

Draft

Acute Exposure Guideline Levels (AEGLs)

for

**Carbon Disulfide
(CAS Reg. No. 75-15-0)**

S=C=S

All individuals and organizations in attendance at the NAC/AEGL meeting on March, 7th-8th, 2003, interested in submitting comments/information regarding the above chemical should send hard copies to

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February 2003

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PREFACE

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

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. Three levels — AEGL-1, AEGL-2 and AEGL-3 — are developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m³]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m³) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m³) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

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EXECUTIVE SUMMARY

Pure carbon disulfide (CS₂) is a colorless, mobile, refractive liquid with a sweetish aromatic odor, similar to chloroform. Commercial and reagent grade products are yellowish with an unpleasant repulsive odor of decaying radish. Owing to its high volatility, low flash point, low autoignition temperature, and the wide range of explosive limits in air, CS₂ poses an acute fire and explosion hazard. The most important industrial use of CS₂ has been in the manufacture of regenerated cellulose rayon by the viscose process.

A wide range of odor thresholds from 0.0243 mg/m³ to 23.1 mg/m³ (0.0078 – 7.4 ppm) for CS₂ were reported. Amoore and Hautala (1983) reported a geometric mean air odor threshold of 0.11 ppm (standard error 0.058 ppm) for CS₂ which was derived from six references without giving detailed information. Leonardos et al. (1969) determined an odor recognition threshold of 0.21 ppm. AIHA (1997), in a critical overview of odor thresholds for chemicals, reported a range of all referenced values from 0.016 – 0.42 ppm. No geometric mean and no “range of acceptable values” for CS₂ were presented, and the use of the 0.21 ppm threshold was rejected because it represented a 100 % recognition concentration. Few data are available with respect to concentrations causing odor annoyance. In one controlled human study (Lehmann 1894), 180 – 240 ppm caused “moderate odor annoyance”, while there were no complaints in a toxicokinetic study at exposure to 10 – 20 ppm (Rosier et al. 1987).

The database is not sufficient to calculate a level of distinct odor awareness (LOA). It must also must be taken into account that strong smelling decomposition products of CS₂ are rapidly formed under the influence of light and air. Therefore, the odor threshold and the hedonic tone of CS₂ will markedly change with the presence and formation of such impurities.

CS₂ is rapidly absorbed from the respiratory tract and distributed throughout the body, the highest concentration occurring in lipid rich tissues. Dithiocarbamates and similar products build up the so called “acid labile” CS₂ by the reaction of CS₂ with NH₂-, SH-, and OH-groups of amino acids, proteins, and amines. While unbound CS₂ is eliminated rapidly after the termination of exposure, the acid labile part shows a longer half life and may accumulate at repeated exposure.

On acute exposure, CS₂ acts on the central nervous system (CNS) in humans and animals. In humans, acute effects on the CNS following CS₂ exposure manifest in dizziness, headaches, autonomic nervous system reactions, nausea, vertigo, vomiting, central paralysis, and narcosis. In animals (rats, mice, rabbits, cats, dogs), acute exposure led to reduced activity but also hyperexcitability, stupor, ataxia, tremors, convulsions, deep narcosis, and finally respiratory arrest and death. Irritation of eyes and mucous membranes occur only at concentrations already affecting the CNS. However, low concentrations without notable effects on the CNS already lead to an inhibition of xenobiotic biotransformation reactions, inhibition of alcohol metabolism via the alcohol/aldehyde dehydrogenase pathway, and alterations of carbohydrate and energy metabolism in the liver.

In several toxicokinetic studies in humans, occasional slight headaches but no other subjective symptoms were reported to occur in some individuals at exposure concentrations in the range of 17 – 51 ppm (Harashima and Masuda 1962; Teisinger and Soucek 1949). Inhibition of biotransformation was observed in humans after 6-hour exposure to 10 ppm CS₂, the lowest concentrations tested (Mack et al. 1974). In rats, 8 hour exposure to 20 ppm, the lowest concentration tested, also inhibited biotransformation of drugs and solvents and caused a decrease of the glycogen content of the liver. All effects were rapidly reversible within about 24 hours and no increase of liver enzymes in serum was observed (Freundt and Kürzinger 1975; Kürzinger and Freundt 1969; Freundt and Dreher 1969; Freundt and Kuttner 1969; Freundt et al. 1976a; Freundt et al. 1974b; Freundt and Schauenburg 1971). In one controlled human study, two volunteers (medical students studying the toxicity of CS₂ during the course of their own PhD) were exposed to concentrations from about 180 ppm to more than 3000 ppm (Lehmann

1894). Exposure to about 500 ppm for 3 hours 50 minutes caused dizziness, anxiety, persisting headaches, temporary impairment of reading ability, lacrymation, and cough attacks. Most of the effects persisted several hours after the end of exposure. Exposure to 2000 ppm for 1 hour caused severe intoxication with difficulty to perform tasks, anxiety, nausea, progressing dizziness, and the beginning of marked central paralysis. After exposure, staggered gait, strong dazed feeling, autonomic nervous system reactions (sudden salivation, increased pulse), vomiting and up to 2 days of feeling ill were recorded. One exposure in one subject to 2180 – 3370 ppm for 1.5 hours caused strong dizziness, nausea, seminarcotic state, and irregular respiration.

The AEGL-1 values are based on studies investigating CS₂ induced inhibition of alcohol metabolism in humans (Freundt et al. 1976b; Freundt and Lieberwirth 1974a). In this controlled study, volunteers were exposed to 20 ppm CS₂ for 8 hours by inhalation and simultaneously or afterwards took in alcoholic beverages to obtain a blood alcohol level of 0.75 ‰. Each person served as its own control. CS₂ exposure caused a 50 – 100 % increase in acetaldehyde in blood compared to conditions without CS₂. The effect occurred when alcohol was taken up during the CS₂ exposure, and similarly when the alcohol uptake started 8 hours after the end of CS₂ exposure. The observed increase of acetaldehyde was asymptomatic, i.e., no “antabuse syndrome” (flush, hypotension, tachycardia) was observed. However, alcohol intolerance has repeatedly been mentioned in workers occupationally exposed to unknown (most probably higher concentrations) of CS₂, and in its guidelines, the German Society for Occupational and Environmental Medicine states alcohol intolerance as a further adverse effect induced by CS₂ (Drexler 1998).

An intraspecies factor of 10 was applied to account for the protection of sensitive population subgroups with an acetaldehyde dehydrogenase (ALDH2(2)) less active than the typical form. The presence of the ALDH2(2) allele (which is common in Asians but rare or absent in Caucasians) results in higher levels of acetaldehyde after ingestion of alcohol compared to persons in which the normal enzyme is present. An additional increase of the acetaldehyde concentration due to exposure to CS₂ may thus lead to an “antabuse syndrome” or aggravate otherwise mild symptoms. Extrapolation was made to the relevant AEGL time points using the relationship $C^n \times t = k$ with the default of $n = 3$ for shorter exposure periods, due to the lack of experimental data for deriving the concentration exponent. For the AEGL-1 for 10 minutes, the AEGL-1 for 30 minutes was applied because the derivation of AEGL values was based on a study with a long experimental exposure period of 8 hours and no supporting studies using short exposure periods were available characterizing the concentration time-response relationship. The derived AEGL-1 values are above the odor thresholds but below concentrations reported to cause moderate odor annoyance (see above).

The derivation AEGL-2 is based on the NOEL of 1000 ppm for behavioral alterations in rats exposed to CS₂ for 4 hours (Goldberg et al. 1964). At the next higher concentration of 2000 ppm, an inhibition of the escape (and also the avoidance) response was observed. A total uncertainty factor of 10 was used. The interspecies uncertainty factor was reduced to 3 because of the similarity of acute effects seen in rodents compared to humans produced by agents affecting the CNS. Moreover, use of a default interspecies uncertainty factor of 10 would have resulted in values which are contradicted by experimental human studies in which no serious or escape-impairing effects were reported during or following 6 – 8 hours of exposure to 80 ppm. An intraspecies uncertainty factor of 3 was applied to account for sensitive individuals because the threshold for CNS impairment is not expected to vary much among individuals. Time scaling was performed according to the regression equation $C^n \times t = k$, using the default of $n = 3$ for shorter exposure periods (30 minutes and 1 hour) and $n = 1$ for longer exposure periods (8 hours), due to the lack of suitable experimental data for deriving the concentration exponent. For the 10-minute AEGL-2 the 30-minute value was used because the derivation of AEGL-2 values was based on a long experimental exposure period (4 hours) and no supporting studies using short exposure periods were available for characterizing the concentration-time-response relationship.

The AEGL-3 was based on a study with rats (Du Pont 1966). In that study, all six animals exposed to 3500 ppm for 4 hours died during or within 2 hours after exposure whereas none of six rats exposed to 3000 ppm died during the exposure or within the 14-day postexposure observation period. A total uncertainty factor of 10 was used. An interspecies uncertainty factor of 3 was applied because the acute effects on the CNS are not expected to vary much between species. Moreover, use of a default interspecies uncertainty factor of 10 would have resulted in values which are contradicted by experimental human studies in which no life-threatening effects were reported during or following 6 – 8 hours exposure to 80 ppm. An intraspecies uncertainty factor of 3 was applied to account for sensitive individuals because the threshold for CNS impairment is not expected to vary much among individuals. Time scaling was performed according to the regression equation $C^n \times t = k$, using the default of $n = 3$ for shorter exposure periods (30 minutes and 1 hour) and $n = 1$ for longer exposure periods (8 hours), due to the lack of suitable experimental data for deriving the concentration exponent. For the 10-minute AEGL-3 the 30-minute value was used because the derivation of AEGL-3 values was based on a long experimental exposure period (4 hours) and no supporting studies using short exposure periods were available for characterizing the concentration-time-response relationship.

SUMMARY TABLE OF AEGL VALUES FOR CARBON DISULFIDE [ppm (mg/m ³)] ^a						
Classification	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour	Endpoint (Reference)
AEGL-1 (Nondisabling)	5.0 (16)	5.0 (16)	4 (12)	2.5 (7.8)	2.0 (6.2)	Increase in blood acetaldehyde in humans with moderate intake of alcohol (Freundt et al. 1976b)
AEGL-2 (Disabling)	200 (620)	200 (620)	160 (490)	100 (310)	50 (160)	NOEL for behavioral changes in rats (inhibition of escape response) (Goldberg 1964)
AEGL-3 (Lethality)	600 (1480)	600 (1480)	480 (990)	300 (930)	150 (470)	No lethality in rats (Du Pont 1966)

^a: Cutaneous absorption may occur. Liquid CS₂ is a severe skin irritant and direct skin contact with the liquid must be avoided.

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1 INTRODUCTION

Pure carbon disulfide (CS₂) is a colorless, mobile, refractive liquid with a sweetish aromatic odor, similar to chloroform. Under the action of light (and air), CS₂ is decomposed with the formation of yellow decay products and a disagreeable odor. Similarly, commercial and reagent grade products are yellowish with a repulsive odor of decaying radish (WHO 1979). The odor was also described as “disagreeable, sweet” (Ruth 1986).

CS₂ is released into the environment from natural sources such as soil, marshes, lakes, and volcanoes. The total global emission of CS₂ and the anthropogenic share of the total emission is not well-known. However, according to more recently modelled scenarios, it is suggested that the majority of CS₂ may be produced through human activity, rather than naturally (Government Canada 2000).

CS₂ was discovered by Lampadius in 1796 by heating a mixture of pyrite (FeS₂) and charcoal. Commercially, CS₂ has been prepared by directing sulfur vapor over glowing coals. In the Western industrial countries, this process has been replaced by the reaction of methane and sulfur at temperatures between 500 and 700 °C and a pressure between 4 and 9 bar (“methane process”). The CS₂ is separated from H₂S and by-products by liquefaction, distillation and treatment with sodium hydroxide. The product thus purified contains a maximum of 0.02 % impurities (BUA 1993).

About 1 million tons of carbon disulfide were produced commercially worldwide in 1984. Since that time, production has been decreasing and was estimated at about 900 000 tons in 1990 (BUA 1993). The most important industrial use of CS₂ has been in the manufacture of regenerated cellulose rayon by the viscose process and of cellophane. CS₂ has also been used for the production of carbon tetrachloride which served as a starting chemical for the synthesis of fluorocarbon propellants and refrigerants (ATSDR 1996). This application has been of declining importance in recent years. Smaller amounts of CS₂ are needed as solvent, e.g. in the purification of sulfur, and for the manufacturing of dithiurams, di- and trithiocarbamates used as fungicides and vulcanization accelerators, for the manufacture of xanthates, which are used as flotation agents in mineral refining processes, and for the synthesis of some other sulfur compounds. CS₂ has also been used for soil fumigation, e.g. in viticulture for fighting vine lice, and in veterinary medicine (BUA 1993; Government Canada 2000).

Chemical and physical properties of carbon disulfide (CS₂) are presented in Table 1. Owing to its high volatility, low flash point, low autoignition temperature, and the wide range of explosive limits in air, CS₂ poses an acute fire and explosion hazard.

TABLE 1: CHEMICAL AND PHYSICAL DATA

Parameter	Data	Reference
Synonyms	Carbon bisulphide, carbon disulphide, carbon sulfide, dithiocarbonic anhydride, sulphocarbonic anhydride	HSDB 2001
Chemical formula	CS ₂	
Molecular weight	76.14 g · mol ⁻¹	ATSDR 1996
CAS Reg. No.	75-15-0	ATSDR 1996
Physical state	Liquid at room temperature	ATSDR 1996
Solubility	2.94 g/L in water (20 °C); soluble in alcohol, benzene, ether	ATSDR 1996; Beauchamp et al. 1983
Vapor pressure	400 mm at 28 °C 300 mm at 20 °C 100 mm at -5.1 °C 40 mm at -22.5 °C	DFG 1975 Weast 1973
Vapor density (air = 1)	2.62	Beauchamp et al. 1983
Liquid density (water = 1)	1.2632 (20 °C)	Weast 1973
Melting point	-111.53 °C	Weast 1973
Boiling point	46.25 °C	Weast 1973
Explosive limits in air	1 – 50 %	Beauchamp et al. 1983
Flash point	-29.62 °C	Beauchamp et al. 1983
Autoignition temperature	100 °C	Beauchamp et al. 1983
Conversion factors (at 25 °C)	1 ppm = 3.114 mg/m ³ 1 mg/m ³ = 0.321 ppm	Calculated according to NRC 2001

2 HUMAN TOXICITY DATA

2.1 Acute Lethality

According to Flury and Zernik (1931), exposure to very high concentrations of CS₂ is followed by acute disturbance of consciousness, delirium, loss of reflexes including loss of pupil reaction, total paralysis, and respiratory arrest. The authors state that exposure to 4800 ppm CS₂ for 30 minutes to 1 hour will immediately or later lead to death, while 3200 – 3850 ppm CS₂ over the same period of time will be life-threatening. The same statement is made by Bittersohl (1972). Furthermore, it is stated that “hyperacute intoxication” with very high concentrations exceeding 10 mg/L (3200 ppm) will immediately lead to loss of reflexes, coma and death. No details or references are presented.

2.1.1 Case Reports

Death has been reported in a community in India following an accidental release of large amounts of CS₂, hydrogen sulfide, and sulfuric acid from a viscose rayon plant (Kamat 1994). Due to the lack of exposure data and the concomitant exposure to other chemicals, no conclusions valid for the derivation of AEGL values can be derived from these data.

Non-inhalation exposure

There are few reports of acute poisonings following oral ingestion. In a fatal case in which the patient had swallowed “a glass” of CS₂, the victim soon became unconscious and died about two hours after drinking the liquid (Davidson and Feinleib 1972). Generally, 30 – 60 mL are reported to be fatal (WHO 1993). However, ingestion of about 18 g CS₂ (about 15 mL) was reported to be lethal in three occasions. Prior to death, spasmodic tremors, prostration, dyspnoea, cyanosis, peripheral vascular collapse, hypothermia, mydriasis, convulsions and coma developed. Death occurred within a few hours (HSE 1981).

2.2 Nonlethal Toxicity

Without giving any references, Bittersohl (1972) states that at about 300 ppm, slight symptoms in humans will occur after several hours of exposure, marked symptoms of intoxication at 400 ppm, severe symptoms at 1150 ppm after 30 minutes, and life-threatening effects at 3200 – 3800 ppm. Furthermore, it is stated that acute intoxications at concentrations higher than 1 mg/L (320 ppm) will lead to narcosis lasting for minutes followed by severe headaches and nausea. No details nor any references are presented in this textbook but some of the data agree with those presented in the summarizing table in Lehmann (1894). This table is also presented by Flury and Zernik (1931) and Lehmann and Flury (1938), and the same values are repeatedly presented by other reports (AIHA 1992; NRC 1984; OSHA 2000).

2.2.1 Case Reports

In an accident, about 30 000 L of carbon disulfide spilled from a broken railroad tank car. As a result of this spill, about 500 people were temporarily evacuated from the adjacent area. Five people were seen at a local hospital, and one of them was admitted. During inspection of the contaminated area, a flash fire occurred in which four people were trapped for a short period of time but no injuries resulted. No further details were reported (NTSB 1998).

Spyker et al. (1982) report of a further accident in which carbon disulfide leaking from a railroad tank car caught fire and was extinguished. An airborne concentration of 20 ppm CS₂ was measured at a site outside the town later during transfer of CS₂ from the leaking tanker, but no measurement data were reported from the town or from the area during emergency operations. About 600 residents of an adjacent area were evacuated. 27 subjects, mostly police and firefighters, who were exposed to unknown concentrations of CS₂ were examined at hospital. Most of the victims complained of headaches (16/27), nausea (14/27), and dizziness (16/27). Burning of throat, lips, and skin (11/27) and shortness of breath or chest pain (4/27) also occurred, two victims complained of impotence, and vomiting was seen in one. Spirometry, single breath CO-diffusing capacity, and arterial blood gases measurements were made in all four victims with shortness of breath or chest pain, and in seven others who appeared clinically to be the most severely ill. Vital capacity and the partial pressure of arterial O₂ were lower at the day of exposure than 9 days later. No significant changes were observed in forced vital capacity, forced expiratory volume, or in diffusing capacity. None of the patients evaluated appeared to have sustained injury lasting beyond the first few postexposure days (Spyker et al. 1982). It is not reported but likely that

these victims were exposed not only to CS₂, but also to the toxic and irritant products of CS₂ burning, especially sulfur dioxide (SO₂) and acid mists.

Following acute exposure to high concentrations of CS₂, fainting and loss of consciousness was observed in about one third of 123 victims in an accidental release of large amounts of CS₂, hydrogen sulfide, and sulfuric acid from a viscose rayon plant in India (Kamat 1994).

Kruse et al.. (1982) report a case in which a healthy 48-year-old man was exposed for 20 minutes to an unknown concentration of CS₂ (possible concentration range 400 – 470 000 ppm) as a result of a laboratory fire. He was unconscious for 10 minutes after the exposure. Later on, serious persistent cerebral deterioration developed. Computerised tomography scanning showed cerebral atrophy, neuro-psychological examination established dementia, and measurement of cerebral flow showed reduced cortical flow in the right hemisphere. Symptoms were still present 21 months after the exposure. The observed encephalopathy may be due to the direct toxic effects of CS₂, or its metabolites, on the brain or to sequelae after anoxia during unconsciousness.

Non-inhalation exposure

A 42-year-old woman who had used CS₂ for a few years to control insects in warehouses accidentally ingested about 5 mL of CS₂ from a used soft drink bottle (Yamada 1977). After 5 hours of induced vomiting, numbness in the lips and nausea and non-induced vomiting occurred. Within 12 hours, abdominal pain, pyrexia, and wave form agitation appeared, and she was hospitalized with conspicuous agitation, hyperesthesia, accentuated tendon reflex of extremities and positive Babinski reaction in lower extremities. 16 hours after ingestion, transient ECG abnormalities were seen (sinus tachykardia and sharp P wave). At the same time, all subjective symptoms of illusion and delusion showed a healthy orientation. Repeated illusion and delusions appeared after discharge. Abnormal EEG, such as sudden group of theta waves of higher potential, light-induced theta waves, etc. was observed 2 d after the accident for about a week. The patient appeared healthy two months later.

2.2.2 Occupational Exposures

Acute effects of exposure to CS₂ are described in occupational medicine and toxicology handbooks and reports. Many serious cases of intoxication have occurred among workers exposed to CS₂ in the cold vulcanization of rubber during the 19th century and later in the viscose rayon production (Davidson and Feinleib 1972). In these reports, exposure concentrations are either lacking, based on estimates but not on measurements, or are stated without any reference. Also, these reports describe cases in which acute symptoms occurred in workers who previously had been exposed for a period of weeks to years to unknown concentrations of CS₂. In view of the chronic effects of CS₂ on the nervous system, it seems likely that such “acute” poisonings were actually acute exposure and acute outbreak of symptoms superimposed on chronic inhalation exposure. Therefore, it is unknown to what extent the effects described are due to acute exposure to CS₂. Eye irritation in workers in the viscose-producing industry has also been described. However, this effect is considered to be mainly caused by hydrogen sulfide which always is present together with carbon disulfide in the viscose production process (BUA 1993; DFG 1997). Additionally, acid mists may also contribute to the effects.

Gordy and Trumper (1938) described six cases of intoxication in workers who had been employed for at least 11 months. Especially in one case, the early effects are likely due to acute poisoning: A 27 year old woman described to be in generally good health had been working in the rayon industry for 6 years as a reeler of artificial silk. One day when she handled incompletely dried viscose, symptoms began with violent headache, faintness, restlessness, weeping, screaming, laughing, and loss of consciousness. After recovering consciousness, the victim felt as “though she had been beaten all over”.

She spit blood and had bloody bowel movements and was semiconscious and stuporous most of the rest of the day. No data regarding the possible concentration of CS₂ were presented. The victim also complained of long lasting effects after this episode. Repeated spells occurred from that day on, lasting about 15 minutes and consisting of headaches and numbness in various parts of the body. Her hands and feet felt as though they were sleep. Additionally, she developed psychotic episodes characterized by auditory hallucinations, vasomotor instability, and disturbance of vision.

In a short notice, Münchinger (1958) briefly summarize their medical and psychic findings in 100 workers in a Swiss viscose factory. The workers were 24 – 66 years old. Exposure duration varied between 1 and 39 years, and the mean CS₂ concentration at the workplace was reported to fluctuate between 5 and 35 mg/m³ (1.6 – 11.2 ppm). Peak or maximum exposures were not reported. About two thirds of the workers complained about subjective symptoms, especially alcohol intolerance, sleep disorders, noticeable tiredness at work, and irritability. About one third each complained of gastrointestinal problems, had pathologic cardiovascular findings or respiratory tract disease. Medical and psychic examination revealed in about two-thirds of the workers alterations of the functions of the autonomic, peripheral and central nervous system compatible with a mild to moderate psychoorganic syndrome. No detailed evaluation was presented.

Alcohol intolerance in subjects exposed to CS₂ has been mentioned in several other reports, and in its guidelines, the German Society for Occupational and Environmental Medicine points to the alcohol intolerance as a further adverse effect induced by CS₂ (Drexler 1998). Freundt et al. (1976b) cite several early reports regarding the development of alcohol intolerance in workers manufacturing rubber or viscose rayon. Reports date back to as early as 1856 and 1910 (Williams 1937), when exposure was likely very high. However, precise data regarding the concentration of CS₂, the amount of alcohol intake, and the temporal relationship were not available. Djuric (1971) noticed that in a group of viscose factory workers exposed to “pretty high concentrations” of CS₂ slight intolerance to alcohol may occur.

