral temporal lobe atrophy in a 60-year-old man after three years of exposure to very high concentrations of methylene chloride (1000 to 3500 mg/m^3; 300 – 1000 ppm) (12).

People exposed to high concentrations of methylene chloride for long periods frequently complain of such symptoms as forgetfulness, concentration problems, insomnia, depression, neurasthenic problems, fatigue, headaches and loss of appetite (45, 62).

Fourteen flooring workers, whose exposure to methylene chloride was measured at between 1400 and 18,400 mg/m^3, had EEG changes in addition to the above symptoms. The EEG changes, slower rhythm and lower amplitude, were seen both immediately after exposure and after a weekend break in exposure (45).

Rats exposed to 245 mg methylene chloride/m^3 (70 ppm) six hours/day for 3 days showed an increase of noradrenalin metabolism in an area of the hypothalamus and a selective reduction of dopamine metabolism in an area of the pons. The same exposure also increased the level of luteotropic hormone in serum after the noradrenalin metabolism in the brain had been manipulated (39).

Other animal studies have revealed changes in amino acid levels and signs of cell death in the brains of rats after three months of continuous exposure to 730 mg methylene chloride/m^3 (210 ppm) (17, 91).

3.9. The peripheral nervous system

When volunteers sat with one thumb in a methylene chloride bath, the thumb became numb and cold after 10 minutes. The slightest movement caused intense pain. The symptoms lasted for about an hour after exposure was terminated (99). Workers exposed to high (not further defined) concentrations of methylene chloride were reported to have complained of paresthesias and numbness in the legs (24).

Workers exposed for a long time to methylene chloride in concentrations of 260 to 350 mg/m^3 (75 to 100 ppm) showed no differences from controls in conduction velocities in the nervus ulnaris or nervus medianus. The other studied neurophysiological variables in these nerves were also unaffected (22).

Rats given single intraperitoneal injections of methylene chloride in doses of 85, 170, 250 and 500 mg/kg showed a dose-dependent reduction in motoric conduction velocity in the nervus ischiadicus (81).

3.10. Reproductive organs

There is no information available.

3.11. Embryos

In a Finnish case-control study of women working in the pharmaceutical industry, the frequency of spontaneous abortions was higher among those exposed to methylene chloride. Women exposed for more than one week during the first trimester had a higher odds ratio (2.8) than women exposed for less than a week (odds ratio 2.0) (104).

Rats and mice were exposed to 4350 mg methylene chloride/m^3 (1250 ppm) 7 hours a day during the 6th to 15th day of pregnancy, and pups were examined for deformities. There were only minor differences between exposed animals and controls. The number of young with an extra ossification center in the sternum was higher in the exposed mice, and the number of young with retarded ossification of the sternum was higher in the exposed rats (94).

In another study, female rats were exposed to 15,700 mg methylene chloride/m^3 (4500 ppm) 6 hours/day, 7 days/week for three weeks before conception and during the first 17 days of pregnancy. The embryos showed no greater frequency of deformities than controls, but had a lower weight (46).

Other female rats were exposed in the same way. Their young were found to adapt more slowly to new environments than controls. These effects could be seen as early as 5 days of age, and lasted in the males until they were 5 months old (16).

When chicken eggs were treated with methylene chloride in doses of 25 to 100 micromol per egg, there was a significant increase in frequency of deformed embryos (33). In another study, in which chicken eggs were treated with methylene chloride in doses up to 14 mg/egg, no teratogenic effects were observed (111).

4. ALLERGY

No allergic effects of methylene chloride have been described.

5. GENOTOXIC EFFECTS

Methylene chloride had mutagenic effect in Ames' tests with Salmonella strains TA 98, TA 100, TA 1535 and TA 1950 (43, 44, 53, 69, 97), on yeast cells (20, 97) and in an experiment with Drosophila (43). However, it has shown no mutagenic effect in several experiments with mammalian cells (5, 19, 43, 56, 82). Methylene chloride caused a slight increase in the number of

sister chromatid exchanges in V79 cells from Chinese hamsters (56). In cultured ovarian cells from Chinese hamsters, however, it caused a large and dose-dependent increase in the number of chromosomal aberrations but no increase in sister chromatid exchanges (105). When embryo cells from Syrian hamsters were exposed to methylene chloride before exposure to adenovirus, they showed a greater increase in the number of transformed cell foci than did cells exposed to the adenovirus only (47).