Vigliani (1954) described findings in Italian viscose rayon factories. From 1940 to 1941, he observed 100 cases of CS₂ poisoning. Outbreaks of poisonings occurred in two plants after war time measures led to bad ventilation, lengthened work shifts up to 12 hours/day, and improper handling. The concentrations in the two plants ranged from a minimum of 0.11 mg/L (35 ppm) in the churn to a maximum of 2.5 mg/L (800 ppm) in the staple bleaching. In the staple rooms, the workers were exposed 4 – 5 hours/day to CS₂ concentrations between 1 and 2 mg/L (320 – 640 ppm). Concentrations higher than 0.5 mg/L (160 ppm) with a maximum of 2 mg/L (640 ppm) were reported to poison workers in 2 to 6 months. In the 100 cases described, symptoms (in decreasing frequency) included polyneuritis, gastric disturbances, headaches, vertigo, sexual weakness, tremors, myopathy, psychoses, extrapyramidal symptoms, opticneuritis, hemiparesis, and pseudobulbar paralysis. Concentrations of 0.40 to 0.50 mg/L (130 – 160 ppm) caused toxicity after 1 or more years of work. Some cases of mild poisoning were also seen in workers exposed to 0.2 – 0.3 mg/L (64 – 96 ppm).

A great number of epidemiological studies on the chronic effects of CS₂ in occupationally exposed workers have been carried out and these studies have been repeatedly reviewed and summarized (ATSDR 1996; Beauchamp et al. 1983; BUA 1993; Davidson and Feinleib 1972; DFG 1975; 1997; Government Canada 2000; HSE 1981; WHO 1979; WHO 2000). A detailed description of the findings from the epidemiological studies is beyond the scope of this document because these studies do not provide data that could be used for the derivation of AEGL.

Briefly, in chronic intoxication with CS₂, almost every organ of the body may be affected. Generalized, subjective symptoms such as tiredness, sleeplessness, headaches, irritability, excitability, nausea, digestive disorders, reduction of libido, neurasthenia and dizziness have been reported. Further effects include gastritis, ulcers, liver disfunction, paresis, paralysis, myopathy, and cardiac arrhythmia.

Exposure to very high concentrations may lead to psychoses, hallucinations, delirium, and dementia. In chronic exposure, most common are polyneuritis with paresthesia, ataxia, reflex disorders and atonia. In the vascular system, hypertonia and arteriosclerosis-like lesions in the vessels of the brain, coronary heart disease, lesions of the kidney, pancreas, and eye may develop. Increased levels of blood lipids have also been reported (DFG 1997).

Neurotoxic effects were described to occur in workers exposed for decades to concentrations lower than 30 mg/m³ (10 ppm). Increased mortality from cardiac infarction, neurotoxicity and changes in blood lipids have been described at concentrations of about 20 mg/m³ (6 ppm) (DFG 1997). A recent exposure-response analysis concluded that the lowest levels associated with reductions in peripheral nerve conduction velocity in CS₂ exposed humans range from 13 - <31 mg/m³ (4 - <10 ppm) (Government Canada 2000).

2.2.3 Experimental Studies

The findings of clinical volunteer studies with controlled exposure are summarized in Table 2 and Table 3.

TABLE 2: NON-LETHAL EFFECTS IN HUMANS FOLLOWING INHALATION OF CARBON DISULFIDE (LEHMANN 1894)			
Sub- ject*	Exposure		Effects/remarks
	Concentration in mg/L (ppm) [#]	Total duration	
I	2 hours: 0.55 (180) 3 ¼ hours: 0.70 (225) 4 ¾ hours: 0.76 (240)	4 hours 45 minutes	Moderate odor annoyance, no subjective symptoms noted
I	1 ¼ hours: 1.35 (435) 2 ½ hours: 1.86 (600) 3 ½ hours: 2.55 (820)	3 hours 30 minutes	Slowly developing tickle in the throat, tingling in right side of head >2 ½ hours: slight dizziness, increasing tickle in the throat with cough; after end of exposure: strong headaches
I	2 ¼ hours: 0.7 (225) 3 ¼ hours: 1.3 (420) 4 hours: 2.3 (740)	4 hours	No symptoms during the first 2 ¼ hours; >2 ¼ hours: as described above; after end of exposure: headaches
I	1 hour: 2.07 (665)	1 hour	Odor annoyance; slight tickle in throat and nose; slight headache after end of exposure
I	2 hours: 2.5 (800)	2 hours	> 30 minutes: tickle in the throat; > 1 hour: feeling of pressure and congestion in the head at the end: slight dizziness; after end of exposure: persisting hiccups, palpitations, increased pulse, unmotivated laughing ("mirth")
I	about 2-3 (640 – 960)	3 hours 30 minutes	Estimated concentration (determination of concentration failed); Unmotivated laughing ("mirth"), followed by dull feeling of pressure in the head; intermittent stinging headaches; after ¾ hours: slight dizziness, temporary marked pain in the head when stepping on the ground; after exposure: congestion at night, feeling dazed next day

TABLE 2: NON-LETHAL EFFECTS IN HUMANS FOLLOWING INHALATION OF CARBON DISULFIDE (LEHMANN 1894)			
Sub- ject*	Exposure		Effects/remarks
	Concentration in mg/L (ppm) [#]	Total duration	
I	2 hours: 3.4 (1100)	2 hours	> 1 hour: dizziness, feeling of pressure in the head – intensifying at movements - and of tension in the face; skin of face feeling hot; tickling throat, increased pulse rate, tingling and paresthesia in arms
I	1 hours: 6.4 (2050)	1 hour	> 20 minutes: feeling of tension and heat in the face, irritant action on pharynx progressing to cough and nausea; persistent hiccups; during further exposure: dizziness gradually progressing to high degree, feeling of beginning central paralysis very distinct, mental capabilities highly impaired, difficulty to perform tasks; vomiting after exposure, disturbed sleep, 2 days of feeling ill
I	½ hours: 6.8 (2180) 1 hours: 10.5 (3370)	1 hour 30 minutes	Last experiment performed At the beginning, some feeling of unease from last exposure; > 30 minutes: strong dizziness, nausea, seminarcotic state, tingling, shallow, irregular respiration with deep gasping in between; After exposure: leg muscle aches, feeling nervous and upset, intermittent headaches for 12 days
II	½ hours: 1.2 (385) 1 hour: 0.8 (260) 1 ½ hours: 1.3 (420)	1 hour 40 minutes	> 15 minutes: feeling of heat in skin of forehead and of tension in the eyes > 30 minutes: slight dizziness, feeling of pressure in the back of the head > 40 minutes: slight cough and feeling of exhaustion at the end: slight lacrymation and burning of eyes
II	1 hour: 1.06 (340) 2 hours: 1.07 (345) 3 hours: 1.1 (350) 4 hours: 1.09 (350)	4 hours	> 45 minutes: slight frontal headache; recovery during 2 hours of lunch break
II	1 hour: 1.05 (340) 2 hours: 0.95 (305) 3 hours: 1.02 (330) 3 ½ hours: 1.1 (355)	3 30 minutes	Exposure continued from morning after 2 hours lunch break (see above); > 15 minutes: increased pulse; slight headache intensifying after 1 hour; feeling of heat in the head; after end of exposure: broken sleep at night, headaches persisting until next evening
II	1 hours: 1.2 (385) 2 hours: 1.1 (355)	2 hours	> 15 minutes: slight dizziness and frontal headache; after end of exposure: headache over the rest of the day
II	2 ½ hours: 1.21 (390)	2 hours 30 minutes	> 45 minutes: dull headache, slight dizziness; tension in the eyes

TABLE 2: NON-LETHAL EFFECTS IN HUMANS FOLLOWING INHALATION OF CARBON DISULFIDE (LEHMANN 1894)			
Sub- ject*	Exposure		Effects/remarks
	Concentration in mg/L (ppm) #	Total duration	
II	1 hour: 1.5 (480) 2 hours: 1.44 (460) 3 hours: 1.62 (520) 3.8 hours: 1.75 (560) mean: 1.57 (505)	3 hours 50 minutes	Immediate feeling of pressure in the head > 15 minutes: slight tickle in the throat and cough > 35 minutes: headaches intensifying > 45 minutes: burning eyes, impairment of reading ability; feeling of heat in the face, lacrymation, pulse still normal > 1 hour: intense feeling of heat in the forehead, cold hands, reading ability improving; > 1 hour 10 minutes: period of cough, feeling of being in an overheated room although temperature at 15 °C (59 F) after end of exposure: headaches persisting until night, irritation of larynx and cough attacks; dizziness, anxiety, intense reddened face, increased pulse rate, then paleness and cold sweat
II	1 ½ hours: 2.2 (710)	1 hour 30 minutes	Severe headache, persisting for 6 hours after exposure
II	½ hours: 3.63 (1165) 1 hour: 3.65 (1170) 1 ½ hours: 3.7 (1190)	1 hour 30 minutes	Immediate feeling of pressure in the head > 20 minutes: intense headache, ≥ 30 minutes: dizzy spell, nausea, vertigo, increased pulse, dizziness re-occurring when trying to walk around; after end of exposure: persistent headaches
II	1/2 hours: 5.75 (1850) 1 hour: 6.67 (2140)	1 hour	Rapidly developing headache and feeling of pressure in the head > 30 minutes: anxiety, increased pulse, marked tickle in the throat, nausea, dizziness; intensifying at continued exposure after end of exposure: staggered gait, strong dazed feeling, sudden salivation with increased pulse; headaches persisting until next morning

*: volume of exposure chamber was 3 m³ for subject I and 36 m³ for subject II.

#: determined at indicated time point

Lehmann (1894) described results from studies with two of his medical students. The two healthy young males were exposed to different concentrations of carbon disulfide vapor in a chamber of 3 m³ (person I) or 36 m³ (person II), respectively (Table 2). Carbon disulfide was evaporated inside the exposure chamber from liquid material by means of a fan. There was no mention of air exchange in the exposure chamber. To determine the concentration of CS₂ in air, air was sucked by means of aspirators (not further described) through two flasks with alcoholic potassium hydroxide solution in which CS₂ was shown to be completely bound as xanthogenate. The xanthogenate formed was determined by titration with iodine-starch. Concentration determinations were mostly carried out at 30 minutes or 1 hour intervals, respectively. It is reported that there were difficulties in maintaining the exposure concentration in the smaller (3 m³) chamber in the first set of experiments while in the second set of experiments, special care was taken regarding the maintenance and control of the exposure concentration. In the course of the whole study, the exposure concentrations were varied between 0.55 mg/L (180 ppm) and 6.67 mg/L (3370 ppm), and the exposures lasted from 1 hour to 4 hours 45 minutes. The data from individual

experiments are presented in Table 2. Signs of respiratory tract irritation (tickle in the throat, dry cough) occurred in most experiments, but always at concentrations which also caused effects on the central nervous system (CNS). In summary, exposure to 0.55 – 0.76 mg/L (180 – 240 ppm) for up to 4 hours 45 minutes caused moderate odor annoyance but no further subjective symptoms. Exposure to 1 – 1.2 mg/L (320 – 385 ppm) caused slight headaches and dizziness after 15 minutes. In the same experiments, temporary burning of eyes and mucous membranes also were noted at the end of exposure after 1 hour 40 minutes. Exposure to 1.4 – 1.75 mg/L (460 - 560 ppm, mean: 505 ppm) for up to 3 hours 50 minutes caused an immediate feeling of pressure in the head, later dizziness, anxiety, persisting headaches, temporary impairment of reading ability, lacrymation, cough attacks, and vasomotor reactions (intensely reddened face, increased pulse rate). About 2.2 - 2.5 mg/L (710 - 800 ppm) caused unmotivated laughter at the end of and after exposure and severe headaches, which, after 1.5 – 3 hours of exposure, lasted for many hours. At about 3.5 mg/L (1125 ppm) symptoms were more severe and occurred more rapidly (within 30 minutes). Exposure for 1 hour to 6.4 mg/L (2050 ppm) in one and 5.75 – 6.67 mg/L (1850 - 2140 ppm) in the other subject caused severe intoxication with difficulty to perform tasks, anxiety, nausea, progressing dizziness, and a very distinct feeling of beginning central paralysis. After exposure, staggered gait, strong dazed feeling, autonomic nervous system reactions (sudden salivation, increased pulse), vomiting and up to 2 days of feeling ill were recorded. One exposure in one subject to 2180 – 3370 ppm for 1.5 hours caused strong dizziness, nausea, seminarctic state, and irregular respiration.

In some toxicokinetic studies with exposure CS₂ concentrations around 20 – 50 ppm, the presence or absence of signs of toxicity was briefly mentioned.

Nine persons who had never previously been in contact with CS₂ were exposed in eleven experiments to 17 – 30 ppm CS₂ (in one case to 51 ppm) for 1 to 4 hours. The concentration of CS₂ in the air was kept constant during the experiment within $\pm 6 \mu\text{g/L}$ (1.9 ppm) and was determined every 15 minutes colorimetrically with diethylamine/copper reagent. The volunteers were reported to be free of symptoms, other than an occasional slight headache (Teisinger and Soucek 1949).

In another toxicokinetic study, five “normal men” were exposed via a plastic face mask to 20 or 25 ppm CS₂ for 1.5 – 2.1 hours. The concentration of CS₂ in the air was kept constant during the experiment within ± 1 ppm and was determined every 30 minutes (no further details reported). None of the subjects noticed any immediate or delayed effects from the vapor exposure and in each case, the blood pressure, heart rate and respiratory rate were normal throughout the experiment (McKee et al. 1943).

In a further pharmacokinetic study, six healthy male volunteers at the age of 27 – 36 years were exposed to concentrations of 10 and 20 ppm CS₂ at rest and to 3 and 10 ppm CS₂ under a 50 W level of exercise (Rosier et al. 1987). The mean inhaled concentrations were within 4.1 % (at 3 ppm), 1.6 % (at 10 ppm), and 3.1 % (at 20 ppm) of the proposed value. Every experiment consisted of four periods of 50-minutes exposure to CS₂ with a resting period of 10 minutes between two consecutive exposures. All volunteers were informed of the practical implications of the experiments. There were no complaints or objective signs of carbon disulfide intoxication after each experiment.

In another toxicokinetic study, 6 male volunteers were exposed through face mask to CS₂ concentrations ranging from 28 – 52 ppm for 0.5 – 2 hours. Their bodies were covered from neck to hip by synthetic resin clothing through which air was blown to collect and determine CS₂ excreted via the skin. No signs of toxicity were reported, apart from a slight headache in three of the six subjects (Harashima and Masuda 1962). Because of the absence of controls, exclusion of the symptom from response to the experimental procedure was not possible.

In a further toxicokinetic study, about 10 persons (probably workers of a factory where CS₂ was used, but no details were reported) were exposed in a 140 m³ chamber up to concentrations of CS₂ of

300 µg/L (96 ppm) for 8 hours and of 445 µg/L (143 ppm) for 5 hours (Demus 1964). The exposure concentration was continuously monitored and reported to deviate no more than $\pm 5\%$ from the nominal concentration. The authors did not report the occurrence of any symptoms of intoxication nor did they explicitly state the absence of such effects.

The inhibition of oxidative N-demethylation of amidopyrine by CS₂ was studied by Mack et al. (1974). Experiments were conducted on healthy male adults aged 21 – 40 years instructed to discontinue drug intake and to restrict alcohol intake a few weeks prior to the experiments. Groups of 4 persons were exposed to 0, 10, 20, 40, or 80 ppm CS₂ for 6 hours. Exposures were carried out in an 8 m³ dynamic exposure chamber (air exchange 8 – 15 times/hour). The CS₂/air mixture entered under uniform pressure through a vent at one edge. The exposure mixture was prepared in a spherical glass mixing vessel by evaporation of liquid CS₂ into a rotametrically metered stream of air. Continuous dropwise addition of CS₂ according to the desired concentration was obtained with an automatic infusion apparatus (Perfusor type 71100, Braun). Constant evaporation was maintained by heating the spherical mixing vessel over a 50° water bath. The CS₂/air mixture was diluted to the desired concentration with ambient air in another larger mixing drum. Permanent circulation of the chamber atmosphere was achieved by a vent in the middle of the roof. The CS₂ concentrations actually prevailing within the chamber were monitored before and during the entire exposure period with an automatically recording infrared analyser (Uras 1, Hartmann & Braun AG) that was mounted outside and connected with the exposure chamber by a glass tube. At the start of each experiment, the individuals received 7 mg/kg b.w. of amidopyrine orally. Metabolites (AAP: 4-aminoantipyrine, N-AcAAP: N-Acetyl-AAP) were assayed in urine sampled 3 – 33 hours after the start of the exposure. A concentration of 10 ppm CS₂ was sufficient to lead to a significant deficit in the excretion of free and total 4-AAP during the exposure. Both the intensity and the duration of the effect showed a well-defined dose-response relationship. The excretion deficit was reversible and compensated for during the subsequent excretion phase. Further experiments with 6-hours exposure to 20 ppm revealed that the effect was no longer detectable at 18 hours after exposure. Exposure to 20 ppm CS₂ 6 hours/day for 5 days produced an inhibitory reaction identical to that seen after a single 6-hours exposure to 40 ppm.

Reports of alcohol intolerance in workers occupationally exposed to CS₂ prompted the investigation of this phenomenon in experimental studies. The influence of inhaled CS₂ on serum parameters was studied in volunteers who also received alcohol (Freundt and Lieberwirth 1974b). Exposures were carried out in an 8 m³ dynamic exposure chamber (air exchange 15 times/hour) as described by Mack et al. (1974). 11 healthy male volunteers aged 20 – 32 years were asked not to take alcohol or medicine several days prior to the experiment. Volunteers (no. in parentheses) were exposed to 0 (11), 40 (5) or 80 ppm (4) CS₂ for 8 hours. Additionally, the volunteers received alcohol (0.57 mL/kg b.w.) in orange juice (3.01 mL/kg b.w.) at the beginning of the exposure and 0.047 mL alcohol/kg b.w. in 0.18 mL/kg b.w. orange juice every 15 minutes until the end of exposure. Standardized meals were served 1.5, 3, and 5 hours after start of exposure. The mean blood alcohol concentration obtained was 0.7 ‰ (range 0.58 – 0.85 ‰, determined by gas chromatography). For the evaluation of serum parameters, the pretreatment values in each group served as control. Alcohol intake alone significantly lowered blood glucose by about 12 %. In alcoholized subjects exposed to 40 ppm CS₂, no significant changes of any serum parameters (cholesterol, calcium, inorganic phosphate, total bilirubin, albumin, total protein, uric acid, urea-N, glucose, LDH, alkaline phosphatase, GOT) were found. However, the blood glucose level was about 13 % lower at the end of the treatment period. At 80 ppm, the decrease in blood glucose reached statistical significance. At this concentration, a significant rise of the serum total bilirubin concentration by 61 % as compared to preexposure also was observed, and the bilirubin concentration just exceeded the normal range. However, a nearly identical bilirubin concentration was observed in the group which only received alcohol. Here, the increase was not significant because the pretreatment level was higher than that observed in the 80 ppm-group. No significant changes were observed in serum parameters (cholesterol, calcium, inorganic phosphate, albumin, total protein, uric acid, urea-N, LDH, alkaline phosphatase, GOT).

In the same study, 4 volunteers were exposed to 20 ppm CS₂ for 8 hours without simultaneous alcohol intake. After exposure, a 30 % decrease of the mean blood glucose level was observed. The decrease did not reach statistical significance. No significant changes were noted on any of the serum parameters mentioned above. When this group subsequently received alcohol (as described above) over a period 16 – 24 hours after the exposure to CS₂, a 108 % rise of the serum total bilirubin concentration to above the upper normal range, and slight non-significant increases (18 – 52 %) in serum albumin, total protein, uric acid, and alkaline phosphatase were observed.

Finally, in this study 4 volunteers were exposed to 20 ppm CS₂ for 8 hours/day for 5 days. Only during the last exposure, alcohol (as described above) was given. During the four days of exposure to CS₂ alone, non-significant decreases in blood glucose levels (up to 12 %) were seen each day. The only significant change observed was a 55 % increase in serum total bilirubin on day 3. After the end of the 5th day, the total serum bilirubin was increased by about 50 % and the blood glucose was significantly decreased by 18 %. However, throughout the study, all blood glucose determinations were within the normal range.

In a related study, the effect of CS₂ on the blood acetaldehyde concentration in alcoholized subjects was investigated (Freundt et al. 1976b; Freundt and Lieberwirth 1974a). 12 healthy male volunteers aged 20 – 32 years were asked not to take alcohol or medicine several days prior to the experiment. Exposure conditions and alcohol intake were performed as described above (Freundt and Lieberwirth 1974b). Acetaldehyde and ethanol were determined by gas chromatographic headspace analysis in blood samples taken from the antecubital vein at hourly intervals. In all experiments, CS₂ exposure had no significant effect on the blood alcohol concentration. The mean blood alcohol concentration obtained was about 0.75 ‰ and remained fairly constant during the experiments. In alcoholized control subjects, the blood acetaldehyde concentration determined was approximately $6 \cdot 10^{-3}$ ‰ (140 µM). During a simultaneous 8 hours exposure of 4 volunteers to 20 ppm CS₂, the blood acetaldehyde concentration rose significantly by about 50 %. Exposure to 40 ppm or 80 ppm CS₂ for 8 hours led to a slight further increase of blood acetaldehyde. In a further experiment with 4 volunteers, administration of alcohol (ca. 0.5 ‰ blood alcohol) for 8 hours, instituted 16 hours (i.e., the next morning) after the end of the 8-hour exposure to 20 ppm CS₂, the blood acetaldehyde concentration reached slightly more than twice the control value. A nearly identical quantitative effect was also seen after repeated exposure to 20 ppm CS₂ 8 hours/day on 5 consecutive days with simultaneous administration of ethanol only at the last day. Under the conditions employed, no signs of an “antabuse syndrome” of alcohol intolerance in any the subjects was noted.

TABLE 3: SUMMARY OF ACUTE NON-LETHAL EFFECTS IN CONTROLLED HUMANS STUDIES FOLLOWING INHALATION OF CARBON DISULFIDE				
Subjects	Exposure duration	Exposure concentration	Effect/remarks	Reference
2 m volunteers	1 hour – 4 hours 45 minutes	180 – 2140 ppm	See separate Table 2	Lehmann 1894
9 volunteers	1 - 4 hours	17 – 30 ppm (one exp: 51 ppm)	Occasional slight headaches, no details reported	Teisinger and Soucek 1949
Not reported	0.5 – 2 hours	38 – 52 ppm	Slight headache in some of the subjects	Harashima and Masuda 1962
6 volunteers	4 x 50 minutes	3 ppm 10 ppm 20 ppm	No complaints or objective symptoms of intoxication after each experiment	Rosier et al. 1987
5 m volunteers	1.5 - 2.1 hours	20 - 25 ppm	No subject noted immediate or delayed effects; normal blood pressure, heart and respiratory rate	McKee et al. 1943
19 m volunteers	6 hours	10 – 80 ppm	≥ 10 ppm: inhibition of aminopyrine metabolism	Mack et al. 1974
11 m volunteers	8 hours	40 ppm 80 ppm	In combination with ethyl alcohol (0.7 %): rise in serum bilirubin, no elevation of hepatic enzymes in serum	Freundt and Lieberwirth 1974b
12 m volunteers	8 hours	20 – 80 ppm	With synchronous or subsequent intake of ethyl alcohol (0.75 %): increase in blood acetaldehyde to twice of control values, no antabuse syndrome	Freundt et al. 1976b
4 trained staff members		0.21 ppm	Odor recognition threshold	Leonardos et al. 1969

Odor perception

Lehmann (1894) described that the odor of concentrated vapors of freshly purified CS₂ resembled that of chloroform while the odor of more diluted vapors from the same samples resembled a mixture of chloroform and decaying radish. The author also reported that exposure of a single volunteer to 0.55 – 0.76 mg/L (180 – 240 ppm) for up to 4 hours 45 minutes caused moderate odor annoyance.

Leonardos et al. (1969) determined the odor recognition threshold for CS₂ under controlled laboratory conditions using a standardized and defined procedure. Carbon disulfide of the highest purity commercially available from large scale production was used. Four trained panel staff members were selected. Prior to exposure to at least five different concentrations in an odor test room, the panel examined the odor over water at various dilutions to become acquainted with the odor type and to develop a common terminology for describing the odor. The order of presentation of concentrations in the test room was on a random basis and observations were separated by a minimum of a 25 minute break. A positive response was indicated for each concentration at which the panelist described the odor of the chemical. The threshold was taken as the lowest concentration at which the panelist could define the odor and that which could be consistently recognized at higher concentrations. The odor threshold represents

that concentration at which all four panelists could positively recognize the odor. A threshold of 0.21 ppm was determined. The value from this study served as the basis for the derivation of the ERPG-1 value for CS₂ of 1 ppm (AIHA 1992).

Amoore and Hautala (1983) reported a geometric mean air odor threshold of 0.11 ppm (standard error 0.058 ppm) for CS₂. Data were derived from six available original literature references which were not explicitly reported.

A wide range of odor thresholds from 0.0243 mg/m³ to 23.1 mg/m³ (0.0078 – 7.4 ppm) for CS₂ were reported in a compilation of data from the industrial hygiene literature (Ruth 1986). The author did not explicitly report from which references the values for individual chemicals were taken. No value regarding irritating concentrations was reported.

In a critical overview of odor thresholds for chemicals, all of the referenced values ranged from 0.016 – 0.42 ppm, but no geometric mean and no “range of acceptable values” for CS₂ were presented (AIHA 1997). The use of the 0.21 ppm threshold (see above) was rejected in this overview because this value represents a 100 % recognition concentration.

Non-inhalation exposure

In instances where CS₂ was swallowed, the following symptoms were reported: spasmodic tremor, Cheyne-Stokes respiration, large pupils, pallor, decreased body temperature, and finally coma. Less serious manifestations included paresthesias, weakness and unsteadiness of arms and legs, and hemiparesis (Davidson and Feinleib 1972).

Liquid CS₂ is a severe skin irritant and vesicant. In workers in the spinning operation of viscose plants, serous and hemorrhagic blisters on the skin of fingers occurred. Recurrent blisters may develop several weeks after cessation of contact (Hueper 1936).

2.3 Developmental/Reproductive Toxicity

Data regarding the reproductive/developmental toxicity of acute exposure of humans to carbon disulfide were not available.

Studies have been carried out on occupational cohorts chronically exposed to CS₂. A detailed description of these findings is beyond the scope of this document, but there are several reviews (ATSDR 1996; Beauchamp et al. 1983; BUA 1993; DFG 1975; 1997, Government Canada 2000; HSE 1981; WHO 1979) from which the following conclusions can be derived. Reports of reduced sperm counts and changes in sperm morphology and of changes in hormone levels that had been presented in earlier studies could not be confirmed in more recent studies. Significant effects in recent studies were found on worker's libido (between 1 and 30 mg/m³; 0.32 and 10 ppm) and potency (above 30 mg/m³). In females, spontaneous abortions and menstruation disorders were described. The concentrations reported range from below 10 mg/m³ (3.2 ppm) to far above 30 mg/m³ (10 ppm). Some evidence for an increase in malformations of the heart and central nervous system has been presented. Two early reports of an increased frequency of spontaneous abortions associated with maternal or paternal employment in the viscose rayon industry could not be confirmed in more recent studies.

2.4 Genotoxicity

In an *in vitro* study, lymphocytes from 25 – 40 years old male volunteers were incubated with CS₂ in gas-tight vessels for 30 minutes (Garry et al. 1990). In the presence, but not in the absence of

metabolic activation system (S-9 from rat liver), CS₂ led to a significant, concentration-dependent increase in the number of sister chromatid exchanges (SCE). Chromosomal aberrations were not increased.

In a further *in vitro* study using WI-38 human lung fibroblasts, the unscheduled DNA synthesis (UDS) was not increased by CS₂ (0.1 – 5 mL/L medium) in the absence of metabolic activation system. In the presence of mouse liver S-9, a slight but significant amount of UDS was observed. Unexpectedly, the positive control substance benzo(a)pyrene failed to induce UDS in this study (Litton Bionetics 1980).

In human sperm exposed to CS₂ *in vitro*, a significant increase in the frequency of chromosomal aberrations and of chromosomal breaks were seen (Le and Fu 1996).

2.5 Carcinogenicity

A mortality cohort study was carried out on 2291 workers with chronic occupational CS₂ poisoning diagnosed during the years 1970 - 1990. The general population of Poland was the reference population. With respect to neoplastic diseases, the analysis in male subjects showed a statistically significant excess of deaths from colon cancer (SMR = 233; 9 cases). All these cases were noted in workers of the two oldest rayon plants and a detailed future analysis is required to derive further conclusions (Peplonska et al. 1996).

Deaths due to neoplasms was compared in a cohort of rayon plant workers and in a cohort of paper mill workers for the period 1967 to 1982. No significant differences were found (Nurminen and Hernberg 1984).

A nested case-control study in a cohort of rubber workers indicated a significantly increased odds ratio for exposure to CS₂ and development of and also death from lymphocytic leukemia when specific exposures in the group to a variety of different solvents other than benzene (jobs with benzene exposure were excluded from the study) were analysed (Wilcosky et al. 1984). However, cautious attention must be paid to a number of factors: The number of cases examined was small, a large number of solvents was looked at in the analysis, many of these solvents were used in mixtures so that identifying single agents was not possible, historical exposure was estimated from the designation “permitted to use” but not from actual use, and confounding factors from non-occupational or other occupational exposures were not taken into account.

A number of epidemiological studies on mortality in workers exposed to CS₂, especially in the viscose rayon industry, have been presented. However, these studies focus on the association between exposure and mortality from cardiovascular diseases, and other findings are poorly described. The available data have been reviewed and summarized (ATSDR 1996; BUA 1993; Government Canada 2000; WHO 1979). Overall, there was no consistent evidence of an increase in mortality from all cancers combined or from cancers at any specific site.

2.6 Summary

In experimental studies, a wide overall range of odor thresholds between 0.0243 mg/m³ and 23.1 mg/m³ (0.0078 – 7.4 ppm) was reported (Ruth 1986). A geometric mean air odor threshold of 0.11 ppm (standard error 0.058 ppm) was reported (Amoore and Hautala 1983). Leonardos et al. (1969) determined a 100 % odor recognition threshold for CS₂ of 0.21 ppm. AIHA (1997), in a critical overview of odor thresholds, reported referenced values ranging from 0.016 – 0.42 ppm, but no geometric mean and no “range of acceptable values” for CS₂ were presented; the use of the 0.21 ppm threshold (see above) was rejected in this overview because this value represents a 100 % recognition concentration. Since CS₂ decomposes rapidly under the influence of air and/or light with the formation of foul smelling decay

products, it is to be expected that the odor detection and recognition threshold of CS₂ will vary widely depending on the purity of the substance and the conditions.

The most sensitive effect following exposure to CS₂ was an inhibition of biotransformation reactions. In an experimental study on oxidative N-demethylation of amidopyrine, exposure to 10 – 80 ppm CS₂ caused a concentration-dependent, reversible inhibition of the urinary excretion of metabolites indicating inhibition of oxidative biotransformation (Mack et al. 1974).

In several experiments, volunteers were exposed to CS₂ in combination with controlled intake of alcohol. The blood alcohol concentration was about 0.7 ‰ representing a level which may be often obtained in “lifestyle activities”. Exposure to 20 – 80 ppm CS₂ for 8 hours caused a 50 % increase in the concentration of acetaldehyde in blood compared to “alcohol only” values of the same subjects. A similar effect was seen when the intake of alcohol started 16 hours after the end of exposure to 20 ppm CS₂, and after an 8 hours/day, 5 consecutive days exposure to 20 ppm CS₂ with alcohol intake only the last day. Under the conditions of the study, there were no complaints about an “antabuse syndrome” or other subjective signs of intoxication (Freundt et al. 1976b; Freundt and Lieberwirth 1974a).

In another experiment, exposure to 80 ppm CS₂ for 8 hours in alcoholized subjects led to a significant 60 % rise of total serum bilirubin. A similar effect was seen in an experiment when the alcohol intake started 16 hours after the exposure to 20 ppm CS₂. Other serum parameters (cholesterol, calcium, inorganic phosphate, albumin, total protein, uric acid, urea-N, glucose) including liver enzymes in serum (LDH, alkaline phosphatase, GOT) were not altered and in the normal range (Freundt and Lieberwirth 1974b).

In two toxicokinetic studies, occasional slight headache but no other symptoms were reported to occur in volunteers exposed to 17 – 51 ppm CS₂ for 0.5 to 4 hours (Harashima and Masuda 1962; Teisinger and Soucek 1949). The volunteers were reported to be free of symptoms in two other toxicokinetic studies at exposures to 3 – 20 ppm CS₂ (Rosier et al. 1987) and 20 – 25 ppm (McKee et al. 1943) for 1.5 – 2.1 hours, respectively.

Only one controlled exposure study is known in which exposure to CS₂ reached concentrations to cause pronounced acute effects on the CNS (Lehmann 1894). In the course of the whole study, the exposure concentrations were varied between 180 ppm and 3370 ppm, and the exposures lasted from 1 hour to up to 4 hours 45 minutes. Signs of respiratory tract irritation (tickle in the throat, dry cough) occurred in most experiments, but always at concentrations which also caused CNS effects. Exposure to 180 – 240 ppm for up to 4 hours 45 minutes caused moderate odor annoyance but no further subjective symptoms. 320 – 385 ppm caused slight headaches and dizziness after 15 minutes. In the same experiments, temporary burning of eyes and mucous membranes also were noted at the end of exposure (1 hour 40 minutes). Exposure to 460 - 560 ppm for up to 3 hours 50 minutes caused an immediate feeling of pressure in the head, later dizziness, anxiety, persisting headaches, temporary impairment of reading ability, lacrymation, coughing attacks, and vasomotor reactions (intensely reddened face, increased pulse rate). About 710 - 800 ppm caused unmotivated laughter at the end of and after exposure and severe headaches which after 1.5 – 3 hours of exposure lasted for many hours. At about 1125 ppm symptoms were more severe and occurred more rapidly (within 30 minutes). Exposure to about 2000 ppm for 1 hour caused severe intoxication with difficulty to perform tasks, anxiety, nausea, progressing dizziness, and the beginning of marked central paralysis. After exposure, staggered gait, strong dazed feeling, autonomic nervous system reactions (sudden salivation, increased pulse), vomiting and up to 2 days of feeling ill were recorded. One exposure in one subject to 2180 – 3370 ppm for 1.5 hours caused strong dizziness, nausea, semiarototic state, and irregular respiration.

Studies in occupationally exposed workers also show that the primary effect of acute intoxication is on the CNS. Often, symptoms such as psychoses remained for a long period of time afterwards (Gordy and Trumper 1938). However, these reports described cases in workers who previously had been exposed for weeks to years. In view of the chronic effects of CS₂ on the nervous system, it seems likely that such “acute” poisonings actually were acute exposure and acute outbreaks superimposed by chronic exposure. No concentrations were reported in such acute cases.

Deaths have been reported following exposure to high concentrations in accidents. Due to the lack of exposure data and the concomitant exposure to H₂S and sulfuric acid, no conclusions valid for the derivation of AEGL can be derived from these data. According to Flury and Zernik (1931), exposure to 4800 ppm CS₂ for 30 minutes to 1 hour will immediately or later lead to death, while 3200 – 3850 ppm CS₂ over the same period of time will be life-threatening. The same statement is made by Bittersohl (1972). Furthermore, it is stated that “hyperacute intoxication” with very high concentrations exceeding 10 mg/L (3200 ppm) will immediately lead to loss of reflexes, coma and death. No details or references are presented in these secondary sources.

Data regarding reproductive/developmental toxicity following acute exposure were not available. Epidemiological studies have provided conflicting evidence of effects on reproduction, spontaneous abortions and malformations. These studies were carried out in workers with chronic exposure to CS₂.

Data on genotoxicity are very limited. CS₂ increased the frequency of sister chromatid exchanges (SCE) *in vitro* in human lymphocytes in the presence, but not in the absence of metabolic activation system. Chromosomal aberrations were not increased (Garry et al. 1990). A further *in vitro* study using WI-38 human lung fibroblasts found no increased unscheduled DNA synthesis (UDS) in the absence of metabolic activation system. In the presence of metabolic activation system, a slight but significant amount of UDS was observed. No valid conclusions can be drawn because, unexpectedly, the positive control substance benzo(a)pyrene failed to induce UDS in this study (Litton Bionetics 1980).

A number of epidemiological studies on mortality in workers exposed to CS₂, especially in the viscose rayon industry, have been presented. However, these studies focus on the association between exposure and mortality from cardiovascular diseases, and other findings are poorly described. Overall, the data base with respect to cancer, is limited. There is no consistent evidence of an increase in mortality from all cancers combined or from cancers at any specific site.

3 ANIMAL TOXICITY DATA

In this TSD, the presentation and discussion of animal toxicity studies has been limited to acute exposure studies and, in addition, to studies with repeated exposure. These were evaluated with respect to the presence or absence of acute toxic effects which are of relevance for the derivation of AEGL values.

3.1 Acute Lethality

Studies were performed on rats, mice, rabbits, and cats. Data are summarized in Table 4.

3.1.1 Rats

Six male CD-rats/group were exposed to 3000 ppm and 3500 ppm CS₂ for 4 hours in a 16-liter exposure chamber (Du Pont 1966). At the higher exposure concentration, analytically determined values as monitored hourly by gas chromatography were about 8.8 % higher than nominal values. Due to instrumental difficulties, no analytical confirmation was performed at 3000 ppm. The data indicate a very steep concentration-response curve for lethality: Whereas all six rats exposed to 3500 ppm died during exposure or within less than 2 hours later, none of 6 rats exposed to 3000 ppm CS₂ died during exposure or within the 14 day postexposure observation period. During exposure, animals suffered from tachypnea, ptosis, incoordination, chromodacryorrhea (release of red fluid from nasolacrimal glands), and gasping. Weight loss, hyperexcitability, and dyspnea were observed 24 hours postexposure. At 3500 ppm, besides the effects described above, salivation, aimless wandering, and prostration were noted. Necropsy of 2 rats exposed to 3500 ppm revealed pleural effusion, dark red and edematous lungs, petechial lung hemorrhages, and pulmonary hyperemia. Changes in other organs were also seen but not reported.

Without further details, a 2-hour LC₅₀ of 25000 mg/m³ (8025 ppm) for rats is reported by Izmerov et al. (1982).

Studies with repeated inhalation exposure

No treatment-related deaths were noted in male and female Fischer rats exposed to either 0, 50, 500, or 800 ppm CS₂ for 6 hours/d, 5 days/week for 2, 4, 8, or 13 weeks as described by Sills et al. (1998b) (see 3.2.2). Lethality occurred in four animals: one male at 50 ppm and one at 500 ppm died within 2 weeks; and two control females died within the 4-week and the 13-week study. Non-lethal effects observed in this study are reported in section 3.2.2 (Moser et al. 1998).

In a subchronic study, Fischer 344 rats and Sprague-Dawley rats (15 m + 15 f/group) were exposed to 0, 50, 300, and 800 ppm CS₂ for 6 hours/day, 5 days/week for at least 89 consecutive calendar days (Toxigenics 1983a; 1983b; 1983c). Both strains of rats and B6C3F1 mice (see 3.1.2) were exposed simultaneously in the same chambers. CS₂ was metered to a vaporization flask. The vapors were swept from the flask by a continuous supply of air and entered the exposure chamber through a turret at the top of the chamber where they were mixed with the chamber air supply. The concentration of CS₂ in the chamber air was monitored by gas chromatography with each test chamber sampled ca. once per hour. Additionally, in selected chambers the build-up and decay rates and the distribution were measured by means of a Miran A infrared analyser. Deviations between nominal and actual time-weighted average CS₂ concentrations were less than 1.5 %. There was no mortality in any group of the Fischer 344 rats during the study. One male Sprague-Dawley rat exposed to 800 ppm was found dead on study day 41 and one male Sprague-Dawley rat exposed to 50 ppm was sacrificed in extremis on study day 50 of the study. Non-lethal effects observed in this study are reported in section 3.2.2.

TABLE 4: SUMMARY OF LETHAL EFFECTS IN ANIMALS AFTER ACUTE INHALATION EXPOSURE TO CARBON DISULFIDE

Species	Exposure	Concentration	Effect/remarks	Reference
Rat	2 hours	25000 mg/m ³ (8025 ppm)	LC ₅₀	Izmerov et al. 1982
	4 hours	3500 ppm	6/6 died	Du Pont 1966
	4 hours	3000 ppm	0/6 died	
	4 hours/d, 5 d/w, 2 weeks	2000 ppm	No death after one exposure; 2/10 died after 10 exposures	Goldberg et al. 1964
	2 hours	0.15 % (1500 ppm)	0/12 died	Savolainen and Järvisalo 1977
	6 hours/d, 5 d/w, 13 weeks	800 ppm	F344 rats: no mortality S-D rats: 1/15 m died at day 41	Toxigenics 1983b; Toxigenics 1983c
	6 hours/d, 5 d/w, 13 weeks	800 ppm	No treatment related deaths	Moser et al. 1998
Mouse	2 hours	10000 mg/m ³ (3210 ppm)	LC ₅₀	Izmerov et al. 1982
	30 minutes	4500 ppm	“average lethal concentration”, 17/30 animals died	Kuljak et al. 1974
	30 minutes/d, 3 days	3000 ppm	21/30 animals died	
	6 hours/d, 2 - 5 days	800 ppm	No death after one exposure; 21/57 died in group on high fat diet; no death in group on normal diet	Lewis et al. 1999
	6 hours/d, 5 d/w, 13 weeks	800 ppm	4/30 died 13 th week	Toxigenics 1983a
	1 hour	220 ppm	LC ₅₀	Gibson and Roberts 1972
Rabbit	6 hours 15 minutes	3220 ppm	2 ½ hours: lying on its side; narcosis at the end; death after 7 d	Flury and Zernik 1931
	6 hours	3000 ppm	4/6 died and 2/6 moribund and euthanized after exposure	PAI 1991
	6 hours/d, 13 days	1200 ppm	Developmental toxicity study 2/24 dams died	PAI 1991
Cat	48 minutes	112 mg/L (36000 ppm)	Lying on its side, convulsions, 1 ¾ hours: narcosis, died after half a day	Lehmann and Flury 1938
	3 hours 8 minutes	≥ 23 mg/L (7400 ppm)	Died during exposure	Lehmann 1894
	2 hours 15 minutes	6450 ppm	40 minutes: lying on its side, convulsions; later narcosis; death after 1 d	Flury and Zernik 1931
	4 hours 15 minutes	3220 ppm	1 ¾ hours: lying on its side, convulsions; after 4 hours: narcosis, death after 1 day	

TABLE 4: SUMMARY OF LETHAL EFFECTS IN ANIMALS AFTER ACUTE INHALATION EXPOSURE TO CARBON DISULFIDE

Species	Exposure	Concentration	Effect/remarks	Reference
Guinea pig	15 minutes	≥ 54 mg/L (≥ 17300 ppm)	Increasing paralysis, death	Lehmann 1894
	30 minutes	≥ 23 mg/L (≥ 7400 ppm)	Died without convulsions	

Studies with non-inhalation exposure

An oral LD₅₀ of 3188 mg/kg b.w. was reported by Izmerov et al. (1982). In another study, following oral administration of undiluted CS₂ to male Sprague-Dawley rats, a LD₅₀ of 1200 mg/kg b.w. was determined (Kanada et al. 1994). No further details were presented in these studies.

After intraperitoneal injection of undiluted CS₂ to male Sprague-Dawley rats, a LD₅₀ of 0.84 mL/kg b.w. (1060 mg/kg) was reported (de Gandarias et al. 1992).

In a further study, the toxicity of CS₂ in Sprague-Dawley rats of different age was compared (Green and Hunter 1985). CS₂ was given intraperitoneally in corn oil vehicle (total: 5 mL/kg i.p.). The 24-hour LD₅₀ was estimated by the “up-and-down” method using groups of three rats each at 1, 5, and 10 day of age (unselected as to sex) and of 20, 30, and 40 days of age (males only). A dose which killed 0/3 or 1/3 was a negative response. In this case, the dosage was increased by 25 %, and the trial was repeated. If 2/3 or 3/3 rats died, the trial was redone at a lower dose. The procedure was duplicated three times after obtaining one positive and one negative response. CS₂ was found least toxic to 20-day-old male rats (LD₅₀ 1545 mg/kg) and most toxic to one-day-old rats (LD₅₀ 583 mg/kg).

3.1.2 Mice

Gibson and Roberts (1972) exposed male Swiss-Webster mice to calculated CS₂ concentrations of 54, 110, 230 and 550 ppm, respectively, for 60 minutes. Treatment was achieved in an exposure apparatus with the following components: compressed fresh air from a cylinder; an evaporation chamber into which CS₂ was introduced through a needle attached to a gas-tight syringe; an infusion apparatus for controlled introduction of CS₂ into the evaporation chamber, and a 4-l glass exposure chamber in an aluminum frame fitted with a rubber seal. Vapors were introduced at the top and the exhaust vent originated near the bottom of the chamber. Fresh food and water were available in the chamber. Air flow was adjusted to 1000 mL/minutes as measured by a Gilmont no.10 flow gauge inserted between the evaporation and exposure chamber. CS₂ infusion rates of 0.005, 0.010, 0.021 and 0.051 mL/minutes were reported to give the calculated concentrations given above. The actual concentrations of CS₂ were not measured. 4 mice occupied the exposure chamber for each exposure. The actual number of animals exposed to a given concentration and the number of exposures were not reported. An “approximate LC₅₀” of 220 ppm was reported. Further data presented indicate a steep concentration-response curve for lethality: Whereas animal lethality precluded time studies of liver function exceeding 2 hours at 230 ppm, such studies could be carried out over 24 hours at 110 ppm.

Izmerov et al. (1982) reported a 2-hour LC₅₀ of 10000 mg/m³ (3210 ppm) for mice. No details were presented.