Methylene chloride is mutagenic in Ames' tests, both with and without metabolic activation with various fractions of rat liver homogenate. The direct mutagenicity is probably related to a bacterial metabolism of methylene chloride to reactive intermediaries. This is the same kind of metabolism that occurs in mammalian cells (44). Experiments with methylene chloride and metabolic activation by rat liver homogenate indicate that increases in both cytochrome P-450 mediated metabolism and glutathione conjugation are associated with increased mutagenicity of methylene chloride (55).

In experiments with rat liver cells in vitro, methylene chloride alkylated neither DNA nor RNA but did alkylate lipids and proteins. Protein and lipid alkylation seemed to increase with biotransformation via both glutathione conjugate and P-450 metabolism (26).

The International Agency for Research on Cancer (IARC) has recently assessed the effects of methylene chloride in short-term tests for genetic activity. They concluded that there was "sufficient evidence" for such activity (51).

6. CARCINOGENIC EFFECTS

Methylene chloride has been studied for carcinogenicity in several animal studies.

In one study, rats and mice were given methylene chloride in drinking water for two years. The highest dose was about 250 mg/kg/day for both species. For the rats, this dose was calculated to be equivalent to 6 hours of inhalation exposure to about 2900 mg/m^3 (830 ppm) (57). Both male and female mice exposed to the highest dose showed toxic effects on the liver. An increase of liver cell tumors, not dose-related, was found in all exposed groups of male mice. However, this increase was comparable with levels in historical controls (96). Rats of both sexes showed a dose-related increase of foci with changed liver cells. Female rats in the highest and in the second-lowest (50 mg/kg/day) of the four dose groups had a higher number of liver cancers and/or benign liver-cell tumors than controls, but the tumor frequencies were comparable to that in historical controls (95). The authors concluded that methylene chloride showed no carcinogenic effects in the experiment.

In a study of Sprague-Dawley rats and Syrian hamsters, the animals were exposed to methylene chloride in concentrations of 0, 1740, 5220 or 12,200 mg/m^3 (0, 500, 1500 or 3500 ppm) 6 hours/day, 5 days/week for two years. The female rats showed a dose-dependent increase of benign mammary tumors. Increased frequencies of benign mammary tumors and malign salivary gland tumors were observed among male rats in the highest exposure group. An increase of salivary gland cancer, not statistically significant, was also noticed in female rats exposed to 5220 mg/m^3 (1500 ppm). The Syrian hamsters showed no increase in tumor frequency as a result of the exposure (19).

In a later study made by the same group, Sprague Dawley rats were exposed as above to concentrations of 0, 175, 700 or 1750 mg/m^3 (0, 50, 200 or 500 ppm). This study is available only in summary (70). An increase of benign mammary tumors, not statistically significant, was observed in females in the highest dose group.

The National Toxicology Program (NTP) in the U.S. has studied the carcinogenicity of methylene chloride for F344 rats and B6C3F1 mice of both sexes. The animals were exposed to methylene chloride 6 hours/day, 5 days/week for two years. Exposure levels were 3500, 7000 or 14,000 mg/m^3 (1000, 2000 or 4000 ppm) for the rats and 7000 or 14,000 mg/m^3 (2000 or 4000 ppm) for the mice. There was a dose-dependent increase in the number of animals with benign mammary tumors among the female rats. The increase was statistically significant even in the lowest exposure group. The number of male rats with such tumors was elevated only in the highest dose group. There was a dose-dependent increase in frequency of lung cancer in both male and female mice. A higher incidence of liver cancer was observed in male mice at both exposures, but females showed an increase only at the higher exposure. The NTP concludes that there is clear evidence that methylene chloride is carcinogenic for both male and female mice and for female rats, and that there are indications that it is also carcinogenic for male rats (75).

There are two published epidemiological studies that include study of cancer mortality among workers exposed to methylene chloride (38, 48, 49, 77). The first work contains a proportional mortality study of 334 deaths among active or retired workers who had been exposed to methylene chloride in a factory. The proportional mortality for all forms of cancer was the same as that observed in unexposed factory workers (38).

The 1013 males who had been exposed to methylene chloride during more than one year of employment in the same factory between 1964 and 1970 were also included in a cohort study. Follow-up through 1984 has revealed no over-frequency of total mortality due to total cancer (41 observed cases), compared either with other industrial workers (53 expected cases) or with the total population of the state of New York (59 expected cases). The observed numbers of different kinds of cancer were also in general lower than expected. Eight workers, however, had died of cancer of the pancreas, compared with about 3

expected. No dose-response relationship could be identified for this high mortality. The exposure conditions in the factory are described in section 3.7 (49).

In another study, the mortality pattern was examined in a cohort of 1271 factory workers who had been exposed to methylene chloride for at least three months between 1954 and 1977. The exposure conditions for this cohort are described in section 3.7. The cohort was followed until 1977, and no over-frequency of mortality from any form of cancer was observed, either in comparison with workers exposed to acetone or in comparison with the total population of the U.S. (77).

An assessment of the carcinogenicity of methylene chloride, based on the studies reviewed above, has recently been published by the IARC. The IARC concludes "there is sufficient evidence for the carcinogenicity of dichloromethane to experimental animals. There is inadequate evidence for the carcinogenicity of dichloromethane to humans" (51).

7. EXPOSURE INDICATORS

7.1. Air concentrations

Methylene chloride in air is generally sampled by adsorption on activated charcoal (72). The air is pumped through two carbon tubes connected in series (150 mg activated charcoal in each), at a speed of 0.01 to 0.21 liters/minute. The first tube is used for the analysis, and the second is a "back-up" or control. With this arrangement it is possible to see that the sampled substance has stayed in the analysis tube and has not, due to overloading or some other reason, leaked into the control tube. For an estimated air concentration of 1750 mg/m^3 (500 ppm), a suitable sample volume is 0.5 to 2.5 liters. After sampling, the methylene chloride is desorbed with carbon disulfide and the solution analyzed by gas chromatography with a flame ionization detector.

The method has been tested with a 1-liter air sample in the range 350 to 10,400 mg/m^3. No source of interference has been identified.

7.2. Biological indicators

7.2.1. Concentrations in alveolar air

During exposure, the concentration of methylene chloride in alveolar air is directly proportional to the amount of uptake (9, 30). The relation between the amount taken up and the concentration in alveolar air after a period of exposure has not been studied systematically. Some authors state that samples of alveolar air taken after termination of exposure can be used to calculate the time-weighted average value for the exposure (29, 83, 88, 101), but the individual results vary over a considerable range.

7.2.2. Carboxyhemoglobin

COHb levels resulting from methylene chloride exposure have been studied both in the laboratory (9, 83, 98, 101) and among occupationally exposed workers (30, 80, 87). Non-smokers have usually been studied, and unless stated otherwise the results reported below apply to non-smokers.

Carbon monoxide is formed within the body; endogenous COHb is normally 0.2 to 0.7%.

The COHb value increases with exposure to carbon monoxide.

Non-smokers who are not occupationally exposed to carbon monoxide can have COHb concentrations around 1 or 2%, while smokers often have levels over 5% (73).

COHb concentrations seem to vary considerably among persons with the same exposure to methylene chloride. In the study made

by Stewart et al in 1972, for example, three persons exposed to 3430 mg methylene chloride/m^3 (986 ppm) for two hours had maximum COHb concentrations of 7.5, 9 and 15% (98).

COHb levels rise during a period of exposure: in both subjects of laboratory experiments and workers occupationally exposed to methylene chloride, the highest levels are reached around the end of a day of exposure (30, 87).

With high exposures to methylene chloride – more than about 870 mg/m^3 (250 ppm) during rest – COHb levels can continue to rise even after termination of exposure. This is probably due to accumulation of unchanged methylene chloride in adipose tissue (30).

The elimination time for COHb in blood is longer after exposure to methylene chloride than after exposure to carbon monoxide, but varies considerably from person to person. For full-day exposures in the laboratory, it is only after exposure to the lowest concentration, 175 mg/m^3 (50 ppm), that there is a return to the initial COHb value within 24 hours after the initiation of the exposure. After exposures to air concentrations of 870 mg/m^3 (250 ppm) or less, 7.5 hours/day for 5 consecutive days, however, COHb values had returned to normal after a weekend without exposure (30, 71). Industrial workers exposed to an average 995 mg methylene chloride/m^3 (286 ppm) the day before sampling, and 637 mg/m^3 (183 ppm) on the day of sampling, had COHb values of 3.3 to 5.3 % at the beginning of work on the study day, and COHb values ranging from 3.6 to 4.9% 24 hours later (87).