Kuljak et al. (1974) exposed mice (sex and strain not reported) to CS₂ in a desiccator through which air mixed with CS₂ was passed at a rate of 1.2 L/hour by means of a vacuum pump. A gas meter and a series of 3 impingers in which the CS₂ was absorbed were placed between the exsiccator and the

vacuum pump. CS₂ was determined by a xanthogenate method. The “average lethal concentration” (LC_m) was determined by straight-line graphic interpolation. Exposure to 4500 ppm (reported to represent the LC_m) for 30 minutes killed 17 out of 30 animals. In a further experiment on mice pretreated with glutathione (100 mg/kg i.p.) two hours prior to exposure to 4500 ppm CS₂ for 30 minutes, 8 of 30 animals died.

Studies with repeated inhalation exposure

Lewis et al. (1999) studied the effects of CS₂ on the development of atherogenic lesions. Female C57BL/6 mice were exposed to 50, 500, or 800 ppm CS₂ for 6 hours/d, 5 days/week for 1, 4, 8, 12, 16, or 20 weeks. The concentration of CS₂ in each Hazleton 1000 exposure chambers was monitored with Fourier Transform Infrared Spectrophotometers every 1.5 minutes in the three chambers used for CS₂ exposure and once every 15 minutes in the control chamber. Actual concentrations were within 3 % of the target concentrations during the study. Immediately after the first exposure, half of each group (i.e., 10 animals) in the 6 subgroups (to be exposed 1, 4, 8, 12, 16, or 20 weeks) were placed on a control diet (standard NIH-07) and half on an atherogenic high fat diet (50 % sucrose, 15 % cocoa fat, 1 % cholesterol, 0.5 % Na-choleate). In the high fat diet groups exposed to 800 ppm, 21 of 60 mice died during the first week of exposure. Not all animals died at the same day, and none died after a single exposure to CS₂ (Lewis, personal communication). Necropsies failed to disclose the cause of death. Subsequently, no further mice died in the 800-ppm group, but there were 3 deaths in the 500-ppm groups at the high fat diet. Animals on the control diet had 5 deaths overall which were dispersed over dose range and exposure time. Non-lethal effects of this study are described in section 3.2.3.

In a subchronic study, B6C3F1 mice (10 m + 12 f/group) were exposed to 0, 50, 300, and 800 ppm CS₂ for 6 hours/day, 5 days/week for at least 89 consecutive calendar days (Toxigenics 1983a). Mice were exposed simultaneously in the same chambers with rats (for experimental details, see 3.1.2). Four mice exposed to 800 ppm were found dead during the study: 2 males on study day 92 and two females on study day 87. Additionally, a female exposed to 300 ppm was discovered missing on day 91. Non-lethal effects observed in this study are reported in section 3.2.3.

Studies with non-inhalation exposure

Male Swiss-Webster mice were given CS₂ in corn oil orally by intubation or intraperitoneally so that each animal received 10 mL/kg of oil-CS₂ solution (Gibson and Roberts 1972). Neither the exact number of animals nor the different CS₂ concentrations used were reported. The median lethal dose of CS₂ (within a 24 hour period) for oral administration was 3020 mg/kg and for i.p. administration 1890 mg/kg.

Without further details, Izmerov et al. (1982) reported an oral LD₅₀ of 2780 mg/kg for mice.

3.1.3 Rabbits

In an unpublished range finding experiment for a reproductive/developmental toxicity study, 6 pregnant New Zealand rabbits were exposed to 3000 ppm CS₂ for 6 hours on the 6th day of gestation (PAI 1991). 4 of 6 animals died during exposure, the 2 others were moribund at the end of exposure and were sacrificed. No gross lesions were observed but the animals exhibited tremors, labored breathing, and apparent anoxia. The four animals which died during exposure did not struggle or convulse prior to death.

Studies with non-inhalation exposure

Without further details, Izmerov et al. (1982) reported an oral LD₅₀ of 2550 mg/kg.

Brieger (1949b) reported that rabbits (no further details given) injected intravenously with 0.5 mL (0.63 g) CS₂ died within 20 minutes.

3.1.4 Cats

Flury and Zernik (1931) reported that individual cats exposed to 3220 ppm CS₂ for 4.25 hours and to 6450 ppm for 2.25 hours became anesthetized during exposure and died after 1 and 7 days, respectively.

3.1.5 Guinea pigs

Lehmann (1894) reported that one guinea pig exposed to at least 7400 ppm died within 30 minutes and another one exposed to at least 17300 ppm within 15 minutes.

Studies with non-inhalation exposure

An oral LD₅₀ of 2125 mg/kg was reported, but no details were given (Izmerov et al. 1982).

Following intraperitoneal administration of 400 mg/kg CS₂, 3 of 4 male guinea pigs died within 24 hours (DiVincenzo and Krasavage 1974).

3.2 Nonlethal Toxicity

Studies with single and repeated inhalation exposure were performed with monkeys, rats, mice, rabbits, dogs and cats. A number of studies with repeated inhalation exposure have reported acute effects in laboratory animals after the first exposure or at the end of the daily exposure period. Nonlethal effects are summarized in Table 5.

TABLE 5: SUMMARY OF ACUTE NON-LETHAL EFFECTS IN ANIMALS AFTER INHALATION EXPOSURE TO CARBON DISULFIDE				
Species, strain, no., sex	Exposure	Concentration	Effect	Reference
Squirrel monkey, 2	2 hours	600 ppm	Rise in electric shock tolerance, diminution of response force	Weiss et al. 1979
Squirrel monkey, 4	18 hours	70 – 200 ppm		
Rat	10 minutes	1660 – 81100 ppm	No overt clinical signs of toxicity, transient slight to moderate weight loss	Du Pont 1981
Rat	4 hours	3000 ppm	0/6 animals died; tachypnea, ptosis, incoordination, gasping, hyperexcitability	Du Pont 1966
Rat, CFE, f	4 hours	2000 ppm	Behavioral alterations	Goldberg et al. 1964

TABLE 5: SUMMARY OF ACUTE NON-LETHAL EFFECTS IN ANIMALS AFTER INHALATION EXPOSURE TO CARBON DISULFIDE

Species, strain, no., sex	Exposure	Concentration	Effect	Reference
Rat	4 hours/day	5 mg/L (1600 ppm)	Exposure well tolerated, no signs of toxicity apart from animals being more subdued	Heubusch and DiStefano 1978
Rat, S-D, 12	2 hours	0.15 % (1500 ppm)	No deaths; slightly somnolent after exposure, recovery within 46 hours	Savolainen and Järvisalo 1977
Rat, Wistar, 4, m	4 hours	1370 ppm	30 % depression of response to electric seizure	Frantik et al. 1994
Rat, Wistar, 8-30, m	4 hours/day, 2 days	4.0 mg/L (1280 ppm)	Myocardial damage only in animals pretreated with phenobarbitone (PB) + noradrenaline (or PB + cold-stress)	Chandra et al. 1972
Rat, Wistar, 7, m	18 hours	2.5 mg/L (800 ppm)	Severe narcosis, reduced cardiac and respiratory rate, straightening of hind limbs, reduced body temperature; uncoupling of oxidative phosphorylation in brain mitochondria	Tarkowski and Sobczak 1971
Rat, Porton, 6, m	15 hours	2.5 mg/L (800 ppm)	Ataxia, tremors, occasional convulsions; 25 % lowering of blood glucose; alterations of brain amino acid metabolism	Tarkowski and Cremer 1972
Rat, Wistar, 7, f	12 hours	2.4 mg/L (770 ppm)	No visible signs of toxicity reported; Brain: Ultrastructural alterations of mitochondria, increase in ATP, decrease in ADP and AMP	Tarkowski et al. 1980
Rat, 6	4 hours/day	800 ppm	No deaths; drowsiness shortly after start of exposure	Battig and Grandjean 1964
Rat, F 344, 9 m, 9 f	6 hours/day, 5 d/w, 2-13 weeks	50 ppm 500 ppm 800 ppm	No treatment-related deaths	Moser et al. 1998
Rat, 18, m	6 or 7 hours	0.15 mg/L (50 ppm) 1.2 mg/L (385 ppm) 2.4 mg/L (770 ppm)	No effect increase in spontaneous motor activity decrease of spontaneous motor activity (ca. 60 %), motor performance, and avoidance reactions	Frantik 1970
Rat, S-D, 4-6, m	4 hours, 8 hours	2 mg/L (640 ppm)	Decrease of brain, adrenal, heart noradrenaline Decrease of adrenal adrenaline Increase of adrenal dopamine	McKenna and DiStefano 1977b

TABLE 5: SUMMARY OF ACUTE NON-LETHAL EFFECTS IN ANIMALS AFTER INHALATION EXPOSURE TO CARBON DISULFIDE

Species, strain, no., sex	Exposure	Concentration	Effect	Reference
Rat, 6, m	4 hours/d; 2 days	2 mg/L (640 ppm)	Decreased noradrenaline concentration in brain; increase in amphetamine-induced stereotypes	Magos et al. 1974
Rat, Porton-Wistar, 4, m	16 hours	2 mg/L (640 ppm)	No obvious sign of toxicity after exposure Brain: increase of dopamine, decrease of noradrenaline	Caroldi et al. 1987
Rat, S-D, 6, m	10 hours/d; 14 days	600 ppm 800 ppm	≥ 600 ppm, each day: narcotic-like stupor during exposure; after 14 hours return to normal levels of alertness and activity 600 ppm, ≥ 9 d: circling behavior, retropulsion 800 ppm, ≥ 4 d: circling behavior, retropulsion	Wilmarth et al. 1993
Rat, Wistar, 14, m	6 hours	500 ppm	Reduced activity level, not strongly irritating or prenarctic	Kivisto et al. 1995
Rat, Wistar, 5 – 15, f	8 hours	20 ppm 100 ppm 400 ppm	Decrease of liver glycogen Increased oxygen consumption No change of serum GOT, GPT, LDH, biliary BSP-clearance; increased hepatic lactate	Freundt and Kürzinger 1975
Rat, Wistar, 5 or 10, f	8 hours	200 ppm	No hepatic damage	Freundt et al. 1974a
Rat, S-D, 4-6, m	8 hours	0.2 mg/L (64 ppm)	Decrease of brain noradrenaline	McKenna and DiStefano 1977b
Rat, Wistar, 5 – 15, f	8 hours	20 ppm	Increase in hepatic microsomal lipid, inhibition of microsomal drug biotransformation	Freundt et al. 1974b; Freundt and Kuttner 1969
Rat, S-D, 20-23 f	6 hours/d, gestation day 6-20	100 ppm 200 ppm 400 ppm 800 ppm	No deaths of dams ≥ 400 ppm: reduced weight gain	Saillenfait et al. 1989
Mouse	20 minutes	11000 ppm	Narcosis; recovery after termination of exposure	Flury and Zernik 1931
Mouse, H, 8, f	2 hours	2600 ppm	30 % depression of response to electric seizure	Frantik et al. 1994

TABLE 5: SUMMARY OF ACUTE NON-LETHAL EFFECTS IN ANIMALS AFTER INHALATION EXPOSURE TO CARBON DISULFIDE

Species, strain, no., sex	Exposure	Concentration	Effect	Reference
Mouse, CD-1, 4-5, m,	30 minutes	120 ppm 580 ppm 2270 ppm 3700 ppm	No effect on behavioral response decreased responding in most mice decreased responding in all mice responding abolished	Liang et al. 1983
Mouse, CD-1, 12, m	30 minutes	2000 ppm 2242 ppm 3700 ppm	Decreased behavioral response in some mice calculated EC ₅₀ abolished response in all mice	Glowa and Dews 1987
Rabbit, 1	2 hours 15 minutes	6450 ppm	50 minutes: lying on its side, convulsions, narcosis, recovery	Flury and Zernik 1931
Rabbit, 1	3 hours	10.4 mg/L (3340 ppm)	Swaying, lying on its side, loss of reflexes, recovery after end of exposure	Lehmann 1894
Rabbit, 1	2 hours 15 minutes	9.3 mg/L (2990 ppm)	Swaying, lying on its side, restlessness, paralysis, nystagmus, recovery	
Rabbit, 1	3 hours 30	7.6 mg/L (2440 ppm)	Swaying, lying on its side, convulsions, paralysis, recovery after about 1 hour	
Rabbit, 2	3 hours	4.3 – 4.7 mg/L (1380 – 1510 ppm)	Variable respiration, restlessness	
Rabbit, 1	9 hours	2.64 mg/L (850 ppm)	No marked symptoms noted, animal takes up food during exposure	
Rabbit, 3	8 hours	1.2 -1.34 mg/L (385 – 430 ppm)	Decreasing respiration rate, no further symptoms	
Rabbit	10 hours	0.2 mg/L (64 ppm)	No signs of acute toxic effects observed	Lehmann 1894
Rabbit	6 hours/d, 13 days	1200 ppm	Developmental toxicity study Dams: reduced weight gain, ataxia, tremors, wheezing, labored respiration	PAI 1991
Rabbit	6 hours/d, 5 d/w, 17 weeks	750 ppm	No signs of acute toxicity observed	Cohen et al. 1959
Dogs, mixed, 8	8 hours/day, 5 days/week 10 – 15 wees	400 ± 102 ppm	During exposure: sleep Immediately after exposure: drowsiness, staggered and stumbled gait, trembling and shaking, restlessness, later excited, noisy Death after 10 – 15 weeks	Lewey et al. 1941
Cat, 1	1 hour 6 minutes	75 mg/L (24100 ppm)	Lying on its side, convulsions after 30 minutes, recovery after end of exposure	Flury and Zernik 1931
Cat, 5	0.5 - 2.5 hours/d 24 – 92 day	8 – 10 mg/L (2570 – 3210 ppm)	Salivation, dyspnoea, restlessness, excitement at first, apathy later, tremor, sometimes coma	Ferraro et al. 1941

TABLE 5: SUMMARY OF ACUTE NON-LETHAL EFFECTS IN ANIMALS AFTER INHALATION EXPOSURE TO CARBON DISULFIDE

Species, strain, no., sex	Exposure	Concentration	Effect	Reference
Cat, 2	2 hours 15 minutes	10.4 mg/L (3340 ppm)	Shaking, repeated vomiting, convulsions, collapse, salivation, slow recovery	Lehmann 1894
Cat, 1	2 hours 30 minutes	9.3 mg/L (2990 ppm)	Shaking, shivering, vomiting, tonic-clonic convulsions, variable respiration	
Cat, 1	3 hours 20 minutes	7.6 mg/L (2440 ppm)	Vomiting, dyspnoea, salivation, tonic convulsions, decreasing respiration rate	
Cat, 1	3 hours	4.7 mg/L (1510 ppm)	Increased respiration, lying on its side, clonic and tonic convulsions, salivation	
Cat, 2	9 hours	2.64 mg/L (850 ppm)	Slow respiration, vomiting, clonic convulsions, lying on its side; recovery after exposure	
Cat, 1	8 hours	1.34 mg/L (430 ppm)	Slow respiration, dozing, defecation, variable respiration rate	
Cat, 1	8 hours	1.2 mg/L (385 ppm)	Slow respiration, no marked effects	
Cat, 1	10 hours	0.2 mg/L (64 ppm)	No toxic effects observed	

3.2.1 Nonhuman primates

Behavioral studies

Aversive thresholds to electric shock stimulation were studied in squirrel monkeys (*Saimiri sciureus*) (Weiss et al. 1979). Individual animals were placed in an exposure chamber and restrained at the waist. The animal faced a T-shaped bar fixed to a strain gauge. A computer-controlled, constant current shock stimulator delivered the aversive stimulus to the electrodes placed on the tail and foot. The strain gauge output was fed to the inputs of a computer. A large force requirement of 300 g enhanced the sensitivity of the experiment to the toxicologic insult. The shock level was raised by 2 % of the total range (60 μ A/step) each time an increment was programmed (every 2 seconds) and reduced by the same amount after each response. The bar had to be released (the force falling to less than 5 g for 100 ms) to initiate a new response, continued application of such a force did not further lower the shock. The concentration of CS₂ in the exposure chamber was monitored continuously by means of Miran-1A[®] infrared spectrophotometer.

Experiments with one monkey revealed a stable performance under control conditions without exposure to CS₂. The aversive threshold rose for the first few minutes and subsequently undulated within narrow limits. This undulating response is explained by the tendency of the animal to wait for the shock level to rise by several steps before emitting a train of responses which lowers the shock level. In a 2 hour exposure to about 600 ppm CS₂, a radically altered response pattern was observed: During the first 30 minutes, the animal responded erratically and tolerated higher shock levels than under control conditions. Long gaps without responding and an inadequate force exerted (responses less than 300 g did not reduce the shock) when the monkey did react contributed to this effect. During the last 30 minutes of exposure, response forces met the criterion as often as in the control session, but the aversive threshold remained

elevated beyond control values suggesting an anesthetic and/or an analgesic effect. This effect was also seen in a second monkey that maintained a shock level 50 % above its own control value.

Additional experiments with lower exposure concentrations over longer periods of time were reported to produce equivalent effects. While the first monkey whose performance is described above displayed a similar response to an exposure of 18 hours to 70 ppm, the highest concentration required to produce such an effect in a group of four monkeys trained as described above was 200 ppm.

3.2.2 Rats

Exposure of 6 rats to 3000 ppm CS₂ for 4 hours resulted in no deaths during exposure or within the 14 day postexposure observation period (Du Pont 1966). During exposure, animals suffered from tachypnea, ptosis, incoordination, chromodacryorrhea (release of red fluids from nasolacrimal glands), and gasping. Weight loss, hyperexcitability, and dyspnea were observed 24 hours postexposure. The data of this study indicate a very steep concentration-response curve since all of six rats exposed to 3500 ppm for 4 hours died during exposure or before 2 hours postexposure (see 3.1.1).

In an upper respiratory tract irritation study, 4 rats/group were exposed head-only to analytically confirmed concentrations of 1660, 8760, 35100, or 81100 ppm CS₂ for 10 minutes. No respiratory rate depression was observed in response to CS₂ exposure. At 1660 ppm but not at higher concentrations, dark red eyes were observed 24 hours to 6 days postexposure. No overt clinical signs of toxicity were noted. However, a slight to moderate transient weight loss (no further data) was observed 24 hours postexposure at all exposure concentrations (Du Pont 1981).

Frantik et al. (1994) studied the inhibition of propagation and maintenance of the electrically evoked seizure discharge in rats and mice. Concentration-effect regressions were determined for 48 common solvents including CS₂ in male Wistar rats. The animals were exposed individually in dynamic 80-liter glass chambers for 4 hours. Sixteen rats – 4 controls exposed to ambient air and 4 at each concentration of solvent – were exposed and measured in one trail, and each trail was repeated at least once for each solvent tested. Three concentrations of solvent were selected in the linear part of the concentration-response curve (between 25 and 75 % of maximum effect, if possible. For some not explicitly named solvents the concentrations had to be lowered to avoid respiratory tract irritancy). Exposure concentrations were measured by gas chromatography, but the exact concentrations used were not reported. Measurements were carried out within 1 minute after removal of the animals from the exposure chamber. A short electrical impulse (0.2 s; 180 V; 50 Hz) was applied through ear electrodes. Of six time characteristics recorded, the duration of tonic extension of hindlimbs was the most sensitive and reproducible response measure in rats. The median of individual control values was subtracted from the values observed after exposure. Group means of differences were corrected for the difference in the simultaneously tested control and converted to relative values, i.e. to percentage of the arbitrary maximum value (8 s). All data were processed using linear regression analysis to estimate the concentration of solvent in air evoking 37 % of the maximum possible effect. In case of CS₂, a concentration of 1370 ppm (one-sided 90 % confidence interval 170 ppm) and a slope of regression of 0.029 %/ppm were calculated. The lowest effect concentration which for most solvents could be proven statistically was 10 %. For CS₂, the EC₁₀ can be calculated as follows: $EC_{10, 4 \text{ hours, rat}} = 1370 \text{ ppm} - 27 \% \div (0.029 \%/\text{ppm}) = 440 \text{ ppm}$.

Behavioral studies

Goldberg et al. (1964) studied the effects of CS₂ exposure on animal behavior in an experimental system (as described in Goldberg et al. 1962). Behavioral training experiments were conducted in plastic chamber with a metal grid floor and a wooden pole with a rough surface attached to the chamber top which served as an escape or safety area. During the training phase, female Carworth

Farms Elias rats aged 30 – 40 days were placed in the chamber for 15 s with no stimulus. Then, a series of electric shocks (100 V, 20 ms, 10 pulses/s) was delivered to the floor for 30 s concurrent with the activation of a buzzer. The stimuli were immediately terminated when the rat successfully climbed the pole as escape area. The response to the shock and the buzzer was considered an unconditioned response (escape response). When the animal had learned to consistently show the proper escape reaction, the stimuli were dissociated and the animal climbed the pole in response to the buzzer alone (conditioned response, avoidance response).

Prior to vapor inhalation experiments, animals were examined for their response to the avoidance and escape stimuli. Effect measurement was done on a quantal basis, i.e., the percentage of rats which showed an inhibition of the response. 8 – 10 rats were used in both control and experimental groups with different chemicals, including CS₂. Rats were exposed 4 hours/d, 5 d/w for 2 weeks to 250, 500, 1000, and 2000 ppm CS₂. Exposure was carried out in a dynamic 200-l exposure chamber at an airflow of approximately 95 L/minutes. Air flows were adjusted so that the actual vapor concentrations as determined with a Zeiss interferometer were within 10 % of the nominal concentrations.

Responses were determined on days 1, 2, 3, 4, 5 and 10 before, during, and 2 hours after removal from exposure. Tests made within two hours after termination of exposure gave maximum effects. Up to 1000 ppm, no effects were seen after one or two exposures. From the third exposure at 1000 ppm, the fourth at 500 ppm and the fifth at 250 ppm CS₂, an inhibition of avoidance response was seen without an accompanying effect on the escape response. At 2000 ppm, an inhibition of the avoidance response was obtained in 50 % of the animals after one and two exposures. Repeated exposure at 2000 ppm resulted in progressive effects on both avoidance and escape response, and the avoidance response showed inhibition in all animals after 10 days. At this concentration, several rats did not escape when the shock was presented, even though they appeared capable. Two animals receiving this concentration died within a few days following the last exposure.

Studies mainly to investigate effects on liver

The acute effects on hepatic energy potential and functions were studied in female Wistar rats (Freundt and Kürzinger 1975; Kürzinger and Freundt 1969). The animals were exposed to 0, 20, 100, 200, or 400 ppm CS₂ for 8 hours. Exposures were carried out in 200-l dynamic exposure chambers as described by Freundt et al. (1974a). During the exposure, the animals had free access to standard food and tap water. A significant, concentration-dependent decrease in the glycogen content of the liver (20 ppm: 68 % of control; 400 ppm: 24 % of control) was observed after exposure to CS₂. An adrenergic mechanism as a cause of the glycogen depletion was disregarded since pretreatment of animals with 2 mg/kg reserpine i.p. 16 hours prior to exposure to 400 ppm CS₂ did not prevent the loss of glycogen. The decrease of liver glycogen was associated with a 68 %-increase of hepatic lactate (only determined at 400 ppm), an increase of hepatic inorganic phosphate levels between 10 % (at 20 ppm) and 40 % (at 400 ppm) and an increased oxygen consumption of hepatic tissue slices *ex vivo* after exposure (only determined at 400 ppm). Furthermore, the exposed animals showed a 20 - 40 % increase in whole-body oxygen uptake, a fall in body temperature by 1.5 °C (at 400 ppm), and a decrease of the body weight. Up to 400 ppm, no changes of the biliary BSP-clearance and of serum activity of GOT, GPT and LDH were found. All parameters were normal 24 hours after exposure to the highest concentration.