With exposure to 175 mg/m^3 (50 ppm) 7.5 hours/day for up to 5 consecutive days, maximum COHb levels have reached 1.9 or 2.9%. The same exposure to 350 mg/m^3 (100 ppm) results in maximum COHb levels of 3.4 or 5.7 % (30, 71). At exposures of 520 and 700 mg/m^3 (150 and 200 ppm) the maximum COHb levels were 5.3 and 6.8%, respectively (30). The industrial workers mentioned

earlier had maximal COHb values ranging from 6.3 to 13% (49). COHb levels were measured for five consecutive days in three workers occupationally exposed to about 120 mg/m^3 (about 33 ppm) (8-hour time-weighted average). The highest value registered was 2.5% (30).

Among workers occupationally exposed to about 350 mg/m^3 (100 ppm) methylene chloride (8-hour time-weighted average), those who did not smoke had an average 3% COHb after a work shift, while smokers with the same exposure had about 6% (80).

As mentioned earlier, uptake of methylene chloride increases as physical exertion increases. At exposure to 350 mg methylene chloride/m^3 (100 ppm), maximum COHb levels are twice as high during medium-heavy or heavy work as during rest. However, heavy work does not yield higher COHb concentrations than medium-heavy work (31). The COHb originating from methylene chloride is additive to that from other sources such as tobacco smoke (31, 101).

As mentioned earlier, the biotransformation of methylene chloride to carbon monoxide seems to be saturated at exposures over about 875 mg/m^3 (about 250 ppm). This is the case for man as well as for rats and hamsters. In rats, carbon monoxide formation from methylene chloride seems to yield a maximum of about 10% COHb (group average) (19, 61). Hamsters can show considerably higher levels, about 25% COHb (group average) (19). Data from a group of non-smoking industrial workers indicate that the average maximum COHb level for such a group probably doesn't exceed 10%. For a corresponding group of smokers, the average maximum COHb level is apparently about 15% (80).

8. EXPOSURE-EFFECT AND EXPOSURE-RESPONSE RELATIONSHIPS

The effects of methylene chloride in animal experiments and on man are summarized in tables 1 and 2.

Table 1. Summary of effects of methylene chloride on experimental animals.

Exposure	Effects (Reference)
14,000 mg/m^3 (4000 ppm)	Increase of liver cancer in female mice after exposure 6 hours/day, 5 days/week for 2 years (75).
13,000 mg/m^3 (3700 ppm)	Cell damage and inflammatory response in lungs of rats after exposure 5 hours/day, 5 days/week for 4 weeks (92).
12,000 to 14,000 mg/m^3 (3500 to 4000 ppm)	Increase of benign mammary tumors in female Sprague Dawley rats after exposure 6 hours/day, 5 days/week for 2 years (19, 75).
12,000 mg/m^3 (3500 ppm)	Increase of malignant salivary gland tumors in male rats exposed 6 hours/day, 5 days/week for 2 years (19).
7000 mg/m^3 (2000 ppm)	Increase of lung cancer in male and female mice and of liver cancer in male mice after exposure 6 hours/day, 5 days/week for 2 years (75).
1750 mg/m^3 (500 ppm)	Increase of benign mammary tumors in female Sprague Dawley rats after exposure 6 hours/day, 5 days/week for 2 years. Cirrhosis in rats after exposure 6 hours/day, 5 days/week for 1 year (19).
350 mg/m^3+ (100 ppm)+ streptococcus aerosol	Increased receptivity to infection in mice simultaneously exposed to both for 3 hours (8).

8.1. Effects of short-term exposure

Among the 33 English workers reported acutely poisoned by methylene chloride, 13 had lost consciousness from the exposure and all the others experienced milder narcotic effects. Four of them experienced effects on respiratory organs. Signs of liver damage were reported in one case, but signs of effects on the heart were not reported in any of the cases (10).

When human volunteers were exposed to 700 mg methylene chloride/m^3 (200 ppm), after 1.5 to 2 hours their results were

significantly worse in a reaction time test for stimuli in the periphery of the vision field and in an eye-hand coordination test given simultaneously. Results in a vigilance test for auditory stimuli were significantly worse after 4 hours of exposure (85). Exposure to about 1000 mg/m^3 (300 ppm) affected results in two psychometric tests, "Critical Flicker Fusion" and an alertness test, after two hours (112).

High (not further specified) short-term exposure to methylene chloride has been reported to irritate mucous membranes of the eyes and upper respiratory passages (24, 50, 68), and lung edema has also been described (18, 50).