Effects of CS₂ on the biotransformation of various xenobiotics were studied by Freundt and Dreher (1969); Freundt and Kuttner (1969); and Freundt et al. (1976a). Female Wistar rats were exposed to 20, 50, 100, 200, and 400 ppm CS₂ for up to 8 hours in an exposure chamber as described (Freundt et al. 1974a). Immediately after termination of exposure, 12 – 20 animals per group were treated with various xenobiotics and the urinary excretion of xenobiotic metabolites was followed. At all concentrations of CS₂ tested, the excretion of the following metabolites was significantly delayed

(indicating inhibition of phase I drug-metabolizing pathways): trichloroethanol and trichloroacetic acid from trichloroethene, 4-OH-antipyrine from antipyrine, acetaminophenol from acetanilide and phenacetin, and 4-aminoantipyrine from aminopyrine. All effects were reversible within 6 – 36 hours. Furthermore, CS₂ led to a concentration-dependent significant increase in the hexobarbital sleeping time in rats which had received 100 mg/kg of sodium hexobarbital i.p.. In contrast, the (phase II) N-acetylation of sulfisomidine and 4-aminoantipyrine and the glucuronidation of 4-OH-antipyrine were not markedly affected up to 400 ppm CS₂. Further investigations revealed that under the conditions of the described exposure CS₂ reversibly increased the hepatic microsomal lipid content, while the microsomal NADPH-cytochrome c-reductase activity and the total microsomal P-450 content remained within the normal range (Freundt et al. 1974b; Freundt and Schauenburg 1971).

The effects of CS₂ on the blood levels of acetaldehyde in ethanol-treated rats were studied in Wistar rats (Freundt et al. 1976b; Freundt and Netz 1973). 4 – 6 female animals per concentration were exposed once to 20 and 400 ppm CS₂, respectively, for 8 hours, or received 12 repetitive exposures to 400 ppm CS₂ at 40-hours intervals (every other day). Exposures were carried out as described by Freundt et al. (1974a). Subsequently, rats were given 2 g/kg ethanol (20 % solution, i.p.; blood level about 2.5 – 3 %) and left exposed to CS₂ for up to 4 hours to the time of blood collection. In the presence as in the absence of CS₂, the blood ethanol concentration decreased linearly and the regression of the blood elimination curves was not significantly different from that of controls. The acetaldehyde concentration in blood rose after administration of ethanol and was about 30 % higher in animals exposed to 20 ppm CS₂. Single or repeated exposure to 400 ppm CS₂ produced a slight additional increase in blood acetaldehyde (up to 1.5-fold of control values). In similar experiments, oral treatment of rats with disulfiram (“antabuse”, 1 g/kg b.w.) increased blood acetaldehyde levels up to 5-fold. Intravenous administration of acetaldehyde to rats treated with 400 ppm CS₂ for 8 hours or with disulfiram revealed that the rate of acetaldehyde elimination from blood was significantly lowered by CS₂ and by disulfiram exposure (control $t_{1/2}$: 1 minutes 45 s; CS₂-treated: 2 minutes 24 s; disulfiram-treated animals: 2 minutes 48 seconds).

In a metabolism study, Kivisto et al. (1995) exposed 7 groups of 2 male Wistar rats/group to 50 ppm CS₂ or 500 ppm for 6 hours. The subgroups received no pretreatment or were pretreated with P450 enzyme inducers or glutathione depletors. Exposures were carried out in a 5 L dynamic exposure chamber (air flow 5 m³/hours) and the concentration of CS₂ was continuously monitored using a Miran 1A infrared analyser. Exposure to 500 ppm was reported to reduce the activity level of the rats. No further details regarding toxic effects were mentioned.

Studies mainly on brain metabolism

Savolainen and Järvisalo (1977) exposed 12 female Sprague-Dawley rats to 0 or 0.15 % CS₂ (1500 ppm) for 2 hours. Additionally, 12 littermate animals were treated with phenobarbitone (PB) in drinking water (0.1 % w/v) for 7 d prior to the experiment. 8 control and 8 PB-treated animals were not exposed to CS₂. Immediately prior to the exposure, animals received ¹⁴C-leucine i.p. No details of the exposure conditions were reported. Immediately after CS₂ exposure, the animals were slightly somnolent, but none of the animals died during the experiment. 1 hour, 4 hours, and 46 hours after exposure, in 4 animals/group the following parameters in brain were determined: leucine incorporation, protein, RNA, activity of acid proteinase, creatine kinase and non-specific cholinesterase were determined. The authors note some minor transient changes in some parameters, but the interpretation of the data is hardly understandable since no statistical evaluation was presented. At the same exposure conditions, CS₂ alone had no effect on liver cytochrome P-450 concentration and transiently lowered 7-ethoxycoumarin O-deethylase (EOD) activity. In rats pretreated with PB, cytochrome P-450 was decreased by 50 % and EOD-activity even more (Järvisalo et al. 1977).

Tarkowski and Cremer (1972) exposed 6 male Porton-strain rats/group to 0 or 2.5 mg/L CS₂ (800 ppm) continuously for 15 hours in a vertical, constant flow chamber as described by Magos et al. (1970). The concentration of CS₂ in the chamber was continuously registered by means of an infrared analyser. As acute signs of poisoning, CS₂-exposed animals suffered from ataxia, tremors and occasional convulsions. At termination of exposure, the animals showed a moderate hypoglycemia, the blood glucose concentration being 25 % lower than that of controls. Additionally, changes in the concentration of amino acids in brain were observed, most notably, exposed animals showed a 70 % increase in glutamine and an increased labelling of brain glutamine from injected [1-¹⁴C]butyrate.

Tarkowski and Sobczak (1971) exposed seven male Wistar rats/group to 0 or 2.5 mg/L CS₂ (800 ppm) continuously for 18 hours in an exposure chamber. The CS₂ concentration was measured colorimetrically. As main symptoms of acute CS₂ poisoning, severe narcosis, reduced cardiac and respiratory rate, straightening of hind limbs and lower body temperature were reported. In brain mitochondria from CS₂ exposed animals, disorders of oxidative phosphorylation (suggesting uncoupling of oxidative phosphorylation) but a decreased ATPase activity were found. However, in a further study in which 6 - 7 female Wistar rats/group were exposed to 0 or 2.4 mg/L CS₂ (770 ppm) for 12 hours, the ATP-content in brain of CS₂-treated rats was 21 % higher while the ADP and AMP contents were lower than in controls (Tarkowski et al. 1980). Some ultrastructural morphological changes with swelling and damage of cristae in the brain mitochondria also were observed. In this study, the authors did not mention the occurrence nor did they explicitly report the absence of visible symptoms of acute poisoning.

Effects on catecholamines

Male Sprague-Dawley rats were exposed to 2000 mg/m³ (640 ppm) for 4, 6, 8, and 8 hours, respectively, in exposure chambers at an air flow of 5 L/minutes (McKenna and DiStefano 1977b). The delivery of CS₂ to the chamber was controlled by a vapor generator flowmeter. The concentration of CS₂ was determined colorimetrically every 30 minutes during exposure by sampling air at the outlet. No signs of toxicity were mentioned to occur during exposure nor did the authors explicitly state the absence of such effects. Exposure to CS₂ caused a time-dependent decrease of noradrenaline (36 % after 8 hours) and a slight transient increase of dopamine in brain. Noradrenaline reapproached control level about 16 hours after exposure. A similar decrease of noradrenaline after 8 hours was seen in the adrenal glands and in the heart. Brain noradrenaline after 8 hours also decreased as a function of CS₂-concentration. 64 ppm was the minimum concentration at which a decrease of noradrenaline could be seen. Similar effects on brain dopamine and noradrenaline following a single exposure to 2000 mg/m³ (640 ppm) for 1 hour or repeated 4 hours/day for 2 days were also described in another study (Magos et al. 1974).

Caroldi et al. (1987) exposed male Porton-Wistar rats to 2000 mg/m³ (640 ppm) CS₂ for 4 hours or 16 hours in a dynamic exposure chamber. The concentration of CS₂ was monitored continuously by an IR-spectrometer. After 16 hours, no obvious signs of toxicity were noted. Similar to the observations described above (McKenna and DiStefano 1977b), CS₂ exposure increased the dopamine concentration in brain and decreased the concentration of noradrenaline in a time-dependent manner.

Studies with repeated inhalation exposure

Behavioral studies

The study of Goldberg et al. (1964) is described at the beginning of this section 3.2.2.

Battig and Grandjean (1964) exposed groups of six rats (about four months old, sex and strain not reported) to 0 or 800 ppm CS₂ for 4 hours/day up to 3 weeks in test cages in a wooden exposure chamber. The chamber was provided with a fresh air supply in which the test substance was vaporized,

and an air circulation system. Samples of the chamber atmosphere analysed at regular intervals by gas chromatography revealed that the initial concentration of 800 ppm during the first 2.5 hours dropped to 550 – 750 ppm and did not decrease further. No animal died during exposure. The rats exposed to CS₂ displayed marked drowsiness from shortly after the start of exposure. The avoidance reaction to painful electric shocks were studied after onset of each exposure. Compared to the corresponding control group, the acquisition curve of the exposed rats rose later and at a lower rate. In the second week, the frequency of avoidance reactions was stable in both groups but was much lower in the group exposed to CS₂.

Frantik (1970) exposed male albino rats (strain not reported) to 0 (n = 42 rats), 0.15 mg/L (n = 18), 1.2 mg/L (n = 18) or 2.4 mg/L (n = 18) CS₂ (0; 50; 385; 770 ppm) for 6 hours/day, 5 days/week for 10 months. A second experiment was carried out with 18 rats per group exposed to 0, 1.2 or 2.4 mg/L CS₂ (0; 385; 770 ppm) for 7 hours/day, 5 days/week from their 7th month of life on. No details regarding incubation conditions were presented. Acute toxic effects on behavioral characteristics and motor capacity were measured 0 – 60 minutes after termination of the daily exposure. At 50 ppm, no effects were observed. At 385 ppm, immediately after the first exposure to CS₂, an increase in spontaneous motor activity was observed. This effect did not re-appear after further exposures. At 770 ppm, changes after the first exposure for 6 hours and especially 7 hours involved reduction of spontaneous motor activity by about 60 %, an inert nature of conditioned avoidance reactions and a decrease in motor performance (maximum speed, static and dynamic endurance). These effects resembled those induced by barbiturates or tranquillizers. They persisted partly for 24 hours and had completely disappeared after 3 days without exposure. After subsequent exposure to the same concentration the pattern was not repeated but instead an activity enhanced compared to control was seen.

Studies on effects on the heart

Chandra et al. (1972) studied the effect of CS₂ on the myocardium of rats. Four groups of 8, 16, or 30 fasted male Wistar rats were exposed in a constant flow inhalation chamber (Magos et al. 1970) to 4 mg/L CS₂ (1280 ppm) for 4 hours/day for 2 days. Additionally, prior to each exposure, rats received no treatment, phenobarbitone (PB), noradrenaline (NA), or both substances. An unspecified number of PB-pretreated rats were exposed to cold stress at 4 °C overnight instead of noradrenaline, and a further unspecified number of PB+NA-pretreated rats received only a single 4-hours exposure to CS₂. Effects on myocardium histology and enzyme activity were determined 0 – 15 days after the second exposure. Treatment with CS₂ alone or combined with PB or NA, respectively, did not result in histological lesions of the myocardium. In a control group pretreated with PB+NA, some slight myocardial lesions were seen. When CS₂ exposure was combined with PB + NA or PB + cold stress, rats developed necrotic lesions resulting in small areas of myocardial fibrosis. Rats pretreated with PB + NA which were given only one exposure to CS₂ also developed myocardium lesions with interstitial edema, cellular infiltrations, and focal hyaline necrosis confined to the papillary muscles and subendocardial region of the left ventricle. In those animals which would develop necrotic lesions, the earliest histochemical changes seen were loss of cytochrome c oxidase and of phosphorylase activity.

Studies on effects on the liver

Effects of CS₂ on the liver were studied in female Wistar rats (Freundt et al. 1974a). Exposure to CS₂ was carried out in 200 L chambers in which 5 animals were placed in wire cages. The CS₂/air mixture was prepared in a glass mixing vessel by evaporating liquid CS₂ into a rotametrically metered stream of air. Continuous dropwise addition of CS₂ according to the desired concentration was obtained with an automatic infusion apparatus. Constant evaporation was maintained by heating the spherical mixing vessel over a 50° C water bath. The prepared CS₂/air mixture was conducted into the exposure chamber directly. The CS₂ concentration was regulated by changing the CS₂ or air volume. The atmosphere in the chamber was changed 12 – 19 times/hour. Continued atmosphere mixing was achieved by a

ventilator in the middle of the roof. The CS₂ concentrations actually prevailing within the chamber were monitored by conventional colorimetric determination of CS₂. Exposure to 200 ppm CS₂ for 8 hours/day for 7 days caused no fatty infiltration of the liver. Similarly, three-day pretreatment with phenobarbital (80 mg/kg i.p.) followed by 8-hours exposure to 20 or 200 ppm CS₂ and a narcotic dose of hexobarbital (100 mg/kg i.p.) caused no appreciable fat accumulation in liver cells and no rise in serum GOT and GPT. In contrast, oral administration of CS₂ (1 mL/kg) caused a moderate accumulation of fat in the liver which became severe and was accompanied by a rise of serum GOT in animals additionally pretreated with phenobarbital.

Neurotoxicity studies

Rats exposed to 5000 mg/m³ CS₂ (1600 ppm) for 4 hours/day for 1 – 6 days showed no signs of toxicity apart from being more subdued. Urination was increased and defecation was decreased. A time-dependent activation of brain tyrosine hydroxylase (TH) was observed. TH activation rose above control after the 2nd day of exposure, reached 140 % of control by the 4th day and declined thereafter (Heubusch and DiStefano 1978).

Wilmarth et al. (1993) exposed male Sprague-Dawley rats to 0, 600 or 800 ppm CS₂ for 10 hours/day for 14 consecutive days. Exposure was carried out in 0.32 m³ steel inhalation chambers. The in-chamber concentration of CS₂ as monitored daily using a Miran Model 1A-CVF IR gas analyser varied by less than 10 %. Both CS₂ concentrations resulted in narcotic-like stupor during exposure. After a 14 hours-recovery period, there was a return to normal levels of alertness and activity. Rats showed a progressive weight loss during the experiment of 14 % at 600 ppm CS₂ and 32 % at 800 ppm, while control animals gained 37 % weight on average. At 800 ppm CS₂, animals began to display retropulsion and circling behavior on day 4 of treatment and developed hindlimb display and signs of mild ataxia by day 7. On day 15, rats displayed a fine whole-body tremor and were severely ataxic or suffering complete hindlimb paralysis. In rats exposed to 600 ppm, circling behavior and retropulsion were noted from day 9. At termination, signs of mild ataxia and moderate hindlimb paralysis were apparent. In the brain of rats exposed to CS₂, an increase in the phosphorylation of endogenous MAP-2 (microtubuli associated protein) and in the autophosphorylation of Ca²⁺-calmodulin-dependent protein kinase II were observed.

In a collaborative NIEHS study, the onset and temporal progression of neurotoxicity as manifested in multiple functional and structural alterations were investigated (Harry et al. 1998; Manuel 1998). Male and female F344 rats (9 rats/sex and time) were exposed to either 0, 50, 500, or 800 ppm CS₂ for 6 hours/day, 5 days/week for 2, 4, 8, or 13 weeks as described by Sills et al. (1998b). Each 2, 4, or 8-week exposure experiment was conducted in duplicate and four 13-week exposures were conducted (group size of 18 rats/sex and dose). Exposures were carried out in Hazleton 1000 exposure chambers with an air flow rate of 400 L/minute. The CS₂ concentration in the chamber was monitored every 15 minutes with Fourier Transform Infrared Spectrophotometers. The actual chamber concentrations were within 3 % of the target concentrations during the study. A summary of the results was presented by Harry et al. (1998). Within 2 weeks of exposure to either 500 or 800 ppm, an increased expression of nerve-growth factor receptor mRNA in the sciatic nerve (indicating alterations in the relationship between axon und Schwann cells) of all animals was found which increased during further exposure (Toews et al. 1998). Neurofilament cross-linking in the spinal cord was observed as early as 2 – 4 weeks at all exposure levels. In erythrocytes, covalent modification of globin was observed at all CS₂ concentrations which was paralleled by spectrin crosslinking (Valentine et al. 1998). Postural abnormalities at all exposure durations, mostly seen at 800 ppm, were described as hunched posture early on, progressing to diminished postural control at the end of the study. Within 2 weeks at 800 ppm, gait abnormalities occurred. At 500 ppm and 800 ppm, from 4 weeks on, neuromotor alterations progressed to a reduction of grip strength of hind and forelimbs (Moser et al. 1998). Axonal swelling, axonal degeneration, and electrophysiological alterations

in the peripheral nerves or the spinal cord occurred at the two highest concentrations in later stages (from 8 weeks on) of the study (Herr et al. 1998; Sills et al. 1998; Valentine et al. 1998).

Studies with non-inhalation exposure

Herr et al. (1992) studied alterations in flash (FEP) and pattern reversal (PREP) evoked potentials in rat brain. Groups of 9 - 22 male Long-Evans rats received a single intraperitoneal dose of 0, 100, 200, 400, or 500 mg CS₂/kg in corn oil or 200 mg/kg i.p. in corn oil daily for 30 days. Acute exposure decreased the amplitude and increased the latency of different FEPs peaks assessed 1 – 4 hours after treatment and decreased the amplitude of PREP peaks 4 hours after treatment. Repeated administration of CS₂ produced similar, but more pronounced effects which could be detected longer than after single acute exposure.

Effects of CS₂ on cardiovascular functions after acute and subacute oral exposure were studied in male Wistar rats (Hoffmann and Klapperstück 1990). 9 – 12 animals per group received 0, 1.66, 3.32 or 6.64 mmol CS₂/kg (0, 126, 253, 506 mg/kg) in *Oleum pedum tauri* (1:10) as a single dose 1 hour prior to the investigations or were treated 5 days/week for 4 weeks followed by the measurements 1 hour after the last administration. No CS₂-related deaths occurred in any group, and heart rate and mean arterial pressure showed no consistent significant changes following acute or subacute exposure. In a second part of the study, groups of rats with a coronary artery ligation were exposed as described. Following artificial induction of cardiac ischemia by coronary artery occlusion, single exposure to 8.3 mmol CS₂/kg (632 mg/kg) significantly reduced the survival rate (29 %) as compared to controls (59 %) (Hoffmann 1987). A similar effect was seen after subacute exposure to ≥ 3.32 mmol/kg.

3.2.3 Mice

Flury and Zernik (1931) reported that mice exposed to 11,000 ppm CS₂ were lying on the side after 15 minutes and were anesthetized after 20 minutes. Quick recovery was seen after the end of exposure.

Lewis et al. (1999) studied the effects of CS₂ on the development of early lesions of atherosclerosis and arterial fatty deposits in C57BL/6 mice (for experimental details, see 3.1.2). Exposure of mice which were fed a standard diet to 500 or 800 ppm CS₂ induced a small but significant increase in the rate of fatty deposit formation under the aortic valve leaflets after 12 weeks. No effects were seen at 50 ppm. In contrast, in animals on a high fat diet, a marked enhancement was observed of the rate of fatty deposit formation in mice at 50, 500 and 800 ppm over the animals on high fat diet alone.

The inhibition of propagation and maintenance of the electrically evoked seizure discharge was studied in rats and mice (Frantik et al. 1994). Concentration-effect regressions were determined for 48 common solvents including CS₂ in female H-strain mice. Two mice were exposed in one dynamic 80-liter glass chamber for 2 hours. 32 mice – 8 controls exposed to ambient air and 8 at each concentration of solvent – were exposed and measured in one trial, and each trial was repeated at least once for each solvent tested. Three concentrations of solvent were selected in the linear part of the concentration-response curve (between 25 and 75 % of maximum effect, if possible. For some not explicitly named solvents the concentrations had to be lowered to avoid respiratory tract irritancy). Exposure concentrations were measured by gas chromatography, but the exact concentrations used were not reported. Measurements were carried within 1 minute after removal of the animals from the exposure chamber. A short electrical impulse (0.2 s; 90 V; 50 Hz) was applied through ear electrodes. Of six time characteristics recorded, the velocity of tonic extension of hindlimbs (i.e., the reciprocal of the latency) was the most sensitive and reproducible response measure in mice. The median of individual control values was subtracted from the values observed after exposure. Group means of differences were corrected for the

difference in the simultaneously tested control and converted to relative values, i.e. to percentage of the arbitrary maximum value (0.5 s⁻¹). All data were processed using linear regression analysis to estimate the concentration of solvent in air evoking 30 % of the maximum possible effect. In case of CS₂, a concentration of 2600 ppm (one-sided 90 % confidence interval 860 ppm) and a slope of regression of 0.008 %/ppm were calculated. The lowest effect concentration which for most solvents could be proven statistically was 10 %. For CS₂, the EC₁₀ can be calculated as follows:

$$EC_{10, 4 \text{ h, mouse}} = 2600 \text{ ppm} - 20 \% \div (0.008 \%/\text{ppm}) = 100 \text{ ppm}.$$

Behavioral studies

The effects of exposure to carbon disulfide were studied on two different behavioral responses in male CD-1 mice (Liang et al. 1983). One response was the interruption of a single light beam passing immediately behind a small hole in the wall of a mouse chamber which was placed in a sealed exposure chamber. The other response was the consecutive interruption of each of three radial light beams spaced around a circular runway. Both responses were maintained under a fixed interval 60-sec schedule of milk presentation. Acute, cumulative concentration-effect functions were determined by step-wise increases in the concentration of CS₂ in the chamber at 30 minutes intervals until responding was abolished. The concentration of CS₂ in the chamber was determined using a thermal conductivity detector and gas chromatography at the end of each interval. Cumulative concentration-effect curves were obtained twice for four or five mice. Each animal served as its own control. Although the level of physical activity required by the two responses was quite different, the acute effects of CS₂ on each were quantitatively similar: a concentration of 120 ppm was without effect, 580 ppm decreased responding in most mice to as low as 0.55 of control levels, 2200 ppm decreased responding in all mice, and 3700 ppm abolished responding. Recovery from these acute effects was slow; following cumulative exposures which abolished responding, recovery to 0.50 of the control level of responding took more than 4 hr, and full recovery required 6 hr. When the daily exposures were repeated for up to five days, the daily control performances before exposure were unaffected indicating that the observed effects of CS₂ did not appear to last as long as 18 hr.