Intravasal hemolysis has been observed after attempted suicide by peroral intake of methylene chloride (15, 90).

Skin contact with liquid methylene chloride gives an intense sensation of cold (50, 99) and pain in the exposed area (99). Methylene chloride can also cause chemical burns on skin (110).

Table 2. Summary of effects of methylene chloride on man

Exposure	Effects (Reference)
1660 mg/m^3 (475 ppm)	Increase of hemoglobin and hematocrit values and increased number of red blood cells in women after long-term exposure (78).
700 mg/m^3 (200 ppm)	Test subjects showed declines in performance on some psychometric tests after 1.5 to 4 hours of exposure (85).
210 to 1660 mg/m^3 (60 to 475 ppm)	Dose-dependent increase of bilirubin level in serum of subjects with long occupational exposures (78).
350 mg/m^3 (100 ppm)	Eight hours of exposure yields an average 3% COHb level in non-smokers. Highest measured COHb values after 7.5 hours of exposure are 3.4 or 5.7% COHb (30, 71).
175 mg/m^3 (50 ppm)	Highest measured COHb values after 7.5 hours of exposure were 1.9 or 2.9% (30, 71).

There are case reports of heart attacks (100) and ECG changes (13) in connection with exposure to high concentrations of methylene chloride.

Subjects exposed to 350 mg methylene chloride/m^3 (100 ppm) 7.5 hours/day for 5 consecutive days had maximum COHb levels of 3.4 or 5.7%, and the COHb value dropped to its original level within 24 hours of the beginning of exposure. Exposure to 175 mg/m^3 (50 ppm) under similar conditions resulted in COHb levels of maximum 1.9 or 2.9%, and returned to initial values within 24 hours of the beginning of exposure (30, 71).

Rats exposed to 245 mg/m^3 (70 ppm) 6 hours/day for three days showed changes in metabolism of some transmitter substances in specific areas of the brain (39).

Mice exposed to 350 mg/m^3 (100 ppm) methylene chloride for 3 hours showed an increased receptivity to infection (8).

8.2. Effects of long-term exposure

At some time between 1964 and 1970, 1013 men had worked in a factory where they were exposed to methylene chloride. A follow-up study of these men from 1964 to 1984 has revealed no over-frequency of mortality due to ischemic heart disease or to total cancer. However, eight cases of pancreas cancer were observed, compared with about 3 expected. The cohort's average exposure to methylene chloride was about 100 mg/m^3 (30 ppm) (49).

Mortality was followed until 1977 in a cohort of 1271 men who had been exposed to methylene chloride during at least three months of employment between 1954 and 1977. The average methylene chloride exposure in different areas of the factory is estimated to have ranged from 490 to 1650 mg/m^3 (140 to 475 ppm). No over-frequency of mortality due to ischemic heart disease or to any form of cancer was observed in the cohort

when compared with expected values generated from the total U.S. population (77).

Persons exposed to high concentrations of methylene chloride for long periods have complained of forgetfulness, concentration problems, disturbed sleep, depression, neurasthenic problems, fatigue, headache and loss of appetite (45, 62, 109). Reported exposure levels in these cases have been 100 to 1400 mg/m^3, more than 1750 mg/m^3 (500 ppm), and 1400 to 18,400 mg/m^3. Fourteen flooring workers exposed to the last-named concentrations all showed EEG changes, even after a weekend without exposure (45). However, no over-frequency of neuropsychiatric symptoms or deterioration of results in psychometric tests was observed in workers exposed to 260 and 350 mg/m^3 (75-100 ppm) (22).

A dose-dependent increase in benign mammary tumors was observed in female rats in two carcinogenicity tests with methylene chloride. Both male and female rats had been exposed to 1750, 5200 or 12,000 mg/m^3 (500, 1500 or 3500 ppm), or to 3500, 7000 or 14,000 mg/m^3 (1000, 2000 or 4000 ppm) for 6 hours/day, 5 days/week for two years (19, 75). In both studies, there was an increased frequency of benign mammary tumors among male rats in the highest dose group. In the first study, males in the highest dose group also showed a higher frequency of salivary gland cancer. Both male and female mice exposed by the same schedule to 7000 and 14,000 mg/m^3 (2000 and 4000 ppm) showed a dose-dependent increase of lung cancer. There was a dose-dependent increase of liver cancers among male mice, but females showed a higher frequency of this cancer form only in the highest dose group (75).