Similar experiments and results were described in a second report of the same study group (Glowa and Dews 1987). A total number of 12 adult male white mice was used in the studies with 5 solvents including CS₂ but the number of animals in each separate part was not presented. Responding (the interruption of a photocell beam located behind a nose-poke hole) was studied under the fixed interval 60-second schedule of milk presentation as above. CS₂ slightly increased rates of responding at concentrations of 100 – 600 ppm, 2000 ppm decreased responding in some mice, and 3700 ppm abolished responding in all mice. Responding did not recover in any of the mice 30 minutes after the end of exposure. The calculated EC₅₀ for decreased responding was 2242 ± 307 (S.D.) ppm CS₂.

3.2.4 Rabbits

Lehmann (1894) conducted a series of experiments with rabbits and cats in which individual animals were exposed to various concentrations of CS₂. A constant stream of air was pressed through a flask containing liquid CS₂ which was held in a water- or icebath at a constant temperature. By varying the temperature in different experiments, the amount of CS₂ vaporizing could be modified. The air containing vaporized CS₂ was mixed with the main air stream which was passed through a gas meter. The air flow rate could be regulated to achieve different mixing ratios and thus CS₂ concentrations in the exposure chamber. The animals were exposed under dynamic exposure conditions in a glass case (no further details reported). The concentration of CS₂ was calculated from the amount of vaporized CS₂ determined by weight and the air flow rate. There were no differences in the acute toxic effects of freshly purified and distilled, colorless CS₂ and of impure yellow technical products with the distinct odor of decaying radish.

No clear signs of acute toxic effects were seen up to 850 ppm. From 1380 ppm upwards, signs of effects on the CNS increased from restlessness and swaying to convulsions, nystagmus, and paralysis finally to narcosis at 6450 ppm (Table 5). All animals recovered after the end of exposure.

Studies with repeated inhalation exposure

4 rabbits were exposed to 1100 ppm CS₂ for 6 hours/day for 12 days (Brieger 1949). The concentration of CS₂ in the exposure chamber was measured by the amount of CS₂ supplied to the chamber and checked by two colorimetric determination methods. The air concentration measured by the amount evaporated was 1218 ± 108 ppm, the chemical determinations showed a value of 1044 ± 104 ppm. Further details of the exposure procedure were not reported. During and at the end of exposure, the ECG showed only minor changes (left axis deviation, changes of T in single leads). The histological examination of the heart showed only minor hyaline changes of individual muscle fibers and, in one animal, circumscribed round cell infiltration.

Cohen et al. (1959) exposed 11 New Zealand white rabbits for 6 hours/day, 5 days/week for 16 weeks to 250 ppm CS₂, followed by 5 weeks of exposure to 500 ppm and further 17 weeks to 750 ppm. Six animals served as controls. Exposures were carried in 25 cubic feet chambers. Inlet and exhaust plenums were installed at the top of the chamber and below the supporting screen. The chamber was provided with one complete air exchange every five minutes. Vaporized CS₂ was introduced into the chamber by bubbling nitrogen through liquid CS₂ and metering the resulting mixture into the air from the central supply stream. The CS₂ concentration in the chamber was determined at the beginning of exposure and subsequently in two-hour intervals with diethylamine-copper reagent by means of a Klett colorimeter. At no time during exposure were any signs of acute toxicity due to exposure observed.

Studies with non-inhalation exposure

Exposure of the skin of rabbits to liquid carbon disulfide caused blisters and ulcers which often resembled severe chemical burns. Severe degenerative changes in the local subcutaneous peripheral nerves have also been described in this study (Hueper 1936).

3.2.5 Dogs

Studies with repeated inhalation exposure

Lewey et al. (1941) exposed 8 dogs to 400 ppm CS₂ for 8 hours/day, 5 d/w for 10 – 15 weeks. The CS₂ concentration in the chamber air was determined colorimetrically by a xanthogenate method using diethylamine/copper reagent. The mean concentration in chamber air was 404 ± 102 (S.D.) ppm (46 analyses). 500 ppm was exceeded significantly on 5 days only. The concentration of CS₂ in the breathing air varied during a day by ± 60 ppm. With respect to signs of intoxication which were obvious in dogs immediately after leaving the exposure chamber, the authors report the following observations: The dogs were drowsy, they staggered and stumbled, trembled and shook, ran restlessly through the room, caving in one leg at one moment and on another the next. The dogs were very thirsty, but did not eat for hours after end of exposure. They slept most of the time during exposure, but were excited and noisy afterwards. During the course of the study, the dogs developed behavioral changes and showed decreased pupillary reflexes after 2 weeks of exposure, followed by loss of cornea reflexes and signs of polyneuropathy with ataxia, tremor, and muscular weakness with loss of power and tendon reflexes. Behavioral changes with aggressiveness also occurred. Retinal angiopathy, possibly as an early sign of arteriosclerosis, developed from the 5th week on. In the heart, significant deviations from the ECG (T wave inversions and accompanying elevation of the RT segment) of normal dogs indicated myocardial derangement. All animals died between week 10 and 15 of exposure.

3.2.6 Cats

Flury and Zernik (1931) reported that a cat exposed to 24100 ppm for about 1 hour showed convulsions during exposure but recovered afterwards. No details were reported.

Lehmann (1894) conducted a series of experiments with rabbits and cats in which individual animals were exposed to various concentrations of CS₂ (see 3.2.4). No differences were observed in the acute toxic effects of freshly purified and distilled, colorless CS₂ and of impure yellow technical products with the distinct odor of decaying radishes.

Signs of slight effects on the CNS with slowed respiration and dozing developed at about 400 ppm (Table 5). Severe signs of toxicity including convulsions became obvious after exposure to 850 ppm for 9 hours and 1510 ppm for 3 hours, respectively. Shivering, shaking, vomiting, and collapse additionally occurred when the concentration was increased up to 3340 ppm for 2 ¼ hours. The 2 cats exposed to this concentration slowly recovered after exposure. However, 2390 ppm caused death in other cats in the same study (Table 4).

Studies with repeated inhalation exposure

Ferraro et al. (1941) exposed 5 cats in a closed 750 litres box. 6 – 7 g of liquid CS₂ was placed in the box on a large dish over a slightly warmed electric stove to facilitate rapid evaporation. It is reported without further details that a concentration of about 8 – 10 mg/L (2600 – 3200 ppm) was present in the box after complete evaporation of CS₂. Animals were exposed to CS₂ for 0.5 – 2.5 hours/day for 24 – 92 days. It is stated that the animals were exposed to varying doses of CS₂ but it is not clear if the animals were always exposed to the above mentioned concentrations. During exposure frequently salivation and dyspnea and occasionally vomiting were observed. Restlessness and excitement were common in the first stages of intoxication, apathy and sometimes coma occurred at more advanced stages. Tremors and muscular jerks occurred frequently. All these symptoms disappeared in a few hours after the animals were taken out of the exposure box.

3.3 Developmental/Reproductive Toxicity

3.3.1 Rats

No studies were available in which animals were exposed only once.

Studies with repeated inhalation exposure

An overview of developmental/reproductive toxicity with rats is given in FIGURE 1.

Saillenfait et al. (1989) exposed 20 – 23 pregnant Sprague-Dawley rats per group to 0, 100, 200, 400, or 800 ppm CS₂ for 6 hours/day during gestational days 6 – 20. CS₂ was introduced into 200-l inhalation chambers designed to sustain dynamic and adjustable air flows (10 – 20 m³/hour). CS₂ vapors were generated by bubbling air through a flask containing liquid carbon disulfide. The required concentrations were obtained by varying the proportion of air passing through the fitted disk of the bubbler. The CS₂ concentration in the chamber was monitored periodically by spectrophotometry of air samples. The number of samples was not given, but chamber concentrations were reported to be within 5 % of nominal. On day 21, maternal and fetal parameters were evaluated, including examination for external, visceral, and skeletal abnormalities. All dams survived the treatment and observation period. No maternal toxicity or adverse effects on the developing embryo or fetus were seen at 100 and 200 ppm. Exposure to 400 or 800 ppm CS₂ resulted in dose-related reduction of maternal weight gain (19 and 48 % reductions in the 400-

and 800-ppm groups, respectively) and fetal body weight (6.6 and 22 % in the 400- and 800-ppm groups, respectively). When gravid uterine weight was subtracted from the dam's body weight gain, the maternal weight was still significantly suppressed indicating maternal toxicity. The number of litters examined were 40, 17, 17, 22, and 22 in the 0-, 100-, 200-, 400-, and 800-ppm groups, respectively. There were no effects on implantations, resorptions, live fetuses, or fetal sex ratio. Fetuses from animals in the 800-ppm exposure group had an increase in unossified sternebrae (16/289 compared to 3/558 in controls), an index of delayed fetal development. A slight increase in club foot at 400 and 800 ppm was noted (1/298 and 7/289 fetuses compared with 0/558 in controls), the difference was not statistically significant. No other effects were seen.

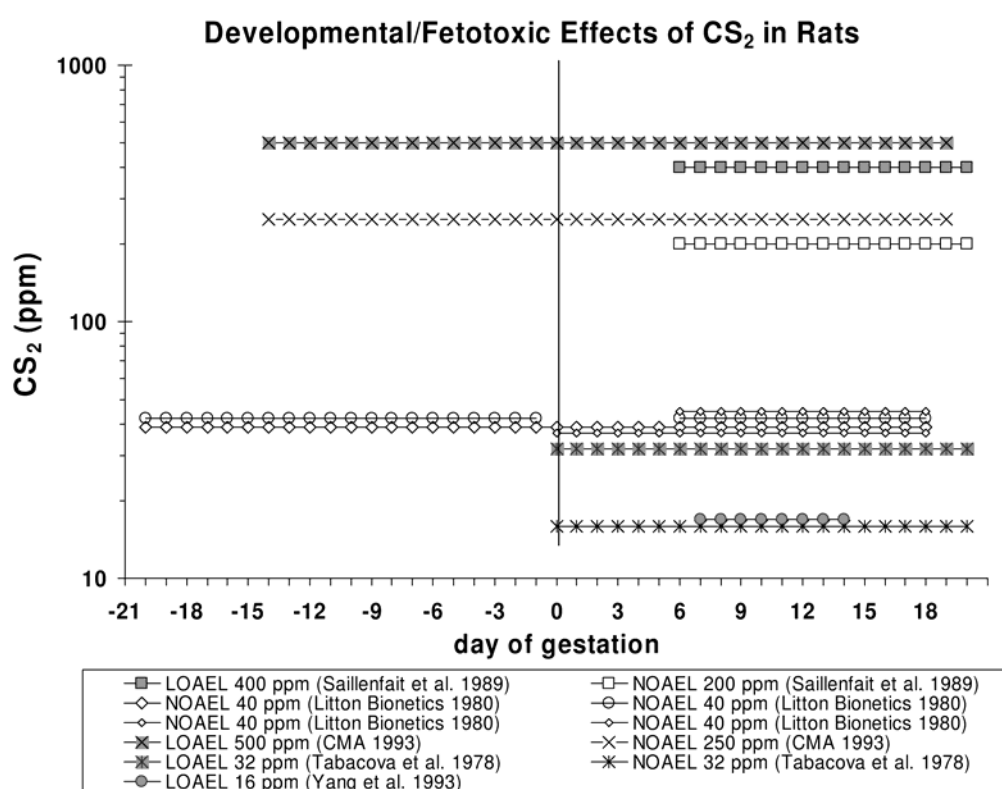


FIGURE 1: OVERVIEW OF DEVELOPMENTAL/FETOTOXICITY STUDIES WITH CS₂ IN RATS

Litton Bionetics (1980) conducted a study for NIOSH in which rats were exposed to 0, 20, or 40 ppm CS₂ for 7 hours/day, 5 days/week for 3 weeks prior to mating. Following mating, groups of rats not exposed pregestationally were exposed to 20 or 40 ppm CS₂ on days 0 – 18 or days 6 - 18 of gestation. Similarly, animals exposed pregestationally were divided into two groups that were exposed to the same concentration as used in the pregestational exposure and exposed during gestation days 0 – 18 or 6 - 18. Control animals were included that were unexposed during the corresponding pregestational and/or gestation period. Chamber concentrations were determined hourly, and the coefficient of variation of the chamber concentration ranged from 8 – 14 %. There was no effect on maternal weight gain and no dose-related effect on maternal organ weights or histology of the liver or kidney. In 12 – 23 litters/group, there

were no significant effects on uterine contents (early or late resorptions, number of fetuses, fetal weight, or fetal length), and no significant external, visceral, or skeletal malformations were observed. There was a slight but nonsignificant increase in resorptions and reduction in live fetuses in two groups (20 ppm, exposed during gestation, and 40 ppm, exposed both pregestationally and during gestation).

In a further study, groups of 15 female CD rats (control: 24) were exposed to 0, 125, 250, and 500 ppm CS₂ 6 hours/day from 14 days prior to mating through day 19 of gestation (CMA 1993; Nemec et al. 1993). The dams were allowed to deliver normally and both pups and dams were observed through day 21 of lactation. Developmental assessment included pinna detachment, palpebral separation, incisor eruption, testicular descent and vaginal patency. No maternal, developmental or reproductive toxicity was observed at 125 or 250 ppm CS₂. A small increase in the length of gestation at 250 ppm was within the range of historical controls. At 500 ppm, signs of slight irritation (clear fluid around the eyes and reddening around the nose) were observed in dams immediately following exposure. A slight decrease in food consumption was observed between days 15 – 20 of gestation in dams exposed to 500 ppm. Difficulty with delivery (dystocia) was observed in 2 dams and total litter loss was observed in 3 dams from the 500 ppm group. Increased pup mortality, decreased pup viability, and decreased mean litter size were also observed in this group.

In a developmental study (Tabacova et al. 1978), 32 female pregnant Wistar rats/group were exposed to 0, 50, 100, and 200 mg/m³ CS₂ (0, 16, 32, 64 ppm), respectively, for 8 hours/day throughout gestation (21 days). No details of the exposure conditions were described. According to a personal communication (Tabacova 1989; cited in US EPA 1998), the CS₂ concentration was measured twice a day using a spectrophotometric method. No data of the chamber monitoring results were available. Behavioral deviations (reduced exploratory activity, increased emotional activity) in open field test were reported to occur in offsprings at all groups exposed to CS₂ but no details were presented. At 64 ppm, preimplantation losses were increased. Exposure to 32 and 64 ppm was reported to significantly decrease fetal weight, to cause a delay in postnatal weight gain, and to cause malformations: The incidence of club foot was 11.9 and 19.2 %, the incidence of hydrocephalus 14.2 and 17.5 % at 32 and 64 ppm, respectively. Both malformations also were seen at 16 ppm (3.1 % and 1.03 %), but the effect was not significant. The authors further reported that, on the whole, the “malformations proved to be relatively mild and compatible with the further life of the progeny”.

Tabacova et al. (1983) further described the results of studies in which F₁ animals which had been prenatally exposed to CS₂ were reared until maturity and mated to produce an F₂ generation. During pregnancy, the F₁ females were again exposed to CS₂ at the same concentrations as the F₀ females throughout gestation. Data were presented for groups exposed to 0, 0.03, 10, 100, and 200 mg/m³ (0.01, 3.2, 32, 64 ppm), respectively. The group exposed to 16 ppm described in Tabacova et al. (1978) was not mentioned. The exposure conditions were not described. It is not clear whether the control groups were handled in the same way as and run concurrently with the exposed groups, and no mention is made of the method of selection of mating pairs or whether sibling pairs were excluded in producing the F₂ generation. In one table, separate control groups are listed for the two lower exposure and the two higher exposure groups. Comparisons were made separately for the 1st and 2nd generation. The authors further mention that direct statistical comparisons between corresponding groups from F₁ and F₂ generations were not performed because of the influence of season and other uncontrollable variables. A number of maternal and developmental effects were reported. The lower exposure levels (0.01 and 3.2 ppm) were reported to be non-toxic and non-teratogenic in the F₁-generation. When pregnant F₁ females were exposed during gestation, increased malformations and alterations in behavioral tests were reported to occur in the F₂-generation at the two lower concentrations.

Behavioral and neurotoxic effects of prenatal exposure to CS₂ in rats were studied also by Lehotzky et al. (1985). Exposures were carried out in a vertical inhalation chamber, no experimental

details or concentration measurements were presented. Pregnant female Lati:CFY rats were exposed to nominal concentrations of 0, 10, 700, or 2000 mg/m³ CS₂ (0, 3.2, 225, 640 ppm) for 6 hours/day from day 7 through 15 of gestation. There is some inconsistency in the data presentation since in a table the lowest concentration is reported as "< 10 mg/m³". After birth, the number per litter was recorded and litters were reduced to 10 pups each by random selection. Postnatal development and behavioral characteristics including auditory startle response, motor coordination, open field behavior and reaction to a conditioned stimulus (jumping on a wooden pole to avoid electric shock after a bell ring) were studied. It is reported that CS₂ caused a dose-related mortality in dams with probably 33 % mortality at 640 ppm, but neither data for the lower concentrations nor the total number of exposed and dead females in each group was reported. Perinatal mortality of pups was reported as 10 %, 20 %, 35 % and 50 % at 0, < 3.2, 225 and 640 ppm, respectively, and motor coordination and performance in open field behavior were reported to be poorer in offsprings from CS₂ exposed dams, but again, detailed data and any statistical evaluation were lacking. The conditioned avoidance response was tested in eight 38-day-old males from every group. The performance in all groups from CS₂ exposed dams was nearly age-appropriate, but the latency of the conditioned avoidance response was significantly lengthened at all concentrations.

Yaroslavskii (1969) exposed groups of 12 Wistar rats throughout pregnancy to 2000 mg/m³ (640 ppm) CS₂ for 2 hours/day in two series of experiments, two groups of 12 and 14 animals, respectively, serving as untreated control. It is reported that some animals produced their young spontaneously, while fetuses were extracted from others at day 19 or 20 of pregnancy. No further details of the experimental procedures or of the incubation conditions were described. The number of corpora lutea was not significantly different between the control and the exposed group. The preimplantation losses in CS₂-exposed animals in the two series of experiments were 3 and 5 times higher, respectively, compared to the corresponding control. A postimplantation loss in CS₂-treated animals was only observed in the first but not in the second series of experiments. The mean duration of pregnancy and the mean fetal weights were not affected by CS₂ treatment.

According to a short English abstract, teratogenic effects in the skeletal system (sternum deletion, coronale enlargement) and the central nervous system (hydrocephalus) were observed when pregnant rats were exposed to 50 mg/m³ (16 ppm) or 150 mg/m³ (48 ppm) CS₂ from day 7 through day 14 of gestation (Yang 1993). No details were presented in the abstract. The full original report was published in a Chinese source in Chinese and was not available for evaluation.

Zenick et al. (1984) studied the effects of CS₂ on the reproductive system of male rats. Two groups of adult Long Evans rats were exposed to 0 or 600 ppm CS₂ for 6 hours/day, 5 days/week for 10 weeks. Exposures were carried out in 60 m³ inhalation chambers. The CS₂ concentration in the chamber was analysed every 10 minutes by gas chromatography/photoionization detection. The average CS₂ concentration was 607 ppm. Mating behavior and semen parameters were evaluated prior to exposure and after 1, 4, 7, and 10 weeks of exposure. The 5th day of exposure of the corresponding week, the exposure period was reduced to 4 hours and animals were mated 1 hour after removal from the exposure chamber. After 1 week of exposure, CS₂ had no effect on body weight gain, mating behavior (mount latency, ejaculation latency, sperm count and motility, semen plug weight). Ejaculation latency was significantly reduced from the 4th week, sperm count from the 7th week, and mount latency after 10 weeks of exposure. Similar alterations were observed in previous study in which copulatory behavior was assessed 8 – 10 hours after exposure (Tepe and Zenick 1984). At termination of the study, plasma levels of testosterone, LH and FSH (and response to HCG-injections) and histopathological evaluations of reproductive organs were performed. No treatment-related effects on hormone levels and on histology of the reproductive organs were noted. Absolute prostate weights were decreased in CS₂ exposed animals, while testis, epididymis, vas deferens, and seminal vesicle weights were not affected. At termination, body weight gain of CS₂ treated animals was 10 % lower. The authors further report that no treatment-related effects on

epididymal sperm counts and reproductive organ weights were seen in a pilot study after exposure to 900 ppm CS₂ for 12 weeks.

Studies with non-inhalation exposure

CD rats (22 – 27 dams/group) were exposed orally to 0, 100, 200, 400, or 600 mg/kg b.w. per day during the period of organogenesis on day 6 – 15. Fetal body weights were decreased in rats exposed to 200 mg/kg and more. At sacrifice (gd 20) all fetuses were examined for gross, visceral, and skeletal malformations. Dams had significantly reduced gestational body weight gain at all dose levels. Mean fetal weight was reduced significantly in the 200, 400, and 600 mg/kg/day litters, but there were no significant differences in the incidence of malformations or resorptions at any dose level. Thus, in rats all of the above doses of CS₂ were toxic to the dam, and the 200 mg/kg/day dose and above produced fetal toxicity in the form of reduced fetal weight (Jones-Price et al. 1984a).

3.3.2 Mice

No studies were available in which animals were exposed only once.

Studies with repeated inhalation exposure

Yaroslavskii (1969) exposed groups of 15 or 20 albino mice throughout pregnancy to 2000 mg/m³ (640 ppm) CS₂ for 2 hours/day, 21 mice served as untreated control. Half the mice were treated for two weeks prior to mating, and some mice from this group received 500 mg/kg i.p. tryptophan daily from day 1 to 19 or 20 of pregnancy. It is reported that some animals produced their young spontaneously, while fetuses were extracted from others at the day 19 or 20 pregnancy. No further details of the experimental procedures or of the incubation conditions were described. The number of corpora lutea was not significantly different between control and exposed animals. The preimplantation loss in CS₂-exposed animals was significantly higher in the group of CS₂ exposed animals (19 %) than in controls (11 %). Postimplantation losses were not observed in untreated controls, but in animals treated with CS₂ (7.4 %), tryptophan (3 %), or both (2.3 %).

3.3.3 Rabbits

No studies were available in which animals were exposed only once.

Studies with repeated inhalation exposure

A developmental study was conducted using New Zealand rabbits (Gerhart et al. 1991; PAI 1991). 24 rabbits/group were exposed by inhalation to 0, 60, 100, 300, 600, or 1200 ppm CS₂ for 6 hours/day on gestation days 6 – 18. Does received a laparotomy and an examination of their uterine contents on gestation day 29. Live fetuses were sexed, weighed and examined externally. All fetuses received visceral, cephalic and skeletal examinations. At 1200 ppm, maternal reduced body weight gain and clinical signs of toxicity including ataxia, lowered food consumption, labored respiration, wheezing, tremors, and abortion with bloody excretion involving the death of 2 animals were observed. No exposure-related signs of maternal toxicity were observed at lower concentrations. Embryotoxic effects were seen in the 600- and 1200-ppm exposure groups. Post implantation loss had a significantly higher incidence in does exposed to 600 or 1200 ppm. Total resorption was observed in 2 of 22 litters of the 600 ppm and 14 of 21 litters of the 1200 ppm exposure group, respectively. Mean fetal body weight was significantly reduced in the 600 and 1200 ppm exposure groups. In the 1200 ppm group, the total incidence of skeletal and visceral malformations was significantly increased; no single malformation accounted for this increase. In the lower dose groups, increases in skeletal malformations were observed in the incidences of

rudimentary 13th ribs, extra ribs, extrathoracic vertebrae, or hypoplastic pubis. The malformations in the lower dose groups did not appear to be dose-related and were within the range of historical control data presented by the authors.