9. RESEARCH NEEDS

There are no studies regarding the effect of methylene chloride on reproductive organs. The substance has been inadequately studied for teratogenic effects on humans. There is a need for

mechanistic studies of the mutagenic and carcinogenic activity of methylene chloride. There are no studies of genotoxic effects on man. More epidemiological studies, on larger exposed populations and with longer follow-up, are needed to evaluate the carcinogenic effects of methylene chloride in man.

10. DISCUSSION AND EVALUATION

Methylene chloride is taken up by the skin much more readily than many other solvents (99, 107).

Methylene chloride can irritate the mucous membranes of the eyes and the upper respiratory passages (10, 24, 50, 62, 68). At high concentrations – high enough to also cause other symptoms of poisoning – methylene chloride may also cause lung edema (18, 50). Rats exposed to 350 mg methylene chloride/m^3 (100 ppm) for three hours were more receptive to infection (8).

Methylene chloride in high concentrations has, like other solvents, a narcotic effect, and can cause loss of consciousness (10, 68, 90). People exposed to high concentrations for long periods have shown symptoms corresponding to the pathological picture of chronic toxic encephalopathy (12, 24, 45, 62, 109). The information in these studies is of limited value, however. Two of the studies are cross-sectional studies with no control group (45, 62), and three of them are case reports (12, 24, 109).

In laboratory experiments, methylene chloride in concentrations of 700 mg/m^3 (200 ppm) or higher has been observed to affect the results of psychometric tests (85). Workers exposed to methylene chloride in concentrations of 260 to 350 mg/m^3 (75-100 ppm) (8-hour time-weighted average) showed no increase in frequency of neuropsychiatric symptoms and did no worse on psychometric tests than controls (22). Rats exposed to 245 mg methylene chloride/m^3 (70 ppm), 6 hours/day for three days, have shown altered metabolism of certain transmitter substances

in specific areas of the brain, but the significance of this is not clear (39).

Methylene chloride was long considered to be relatively atoxic, and the exposure limits were high in relation to those for many other solvents. When Stewart et al discovered in 1972 (98) that methylene chloride was biotransformed to carbon monoxide, exposure limits began to be reduced. In Western European countries the exposure limit for carbon monoxide has been set to avoid COHb values over 5% (73).

Exposure to 350 mg methylene chloride/m^3 for 7.5 hours/day during 5 consecutive days has yielded a maximum 3.4 or 5.7% COHb in non-smokers. COHb levels returned to normal during a weekend without exposure. A work week of exposure to 175 mg/m^3 results in a maximum COHb value of 1.9 or 2.9%. COHb values then return to normal within 24 hours of the beginning of exposure (30, 71). Industrial workers (non-smokers) exposed to about 350 mg/m^3 (100 ppm) (8-hour time-weighted average) had an average 3% COHb at the end of a work day (80). The halving time for COHb is longer with exposure to methylene chloride than with exposure to pure carbon monoxide (30, 83, 87, 98, 101). Carboxyhemoglobin from methylene chloride is additive to COHb from other sources (31, 101). There are case reports of heart attacks (100) and ECG changes (13) in connection with exposure to high concentrations of methylene chloride. Long-term exposure to an average 100 mg/m^3 (30 ppm) or to 500 to 1650 mg/m^3 (140 to 475 ppm) has not been shown to correlate with increased mortality due to heart disease (49, 77). No abnormalities were seen in 24-hour ECGs of workers exposed to the latter concentrations (79).

Methylene chloride is mutagenic in Ames' tests (43, 44, 53, 69, 97) and for yeast cells (20, 97) but in general not for mammalian cells (5, 19, 43, 56, 82). Rats exposed to methylene chloride by inhalation, 6 hours/day, 5 days/week for two years, showed a dose-dependent increase of benign mammary tumors (in

females) in two studies (19, 75). This increase was seen at the lowest concentrations: 1750 and 3500 mg/m^3 (500 and 1000 ppm) respectively. Male rats in the two studies showed a higher frequency of these tumors at the highest air concentrations: 12,000 and 14,000 mg/m^3 (3500 and 4000 ppm). In one of the studies, the number of male rats with salivary gland tumors was also higher at the highest air concentration (19). Male and female mice exposed as above to 7000 or 14,000 mg methylene chloride/m^3 (2000 or 4000 ppm) showed a dose-dependent increase in frequency of lung cancers. There was an increase of liver cancer in male mice in both exposure groups, and in females in the high-dose group (75).