Litton Bionetics (1980) conducted a developmental study for NIOSH. Rabbits were exposed to 0, 20, or 40 ppm CS₂ for 7 hours/day, 5 days/week for 3 weeks prior to mating. Following mating, rabbits were exposed to 20 or 40 ppm on days 0 – 21 or days 7 - 21 of gestation. Similarly, animals exposed pregestationally were divided into two groups that were exposed to the same concentration as used in the pregestational exposure and exposed during gestation days 0 – 21 or 7 - 21. Control animals were included that were unexposed during pregestational and gestation periods. Chamber concentrations were determined hourly, and the coefficient of variation of the chamber concentration ranged from 8 - 14%. There was a high level of mortality in rabbits, which was not exposure related, and which makes interpretation of the rabbit study difficult. There was no effect on maternal body weight, organ weight, or histology of the liver or kidney. In 15 - 18 litters examined per group from the groups that were not exposed pregestationally, there was no effect on uterine contents and no increase in external, visceral, or skeletal abnormalities.

Studies with non-inhalation exposure

New Zealand White Rabbits CS₂ (23 – 28 dams/group) received 0, 25, 75 or 150 mg CS₂/kg b.w. each day on gestational days 6 to 19. At sacrifice on gestational day 30, all fetuses were examined for gross, visceral, and skeletal malformations. Significant maternal toxicity and increased maternal liver weight were observed at 75 and 150 mg/kg. A significantly increased percentage of resorptions/litter was seen at all dose levels (12.3 %, 32.5 %, 41.6 % and 61.2 % resorbed in the vehicle through high-dose, respectively). At 25 mg/kg, this effect occurred without apparent adverse maternal effects. The percent fetuses malformed per litter was significantly increased above vehicle controls (5.7 %) only at the highest dose (19.5 %) (Jones-Price et al. 1984b).

3.4 Genotoxicity

Genotoxicity tests with carbon disulfide were reviewed and summarized (ATSDR 1996; Beauchamp et al. 1983; BUA 1993).

No mutagenic activity, with or without metabolic activation (S-9 from rat and from hamster liver), of CS₂ was observed in bacterial test systems using *S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 (Donner et al. 1981; Haworth et al. 1983; Hedenstedt et al. 1979). Similarly, findings in *E. coli* WP2 uvr A were negative (Donner et al. 1981).

In a host-mediated assay using CD-1 mice with *S. typhimurium* TA 98, no increase in the mutation rate was observed after treatment of female and male mice to 20 ppm CS₂. At 40 ppm, no effect was seen after treatment of females. In males exposed to 40 ppm CS₂, bacteria showed a slight increase in the mutation rate (2.5 fold over background), but due to the small recovery rate of bacteria, the result was considered doubtful (Litton Bionetics 1980).

No mutagenicity was observed in a sex-linked recessive lethality assay in *Drosophila melanogaster* after feeding sucrose solution with 200 – 1000 ppm CS₂ or 7 hour of exposure to 20 – 40 ppm CS₂ in air (Donner et al. 1981; Beauchamp et al. 1983). However, there was no positive control and no data on measured concentrations of CS₂ in food in the feeding experiment and the exposure concentration in the inhalation study was very low.

Exposure of rats to CS₂ at concentrations of 60 and 120 mg/m³ (20 – 40 ppm) 7 hours/day for up to 5 days did not increase the frequency of chromosomal aberrations in bone marrow cells (Litton Bionetics 1980).

When male rats were exposed to 20 – 40 ppm CS₂ for 7 hours/day for 5 d, there was no significant increase in dominant lethal mutations. Also there, there was no dose-related increase in sperm abnormalities in rats or mice at the same exposure. However, no evaluation is possible since there was a lack of a positive response in positive control rats in this study (Litton Bionetics 1980).

3.5 Carcinogenicity

A/J-mice were exposed to CS₂ 6 hours/day, 5 days/week for 6 months (Adkins et al. 1986). At 900 mg/m³, the number of lung adenomas was slightly, but significantly increased compared to number in the corresponding controls, but not to that in the historical controls. The frequency of carcinomas in the lungs and other organs was not increased. It must be noted that the rate of spontaneously occurring lung adenomas is high in this specific strain of mice and that known carcinogens show a considerably higher increase in lung adenomas. On the other hand, only one concentration was tested and the test duration was relatively short.

The results of a long-term study sponsored by the National Cancer Institute (NCI) with rats and mice administered CS₂ by gavage were considered inadequate for the evaluation of carcinogenicity because of poor survival of both species (Beauchamp et al. 1983).

No further data from experimental carcinogenicity studies were available.

3.6 Summary

As in humans, the observed acute toxic effects in animals are mainly on the CNS. Irritation of eyes and/or mucous membranes occur at concentrations that already have effects on the CNS.

With respect to lethality, the data for rats indicate a steep concentration-reponse curve: Whereas none of 6 rats survived a 4-hour exposure to 3500 ppm, all 6 rats survived at 3000 ppm (Du Pont 1966). No rats died after exposure to 2000 ppm for 4 hours (Goldberg et al. 1964) or to 1500 ppm for 2 hours (Savolainen and Järvisalo 1977).

In rabbits, death occurred in animals after single exposures to 3000 ppm or 3200 ppm for 6 hours (Flury and Zernik 1931; Lehmann 1894; PAI 1991). Individual cats died after exposure to 6450 ppm for 2 ¼ hours or after exposure to 3200 ppm for 4 ¼ hours (Lehmann 1894).

For mice, LC₅₀ values were reported of 3210 ppm (2 hours) (Izmerov et al. 1982) and of 4500 ppm (30 minutes) (“average lethal concentration”, Kuljak et al. 1974). A further LC₅₀ of 220 ppm (1 hour) (Gibson and Roberts 1972) is exceedingly low. The concentrations in this study were not measured, and the data are in contrast with other observations regarding lethal effects in this and other species in acute and in repeated exposure studies. It is likely that this value is erroneous¹ and no conclusions will be drawn from it.

¹ A higher sensitivity of the mouse strain used can be ruled out since the oral and i.p. LD₅₀ (3020 and 1890 mg/kg, respectively) also presented in the study are in accordance with data from other studies.

No treatment related deaths were observed in rats and mice following repeated exposures for at least 2 weeks to 800 ppm CS₂ (Toxigenics 1983a,b,c; Lewis et al. 1999; Moser et al. 1998; Wilmarth et al. 1993). In one study with mice, about 30 % of the those animals died which were given a high fat diet after the 1st exposure to 800 ppm CS₂ (Lewis et al. 1999). Necropsy did not reveal the cause of death in these animals. This observation deserves further investigation.

At non-lethal concentrations, acute effects on the nervous system including neurobehavioral alterations, alterations of catechol amine levels, and effects on the liver have been studied.

In squirrel monkeys, limited data from one study (Weiss et al. 1979) show behavioral alterations in response to an aversive electric shock during exposure to 600 ppm for 2 hours. In the first phase of exposure, the animal tolerated a higher shock level and showed long gaps without responding. During the last 30 minutes, the animal responded as often as during the control phase but the aversive threshold was elevated suggesting an anesthetic and/or an analgesic effect. This effect occurred similarly in a second animal. When the exposure period was extended to 18 hours, effects were seen in 4 monkeys at concentrations between 70 and 200 ppm. The results of these experiments are, however, not described in detail.

In rats, effects on the CNS were observed in several studies. 500 ppm for 6 hours reduced activity, but were reported as not strongly irritating or prenarctic (Kivisto et al. 1995). A little higher concentration of 600 ppm but longer exposure period of 10 hours caused narcotic-like stupor (Wilmarth et al. 1993). The effect of exposure time is obvious in three studies in rats exposed to 770 – 800 ppm: No visible signs of toxicity were reported after 12 hours; ataxia, tremors, occasional convulsions occurred after 15 hours, and severe narcosis was seen after 18 hours (Tarkowski et al. 1980; Tarkowski and Cremer 1972; Tarkowski and Sobczak 1971). Rats exposed to 1500 ppm for 2 hours or to 2000 ppm for 4 hours were slightly somnolent or more subdued, but exposure was reported to be otherwise well tolerated (Heubusch and DiStefano 1978; Savolainen and Jarvisalo 1977).

At 640 – 800 ppm, metabolic and/or ultrastructural alterations in rat brain were observed such as changes in amino acid concentrations (Tarkowski and Cremer 1972), mitochondrial swelling and disorders of oxidative phosphorylation (Tarkowski et al. 1980; Tarkowski and Sobczak 1971), and raised dopamine/noradrenaline ratio. The latter effects was also demonstrated in heart and in adrenal glands. The lowest concentration of CS₂ at which a decrease of noradrenaline in brain was observed was 64 ppm (8 hours exposure) (Magos et al. 1974; McKenna and DiStefano 1977b).

The inhibition of propagation and maintenance of electrically evoked seizure discharge in rats was studied by Frantik et al. (1994). The duration of tonic extension of hindlimbs served as the most sensitive and reproducible effect. The concentration of CS₂ evoking 37 % of maximum response was 1370 ppm. By means of linear regression analysis, an EC₁₀ of 440 ppm was calculated. In mice, 30 % of maximum possible effect were seen at 2600 ppm and the calculated EC₁₀ was 100 ppm.

In rats, acute exposure to 2000 ppm for 4 hours caused an inhibition of the escape and avoidance response in a pole climbing test in 12 % and 50 % of the animals, respectively; no such effects were seen after one 4-hour exposure to 1000 ppm (Goldberg et al. 1964). In a neurobehavioral study in mice, a decreased response (determination of activity in response to milk presentation as stimulus) was seen after 30 minutes exposure to 580 ppm in some animals. Response was decreased in all mice at 2200 ppm and abolished at 3700 ppm (Liang et al. 1983). The calculated EC₅₀ for decreased responding was 2242 ppm (Glowa and Dews 1987).

It is likely that these inhibition of responses are related to the narcotic effects of CS₂ which are described in other studies following acute exposure at similar and lower concentrations (see above). Battig

and Grandjean (1964) also reported that rats were drowsy shortly after a 4-hours exposure to 800 ppm (actual concentration measured was between 550 and 800 ppm). Frantik (1970) described a reduction in spontaneous motor activity, a decrease in motor performance, and an inert nature of conditioned avoidance reactions in rats after a single exposure to 770 ppm for 6 or 7 hours. The effects completely disappeared after 3 days without exposure and were not recurring after further exposures.

However, Goldberg et al. (1964) also described that the response to 2000 ppm became more pronounced after further exposures for up to 10 days and that the effects were then seen at lower concentrations down to 250 ppm. This could indicate a cumulative effect of CS₂. In view of the rapid elimination of free CS₂ (see 4.1.2), this seems unlikely. More conceivably, the results could be explained as the onset of first chronic effects related to structural damages in the nervous system which are seen after about 2 weeks of exposure in other studies (see below).

In the NIEHS study, 10 daily exposures to 800 ppm and 500 ppm for 6 hours/day in 2 weeks caused an increased expression of nerve-growth factor receptor mRNA (indicating alterations in the relationship between axon and Schwann cells) (Toews et al. 1998). Neurofilament cross-linking in the spinal cord was observed 2 - 4 weeks after exposure to 50, 500, and 800 ppm (Valentine et al. 1998). Gait abnormalities occurred at 800 ppm after 2 weeks, and neuromotor alterations which progressed to a reduction of grip strength of hind and forelimbs occurred at 800 and 500 ppm from 4 weeks on (Moser et al. 1998). Unfortunately, parameters were determined only from two weeks of exposure on so it is unknown if some of the described alterations may occur earlier.

Effects on liver metabolism of rats were observed at concentrations as low as 20 ppm. A reversible concentration-dependent breakdown of hepatic glycogen was observed following exposure of rats to 20 – 400 ppm CS₂ for 8 hours (Freundt and Kürzinger 1975; Kürzinger and Freundt 1969). The decrease of glycogen was accompanied by an increase of hepatic lactate and inorganic phosphate levels, an increased oxygen consumption of whole body and of hepatic tissue in slices *ex vivo*. No increase of liver enzymes (GOT, GPT, LDH) in serum was found. In the same concentration range, CS₂ exposure was followed by a reversible inhibition of phase-I biotransformation reactions as indicated by a delay in the excretion of several drugs and solvents (Freundt et al. 1976a; Freundt and Dreher 1969; Freundt and Kuttner 1969). In alcoholized rats, exposure to 20 ppm CS₂ led to a 30 % increase in blood acetaldehyde concentration and to a prolongation of the half-life of elimination of acetaldehyde from blood (Freundt et al. 1976b; Freundt and Netz 1973).

All developmental/reproductive toxicity studies were performed with repeated exposure to CS₂ during selected phases of embryonal development or during the whole period of gestation (and in some studies including pregestational exposure). No studies were available in which developmental/reproductive toxicity was investigated after a single exposure. CS₂ showed embryotoxic/fetotoxic and teratogenic effects in developmental toxicity studies at doses of low or no maternal toxicity. In rats, a slight weight reduction in fetal weight (6 %) was seen at 400 ppm and a 22 % reduction at 800 ppm in one study with exposure during gestational days 6 - 20, both concentrations reduced maternal weight (Saillenfait et al. 1989). When rat dams were exposed 14 days prior to mating through gestation day 19 to 500 ppm, fetotoxicity was observed, difficulty with delivery and total litter loss occurred in some dams (CMA 1993; Nemec et al. 1993). Results from further studies with rats (Lehotzky et al. 1985; Hinkova and Tabacova 1978; Yang 1993) reporting teratogenic effects and/or behavioral alterations in offsprings of dams exposed to lower concentrations (16 ppm) of CS₂ cannot be evaluated due to insufficient presentation of data. In rabbits (dams exposed 6th – 18th d of gestation), post implantation loss was increased and fetal body weight decreased at 600 ppm CS₂; teratogenic effects were observed at 1200 ppm (Gerhart et al. 1991; PAI 1991).

CS₂ was not mutagenic in bacterial test systems with and without metabolic activation (Donner et al. 1981; Haworth et al. 1983; Hedenstedt et al. 1979). In a host-mediated assay with male rats, a slight increase in the mutation rate of *S. typh.* TA 98 was reported (Litton Bionetics 1980). No increase of chromosomal aberrations were seen in bone marrow of rats *in vivo* and in a dominant lethal assay (Litton Bionetics 1980); however, the exposure concentrations were low (20 – 40 ppm). Overall, the database with respect to mutagenicity of CS₂ is insufficient for evaluation.

The carcinogenicity of CS₂ cannot be assessed. A screening study of lung tumor induction in A/J-mice showed a slight but significant increase in lung adenomas but not carcinoma (Adkins et al. 1986). No adequate carcinogenicity studies were available.

4 SPECIAL CONSIDERATIONS

4.1 Metabolism and Disposition

4.1.1 Human data

As shown in controlled exposure studies, CS₂ is rapidly and extensively absorbed through the respiratory tract. Unmetabolized CS₂ is mainly excreted via the lungs. Uptake through the skin was demonstrated from aqueous solutions of CS₂.

In a pharmacokinetic study (Teisinger and Soucek 1949), 9 persons were exposed to 17 – 30 ppm CS₂ (in one case to 51 ppm) for 1 – 4 hours. The concentration of CS₂ in the air was kept constant during the experiment within $\pm 6 \mu\text{g/L}$ (1.9 ppm) and was determined every 15 minutes colorimetrically with diethylamine/copper reagent. In the first 15 minutes of exposure, about 80 % of inhaled CS₂ was retained. After 45 minutes and until the end of exposure, uptake decreased to about 40 %. The percentage absorbed did not depend on the concentration in the inhaled air. The blood:air coefficient of CS₂ after 90 – 120 minutes was 2.2 on average. At the end of exposure, the concentration of CS₂ in blood fell rapidly to 25 % of the value present at the end of exposure within one hour and CS₂ disappeared from blood after two hours. Only a small portion (about 5 %) of CS₂ was eliminated by the lungs, and this elimination was largely completed two hours after termination of exposure. Only minor amounts of unchanged CS₂ could be detected in urine.

In a further study volunteers inhaled 38 – 52 ppm CS₂ through face masks for 0.5 – 2 hours (Harashima and Masuda 1962). During the first 10 minutes of exposure, on average 51 % of the inhaled CS₂ was exhaled in breath, and this percentage increased to 65 % after 40 minutes when about equilibrium was reached. After exposure ceased, the concentration of CS₂ in exhaled breath declined rapidly with a half-life in the order of 10 minutes. There was a high variation between individuals in the actual amount of absorbed CS₂ that was exhaled after exposure (8 – 48 %, average 23 %). Less than 1 % of unchanged CS₂ was excreted through the skin.

Herrmann et al. (1982; 1983; 1985; 1989) conducted a series of toxicokinetic studies on inhalational uptake of CS₂ in non-exposed and occupationally exposed workers. Up to 12 test persons were exposed to 6 – 108 mg/m³ (1.9 – 35 ppm) CS₂ via face mask. CS₂ concentrations were monitored by gas chromatography. Retention was determined in 5 minutes intervals. During the first 5-minutes interval, individual retention ranged from 47 – 80 %. After 30 minutes of exposure, individual retention values decreased to 38 – 71 % (n=11; mean retention 48.7 %). Regression analysis revealed that the retention increased significantly but slightly with increasing exposure concentration. Moderate exercise (100 W) decreased the retention after 30 minutes to 15 – 37 %. In a further experiment with constant light exercise (25 W), the initial retention of about 50 % dropped to about 33 % after 30 minutes and was constant thereafter to the end of exposure after 4 hours. Demus (1964) obtained similar mean retention values of 51.6 % (range 43.5 – 60 %, n=11 individuals) after 30 minutes, 36.8 % (26 – 43.5 %) after 2 hours and 31.7 % (20 – 40 %) after 5 hours at CS₂ exposure concentrations of 53 – 445 $\mu\text{g/L}$ (17 – 142 ppm).

Interindividual variation in the uptake of CS₂ by inhalation proved significantly influenced by the amount of body fat. In a study (Rosier et al. 1987), 6 male human volunteers were exposed to 10 and 20 ppm CS₂ at rest and to 3 and 10 ppm under light physical exercise (50 W) for four consecutive periods of 50 minutes each. At rest, the retention values were about 40 % at 10 ppm and 20 ppm. At physical exercise, the retention values decreased to about 28 % at 3 ppm and 10 ppm. The most important fraction of the interindividual variation observed could be explained by the differences in percentage of body fat. During exposure, only an apparent steady state was reached. The exhaled concentration of CS₂ followed over 180 minutes after the end of exposure could be described by means of a biphasic elimination. There

was an initial very fast decrease with a half-life of 1.1 minutes followed by a second slower decrease with a half-life of 109.7 minutes. The total amount of CS₂ being exhaled in 180 minutes varied from 5.4 to 37.9 %. Again, it could be shown that interindividual differences in body fat significantly determined this parameter.

Studies regarding the distribution of CS₂ in humans were not available. Limited data are available on the metabolism of CS₂ in humans. In vitro studies have shown that CS₂ combines with amino acids in human blood, and the so called “acid labile” CS₂ (see 4.1.2) is mainly (90 %) found in the erythrocytes (Lam and DiStefano 1983; 1986).

Metabolites of CS₂ are primarily excreted via the kidney. Several sulfur-containing urinary metabolites were identified including thiourea, 2-thio-5-thiazolidinone, and 2-thiothiazolidine-4-carboxylic acid (TTCA). These substances are formed by the reaction of CS₂ with glutathione, cysteine, glycine, and other amino acids. Less than 5 % of the CS₂ taken up is excreted as TTCA. However, the excretion of TTCA is linearly correlated with the CS₂ exposure occurring at today’s workplaces. Therefore, this parameter is used in biological monitoring (Drexler 2000). Recently, from the urine of workers exposed to CS₂, 2-thioxothiazolidin-4-carboxylglycine (TTCG) was identified as a metabolite of CS₂. This compound is suggested to be a precursor of TTCA (Amarnath et al. 2001).

4.1.2 Animal data

A number of studies have shown that CS₂ is rapidly absorbed through the respiratory tract. Absorption of gaseous CS₂ through the skin of rabbits was also demonstrated (Cohen et al. 1958).

The toxicokinetics of CS₂ in rats was studied as part of the collaborative NIEHS study (Moorman et al. 1998) (see 3.2.2). Male and female F344 rats were exposed nose-only 50, 500, and 800 ppm CS₂ for 180 minutes and blood samples were taken 4, 8, 15, 30, 60, and 180 minutes after the start of exposure. Values for kinetic parameters were calculated from the fits of a two-compartment model to the blood concentration versus time. At 50 ppm, the blood concentration of CS₂ was at the limit of quantification in males after 180 minutes (0.8 µg/mL) and below at all other time points and throughout in females. At 500 and 880 ppm, uptake in blood was found to be rapid with a half-time of 6 – 9 minutes. The concentration in blood at 180 minutes increased proportionally with dose and was significantly (about 40 %) lower in females than in males. No true steady-state during the exposure was reached.

In the same study, the distribution and elimination kinetics from blood were determined following single intravenous administration of CS₂ (50 mg/kg) into the tail vein. Both parameters were modeled using a two compartment model with first order elimination from the central compartment. The apparent total volume of distribution was 4.2 L/kg, the terminal elimination half-life was 24 minutes, and the total clearance was 112 mL/minutes/kg.

Finally, in this study experiments were conducted with rats exposed via inhalation to 50, 500, and 800 ppm, respectively, for up to 13 weeks. In males, blood concentrations of CS₂ remained relatively constant throughout but decreased in females with increasing duration of the study. Non-linear kinetics was observed: At all time points, the CS₂ concentration in blood of the 500 and 800 ppm males and females were significantly (about 1.5 – 2 times) higher compared to the 50 ppm group than would be expected by linear dose proportionality. Non-linear kinetics was also observed in the excretion of the metabolite thiazolidine-2-thione-4-carboxylic acid (TTCA) in urine of repeatedly exposed rats. The total excretion of TTCA during 18 hours was not different between animals exposed to 500 and 800 ppm (except for males after 2 weeks). The excretion of TTCA in the 50 ppm group was lower than that in the two other groups exposed to CS₂, but the difference was less than would be predicted by dose proportionality. Taking together, these results indicate that uptake may be more efficient at higher

concentrations or, more likely, metabolism and elimination pathways become saturated at the higher concentrations.

In a study with rabbits, blood equilibrium concentrations of CS₂ were reached after exposure to 20 – 150 ppm for 1.5 – 2 hours. After the end of exposure, 15 – 30 % of the CS₂ absorbed was excreted through the lungs and less than 0.1 % via the kidney. In rats exposed to 60 – 350 ppm CS₂, the substance was rapidly eliminated during the first 6 – 8 hours after exposure. 20 hours after exposure, low concentrations of CS₂ could still be detected in brain, liver, and kidney (Beauchamp et al. 1983).

Unmetabolized CS₂ is largely excreted via the lungs, but most of the CS₂ taken up is metabolized and eliminated in the form of various metabolites by the kidney.