Two cohorts, one comprising about 1,000 workers exposed to an estimated average 100 mg methylene chloride/m^3 (30 ppm) and the other of 1,250 workers with estimated exposures of 200 to 1650 mg/m^3 (60 to 475 ppm), showed no over-frequencies of total cancer (49, 77). In the first of these cohorts, however, there was an increased mortality from cancer of the pancreas (8 cases vs. about 3 expected).

The risk of effects on the central nervous system or other organs, and the risk of COHb levels over 5% (for non-smokers), is probably small if exposure to methylene chloride is below about 350 mg/m^3 (100 ppm) (8-hour time-weighted average). Methylene chloride is mutagenic in tests with bacteria. The IARC has reviewed the reports of cancer tests and stated that methylene chloride is carcinogenic to experimental animals (51). Methylene chloride should therefore be, at least for the present, regarded as though it were carcinogenic also for man.

In discussions of occupational exposure limits for methylene chloride, its possible carcinogenic effects should be given primary consideration. Its effects on COHb levels and on the central nervous system should also be taken into consideration.

11. SUMMARY

I. Lundberg: Methylene chloride. 76. Nordic expert group for documentation of exposure limit values.

The literature concerning health effects relevant to a hygienic standard for methylene chloride has been reviewed. Methylene chloride is biotransformed to carbon monoxide and carboxyhemoglobin is formed. Exposure to methylene chloride may impair the performance in psychometric tests. The present exposure limit value is based on these effects.

Methylene chloride is a mutagen in bacterial test systems and is also carcinogenic to experimental animals. The possible carcinogenic effects of methylene chloride must be of prime concern when a new exposure limit value for methylen chloride is discussed. The formation of carboxyhemoglobin and the neuropsychiatric effects should also be considered.

A Swedish version is available; 113 references.
Key words: Methylene chloride, exposure limit value, cancer, carboxy-hemoglobin, neuro-psychiatric effects.

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APPENDIX I. Occupational exposure limits for airborne methylene chloride.

Country	mg/m^3	ppm	Year	Note	Ref
BRD	360	100	1985		5
Denmark	175	50	1985	S	2
Finland	350 525	100 250	1981 -"-	 15 min	10
France	360 1800	100 500	1986 -"-	8 tim 15 min	11
Iceland	350	100	1978		8
Netherlands	350 1750	100 500	1986 -"-	 STV	7
Norway	245	70	1984		1
Soviet Union	50		1978		6
Great Britain	350 870	100 250	1986 -"-	 STEL	4
Sweden	250 500	70 150	1985 -"-	S STV	3
USA (ACGIH)	175	50	1986-87	C	9

C= suspected carcinogen

S = skin

STV = short-term value

STEL = short-term exposure limit (10 min)

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Appendix. Documents published by the Nordic Expert Group:

Acetaldehyde	69	Arbete och Hälsa	1986:25
Acetone	72	"	1986:39
Acrylates and methacrylates	42	"	1983:21
Acrylonitrile	55	"	1985: 4
Allyl alcohol	65	"	1986: 8
Ammonia	71	"	1986:31
Arsine	73	"	1986:41
Asbestos	39	"	1982:29
Benomyl	50	"	1984:28
Benzene	20	"	1981:11
Boric acid and borax	13	"	1980:13
1-Butanol	17	"	1980:20
Cadmium	27	"	1981:29
Carbon monoxide	12	"	1980: 8
Chlorine and chlorine dioxide	11	"	1980: 6
Chloromequat chloride	52	"	1984:36
Chlorophenols	54	"	1984:46
Chromium	8	"	1979:33
Cobalt	32	"	1982:16
Copper	18	"	1980:21
Cyclohexanone and cyclopentanone	63	"	1985:42
n-Decane and n-undecane	75	"	1987:25
Deodorized kerosene	61	"	1985:24
Diisocyanates	9	"	1979:33
-"-	58	"	1985:19
Dimethylformamide	38	"	1982:28
Dioxane	28	"	1982: 6
Epichlorohydrin	19	"	1981:10
Ethylbenzene	67	"	1986:19
Ethylene glycol	14	"	1980:14
Ethyleneglycolmonoalkyl ethers and their acetates	62	"	1985:34
Ethylene oxide	29	"	1982: 7
Formaldehyde	1	"	1978:21
-"-	37	"	1982:27