The metabolism of CS₂ involves the reaction with NH₂, SH- and OH-groups on one hand and the reaction with the microsomal mixed-function oxidase cytochrome P-450 on the other (FIGURE 2). The reaction of CS₂ with NH₂- and SH-, and OH-groups leads to the formation of the so called “acid labile” pool of bound CS₂. This pool consists of dithiocarbamates, trithiocarbamates, and related sulfur containing products. Dithiocarbamates are the first reaction products of CS₂ with the NH₂-residues of amino acids, proteins, and catecholamines. Due to the reversible reaction, it is not possible to strictly distinguish between “free” and “acid labile” CS₂ quantitatively (McKenna and DiStefano 1977a).

McKenna and DiStefano (1977a) studied the distribution of free and acid-labile CS₂ in rats following inhalation of 2 mg/L (640 ppm) CS₂. The concentration of free CS₂ reached (liver, kidney, heart, muscle) or approached (brain) a steady state level within 4 to 5 hours of exposure in all tissues studied with the possible exception of fat. In contrast, the tissue level of “acid-labile” CS₂ continued to increase until the end of exposure. The highest concentration of free CS₂ was found in fat followed by adrenal glands and liver. Except for fat and blood, 40 – 90 % of the total CS₂ in the tissues was found as “acid labile” metabolites. In most tissues (adrenals, kidney, brain, muscle, heart), the concentration of “acid labile” CS₂ was higher than that of free CS₂. The concentration of free CS₂ declined rapidly after the end of exposure, while the “acid labile” CS₂ was removed slowly. In brain, approximately one-third was detectable 16 hours after the exposure. In another study with rats exposed to 640 ppm CS₂ for up to 4 hours, the half-life of elimination of free and “acid labile” CS₂ from blood could be described by a two-exponential, first order process (Lam and DiStefano 1982). However, the half-times greatly differed for free CS₂ (about 9 and 55 minutes) and “acid labile” CS₂ (2.2 and 42.7 hours). When rats were repeatedly exposed over several days to 120 mg/m³ (40 ppm) for 8 hours/day, the “acid labile” CS₂ in blood continuously increased with each exposure while the free CS₂ level remained relatively constant. By the 6th to 7th exposure, the “acid labile” CS₂ concentration was about 2.5 times that after the 1st exposure and about 3 times higher than the concentration of free CS₂ (Lam and DiStefano 1983).

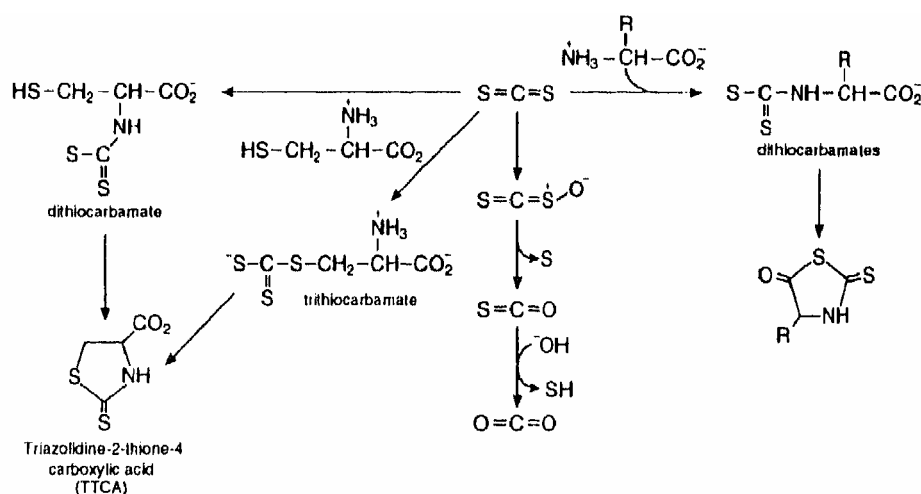


FIGURE 2: PRINCIPLES OF METABOLIC PATHWAYS FOR CARBON DISULFIDE (SOURCE: Moorman et al. 1998)

Studies with low-molecular weight dithiocarbamates such as diethylthiocarbamates have shown that CS₂ can be released *in vivo*. Therefore, it seems likely that the formation of thiocarbamates from CS₂ and endogenous NH₂-groups is at least partially reversible, and that amount of CS₂ which is slowly eliminated with long half-life may be derived from this pool. On the other hand, subsequent reactions of thiocarbamates may lead to long-lived protein modifications. Cross-linking of globin and spectrin in erythrocytes and of neurofilaments in spinal cord has been demonstrated in rats after repeated exposure to 50 ppm CS₂ by inhalation or repeated i.p. injection of 2 mmol/kg (150 mg/kg) (Erve et al. 1998; Valentine et al. 1993; 1997; 1998).

The cytochrome P-450 dependent oxidation of CS₂ is probably catalyzed by the alcohol inducible isoenzyme. In the first step, an active sulfur atom and carbonyl sulfide (COS) are released. COS is further metabolized mainly by carboanhydrase to carbon dioxide and hydrogen sulfide (Chengelis and Neal 1980; 1987). Sulfur and sulfide are finally oxidized to sulfate entering the endogenous sulfate pool. The reactive sulfur also binds to macromolecules including cytochrome P-450 dependent monooxygenases. This reaction is held responsible for the inhibition of P-450 dependent monooxygenases which has been observed in many studies after exposure to CS₂ *in vivo* and *in vitro* (e.g., Dalvi et al. 1975; Dalvi and Neal 1978; Freundt et al. 1974b) and for the hepatotoxicity of CS₂ in phenobarbital pretreated rats (Chengelis 1988).

The extent to which CS₂ is metabolized by the P-450 dependent pathway is not clear. The sulfur-containing metabolites which are excreted in urine of humans (see 4.1.1) and animals derive from reaction products of CS₂ with amino acids. In a study in rats exposed to 50 or 500 ppm CS₂ for 6 hours, pretreatment with P-450 enzyme inducers (phenobarbital, ethanol, 3-methylcholanthrene) had no effect on the excretion of TTCA. On the other hand, administration of substances which deplete the level of tissue glutathione (phorone, diethyl maleate, buthionine sulfoximine) at least initially decreased the excretion of TTCA (Kivisto et al. 1995).

4.2 Mechanism of Toxicity

The acute exposure to CS₂ primarily manifests in rapidly occurring effects on the nervous system. High exposure to CS₂ in humans results in dizziness, headaches, autonomic nervous system reactions, nausea, vertigo, vomiting, central paralysis, and finally narcosis and death. In animals, death after acute inhalation of CS₂ also occurs due to effects on the CNS with deep narcosis and finally respiratory arrest. Pulmonary congestion with hyperemia and hemorrhages were also seen in animals after lethal intoxication. Signs of toxic effects on the CNS (narcosis, stupor, ataxia, tremors, convulsions, reduced activity but also hyperexcitability) also are predominant at lower concentrations.

The formation of “acid labile” CS₂, especially dithiocarbamates from the reaction of CS₂ and amino groups (e.g., of free or protein-bound amino acids), may contribute to the toxicity of CS₂. Low-molecular weight dithiocarbamates are chelators of transition metal ions (e.g. Fe²⁺, Cu²⁺, Zn²⁺), and this may lead to the inhibition of enzymes for which these ions are essential. The inhibition of acetaldehyde dehydrogenase by dimethyl- and diethyldithiocarbamates and their corresponding disulfides (thiram, disulfiram, also known as “antabuse”) in humans and animals *in vivo* is well known (Freundt and Netz 1973; 1977; Fried 1980). This inhibition seems also of relevance in case of CS₂ since an increase in blood acetaldehyde after intake of alcohol and exposure to CS₂ was demonstrated in humans and experimental animals.

The inhibition of xenobiotic biotransformation is likely to be related to the P-450 dependent biotransformation of CS₂ by which reactive atomic sulfur is formed. It is not known whether the effects on the carbohydrate metabolism (depletion of glycogen, accumulation of hepatic lactate) are also related to this reaction.

With respect to long-term toxicity, the formation of reactive thiocarbamates also seems to play a role in the development of lesions in nervous system. It has been postulated that the axonal degeneration that underlies the neuropathy caused by CS₂ is the result of the reaction of CS₂ with protein amino groups to yield initial adducts (dithiocarbamate derivatives). Covalent binding of CS₂ with the formation of thiocarbamates and subsequent cross-linking of neurofilaments was demonstrated in rats after subacute to subchronic exposure (Erve et al. 1998a; Erve et al. 1998b; Harry et al. 1998). Progressive crosslinking of the neurofilament is postulated to occur during its transport along the axon, and covalently crosslinked masses of neurofilaments may occlude axonal transport at the nodes of Ranvier, ultimately resulting in axonal swelling and degeneration (Government Canada 2000).

The mechanisms by which CS₂ may lead to arteriosclerotic changes and cardiotoxicity remain to be elucidated.

4.3 Other Relevant Information

4.3.1 Interspecies variability

The limited database with respect to lethality does not show marked species differences. LC₅₀ values for comparison are missing. The LC₅₀ for mice reported by Gibson and Roberts (1972) is exceedingly low and contrasts with all other observations regarding lethal effects in this and other species in acute and in repeated exposure studies. It is likely that this value is erroneous and no conclusions can be drawn from it.

The data for rats and rabbits indicate a steep concentration-response curve for lethality at a similar concentration:

- all rats (6/6) died at 3500 ppm exposed for 4 hours (Du Pont 1966),
- no rat (0/6) died at 3000 ppm exposed for 4 hours (Du Pont 1966);
- several rabbits died at 3000 ppm or more exposed for 6 hours (Flury and Zernik 1931; PAI 1991);
- no rabbit died at exposure to
 - 6450 ppm for 2 hours 15 minutes
 - 3340 ppm for 3 hours
 - 2990 ppm for 3 hours 30 minutes
 - 2440 ppm for 3 hours 30 minutes (Flury and Zernik 1931; Lehmann 1894)

Cats could be more sensitive than rabbits, but the database is too restricted to allow firm conclusions.

Non-lethal effects on the CNS in different species are seen at similar exposure concentrations and exposure duration. In humans, such effects have also been observed in a controlled exposure study and in case of accidents. Effects on liver metabolism (inhibition of biotransformation, CS₂ induced additional increase of acetaldehyde blood levels after alcohol intake) without concomitant signs of liver damage have also been seen in humans and rats.

4.3.2 Intraspecies variability

Green and Hunter (1985) observed some variability with age in the acute lethal toxicity of CS₂ in rats. Following i.p. administration at 1, 5, and 10 days of age (unselected as to sex) and of 20, 30, and 40 days of age (males only), CS₂ was least toxic to 20 days old male rats (LD₅₀ 1545 mg/kg) and most toxic to one day old rats (LD₅₀ 583 mg/kg). The toxicity to adult male rats of the same strain (Sprague-Dawley) determined in another study (de Gandarias et al. 1992) fell within this range (LD₅₀ i.p. 1060 mg/kg).

No data on humans or experimental animals were available regarding the susceptibility to CS₂ at higher age. With respect to the narcotic effect of CS₂, it seems reasonable to assume a higher susceptibility with increasing age. For volatile anesthetics, it is well known that elderly are more susceptible. The effectiveness of volatile anesthetics is usually reported as MAC, i.e. the minimum alveolar concentration required to prevent movement in response to surgery in 50 % of persons. Data from a meta-analysis indicate that the lg₁₀-MAC for the medically used inhaled anesthetics decreases with about 6 % per decade of age (for age > 1 a) (Mapleson 1996). Besides the elderly, newborn including prematures, and pregnant women are more sensitive to anesthetics than older infants, toddlers, children, and adults. The total range of sensitivity is 2 – 3 fold (NRC 2001). The acute effects on the nervous system in humans and animals of a single exposure to CS₂ seem compatible with an anesthetic effect. This does not hold true of other acute effects and of effects after repeated exposure to CS₂.

In the studies of Freundt et al. (1976b), the effect of CS₂ exposure on the blood acetaldehyde level was observed not only when the alcohol was taken in during CS₂ exposure but similarly when the alcohol intake only started 16 hours after the end of CS₂ exposure. In view of the rapid elimination of "free" CS₂, it is likely that the effect is mediated not by CS₂ itself but by CS₂-metabolites. Animal

experiments have shown that the CS₂-derived thiocarbamates (the “acid labile” CS₂) are slowly eliminated (see 4.1.2).

Several dithiocarbamates and their corresponding disulfides have been shown to increase the concentration of acetaldehyde in blood of alcohol-treated humans and experimental animals by inhibition of the acetaldehyde dehydrogenase (ALDH). Diethyldithiocarbamate, the reduced form of tetraethylthiuramdisulfide, does not inhibit ALDH *in vitro* but is as efficient as disulfiram *in vivo*. This suggests that dithiocarbamates can rapidly undergo redox reactions *in vivo*. Alcohol intake following administration of tetraethylthiuramdisulfiram (better known as “antabus”) leads to an increase in blood acetaldehyde with an unpleasant alcohol intolerance syndrome involving vasodilation, facial flushing, increased heart and respiration rate, lowered blood pressure, nausea, and headache (Eriksson 2001; Freundt and Netz 1977; Fried 1980).

Ethanol is mainly oxidized by a mitochondrial acetaldehyde dehydrogenase known as ALDH2. A mutation in the gene for ALDH2 results in synthesis of an enzyme ALDH2(2) which is less active. The distribution of this allele shows ethnic differences and has a high frequency in Asians. The presence of the ALDH2(2) allele results in an excessive production of acetaldehyde after ingestion of alcohol. Persons heterozygous in ALDH2(2) frequently show a mild “antabuse syndrome” with facial flushing quickly after the ingestion of alcohol. Homozygous individuals are even more susceptible (O’Brien 2001). In a Japanese study, all individuals with homozygous atypical ALDH2(2)/ALDH2(2) and most of those with heterozygous atypical ALDH2(1)/ALDH2(2) were alcohol flushers, while all of the usual ALDH2(1)/ALDH2(1) were nonflushers (Shibuya 1993).

5 DATA ANALYSIS FOR AEGL-1

AEGL-1 is the airborne concentration (expressed as ppm or mg/m³) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

5.1 Summary of Human Data Relevant to AEGL-1

Leonardos et al. (1969) reported a 100 % recognition threshold for CS₂ of 0.21 ppm. AIHA (1997), in a critical overview of odor thresholds, reported referenced values ranging from 0.016 – 0.42 ppm. No geometric mean and no “range of acceptable values” for CS₂ were presented. The use of the 0.21 ppm threshold from Leonardos et al. (1969) was rejected in the AIHA-overview because this value represents a 100 % recognition concentration. Since CS₂ decomposes rapidly under the influence of air and/or light with the formation of foul smelling decay products, it is to be expected that the odor detection and recognition threshold of CS₂ will vary widely depending on the purity of the substance and the conditions.

There is a large span between the concentration range at which the odor may be perceived and concentrations at which other effects of CS₂ become noticeable. Hence, the odor would have warning properties at concentrations that are unlikely to represent any health hazard at acute exposure. This may be more important since irritation occurs only at concentrations of CS₂ that already have depressant effects on the CNS, and therefore, irritation offers no warning.

Inhibition of alcohol metabolism was observed in volunteers exposed to CS₂ in combination with controlled intake of alcohol. The blood alcohol concentration was about 0.7 ‰ representing a level which may be often obtained in “lifestyle activities”. Exposure to 20 ppm CS₂ for 8 hours caused a 50 % increase in the concentration of acetaldehyde in blood compared to “alcohol only” values of the same

subjects. A similar effect was seen when the intake of alcohol started 16 hours after the end of exposure to 20 ppm CS₂, and after an 8 hours/day, 5 consecutive days exposure to 20 ppm CS₂ with alcohol intake only the last day. Under the conditions of the study, there were no complaints about an “antabuse syndrome” or other subjective signs of intoxication (Freundt et al. 1976b; Freundt and Lieberwirth 1974a). However, acetaldehyde dehydrogenase is a polymorphic enzyme and subjects with a less active form ALDH(2)2, which is frequent in Asians but rare or absent in Caucasians, are more susceptible to develop an “antabuse syndrome” after alcohol intake (O’Brien 2001). Additionally, alcohol intolerance has repeatedly been mentioned in workers occupationally exposed to unknown (most probably higher concentrations) of CS₂, and in its guidelines, the German Society for Occupational and Environmental Medicine states alcohol intolerance as a further adverse effect induced by CS₂ (Drexler 1998).

Other effects seen at similar concentrations involved an inhibition of oxidative N-de-methylation in humans exposed to 10 – 80 ppm was observed by Mack et al. (1974). However, no signs of liver damage as judged by serum parameters were observed at exposures between 20 and 80 ppm (Freundt and Lieberwirth 1974b).

In two toxicokinetic studies, occasional slight headache but no other symptoms were reported to occur in volunteers exposed to 17 – 51 ppm CS₂ for 0.5 to 4 hours (Harashima and Masuda 1962; Teisinger and Soucek 1949). The volunteers were reported to be free of symptoms in two other toxicokinetic studies at exposures to 3 – 25 ppm CS₂ for about 1 – 2 hours (Rosier et al. 1987; McKee et al. 1943). In a further toxicokinetic study in which volunteers were exposed to 17 – 142 ppm for up to 5 hours, the authors did not report any symptoms nor did they explicitly state the absence (Demus 1964).

5.2 Summary of Animal Data Relevant to AEGL-1

Several studies in rats describe effects on hepatic metabolism similar to those observed in humans. An increase in blood acetaldehyde levels occurred in alcohol-treated rats following CS₂ exposure to 20 or 400 ppm; the observed increases were lower than those obtained with disulfiram (“antabuse”) in similar experiments (Freundt et al. 1976b; Freundt and Netz 1973). The same concentration range led to a temporary depletion of hepatic glycogen accompanied by an increase in hepatic lactate and oxygen consumption, and to an inhibition of phase-I biotransformation reactions (Freundt and Dreher 1969; Freundt and Kürzinger 1975; Freundt and Kuttner 1969; Kürzinger and Freundt 1969). Signs of liver damage were not observed in rats exposed to CS₂ alone but after pretreatment with phenobarbitone (Chengelis 1988; Freundt et al. 1974a).

5.3 Derivation of AEGL-1

The AEGL-1 was based on an increase of acetaldehyde blood level in a controlled study in humans (Freundt et al. 1976b; Freundt and Lieberwirth 1974a). Exposure to 20 ppm CS₂ for 8 hours caused a 50 – 100 % increase in the blood acetaldehyde level when the subjects simultaneously or afterwards had taken in low to at most moderate amounts of alcohol (0.7 ‰ blood alcohol). The observed increase of the acetaldehyde level was not accompanied by an “antabuse syndrome” in healthy subjects. An uncertainty factor of 10 was applied to account for the protection of sensitive population subgroups, i.e., with an acetaldehyde dehydrogenase (ALDH2(2)) that is less active than the typical form (see 4.3.2 and 5.1).

Time scaling using the equation $C^n \times t = k$ was done to derive the other exposure duration specific values. Due to a lack of a definitive data set, a value of $n = 3$ was used in the exponential function for extrapolation from the experimental period of 8 hours to shorter exposure periods as described in NRC (2001). For the 10-minute AEGL-1 the 30-minute value was applied since the derivation was based on a

long experimental exposure period of 8 hours and no supporting studies using short periods were available for characterizing the concentration-time relationship.

The calculated values are listed below.

TABLE 6: AEGL VALUES FOR CARBON DISULFIDE					
AEGL Level	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour
AEGL-1	5.0 ppm (16 mg/m ³)	5.0 ppm (16 mg/m ³)	4 ppm (12 mg/m ³)	2.5 ppm (7.8 mg/m ³)	2.0 ppm (6.2 mg/m ³)

Support for this AEGL-1 comes from observations in toxicokinetic studies in humans. Slight headaches but no further symptoms were reported in individuals exposed to 17 – 51 ppm CS₂ for up to 4 hours (Harashima and Masuda 1962; Teisinger and Soucek 1949), and volunteers were reported to be free of symptoms at exposures to 3 – 25 ppm CS₂ for up to 2 hours (Rosier et al. 1987; McKee et al. 1943). Furthermore, exposure to 10 – 80 ppm for 6 – 8 hours caused transient alteration of xenobiotic biotransformation but no hepatotoxicity (Freundt et al. 1974b; Freundt and Lieberwirth 1974b; Mack et al. 1974).

The derived AEGL-1 values are above the 100 % odor recognition threshold of 0.21 ppm reported by Leonardos (1969) and the range of odor thresholds of 0.016 – 0.42 ppm (AIHA (1997)). Few data are available with respect to concentrations causing odor annoyance: In the study of Lehmann (1894), 180 – 240 ppm caused “moderate odor annoyance”, while there were no complaints in a toxicokinetic study at exposure to 10 – 20 ppm (Rosier et al. 1987). Thus, the calculated AEGL-1 values can be assumed not to cause moderate odor annoyance.

The database is not sufficient to calculate a level of distinct odor awareness (LOA). It must also must be taken into account that strong smelling decomposition products of CS₂ are rapidly formed under the influence of light and air. Therefore, the odor threshold and the hedonic tone of CS₂ will markedly change with the presence and formation of such impurities.

6 DATA ANALYSIS FOR AEGL-2

AEGL-2 is the airborne concentration (expressed as ppm or mg/m³) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects, or an impaired ability to escape.

6.1 Summary of Human Data Relevant to AEGL-2

Controlled exposure to 80 ppm for 8 hours was reported to be well tolerated in humans (Freundt et al. 1976b).

Only one controlled exposure study is known in which exposure to CS₂ reached concentrations to cause pronounced acute effects on the CNS (Lehmann 1894). In the course of the whole study, the exposure concentrations were varied between 180 ppm and 3370 ppm, and the exposures lasted from 1 hour to up to 4 hours 45 minutes. Signs of respiratory tract irritation (tickle in the throat, dry cough) occurred in most experiments, but always at concentrations which also caused CNS effects. Exposure to

180 – 240 ppm for up to 4 hours 45 minutes caused moderate odor annoyance but no further subjective symptoms. 320 – 385 ppm caused slight headaches and dizziness after 15 minutes. In the same experiments, temporary burning of eyes and mucous membranes also were noted at the end of exposure (1 hour 40 minutes). Exposure to 460 - 560 ppm (mean: 505 ppm) for up to 3 hours 50 minutes caused an immediate feeling of pressure in the head, later dizziness, anxiety, persisting headaches, temporary impairment of reading ability, lacrymation, cough attacks, and vasomotoric reactions (intense reddened face, increased pulse rate). About 710 - 800 ppm caused unmotivated laughter at the end of and after exposure and severe headaches which after 1.5 – 3 hours of exposure lasted for many hours. At about 1125 ppm, symptoms were more severe and occurred more rapidly (within 30 minutes). Exposure to about 2000 ppm for 1 hour caused severe intoxication with difficulty to perform tasks, anxiety, nausea, progressing dizziness, and the beginning of marked central paralysis. After exposure, staggered gait, strong dazed feeling, autonomic nervous system reactions (sudden salivation, increased pulse), vomiting and up to 2 days of feeling ill were recorded. One exposure in one subject to 2180 – 3370 ppm for 1.5 hours caused strong dizziness, nausea, seminarcotic state, and irregular respiration.

In this study, the effects were described in detail and analytical determinations of the exposure concentrations were performed. However, only two volunteers were exposed, one of them in a small chamber of only 3 m³, and the author reported that there were difficulties in maintaining the exposure concentration in this set of the experiments. Therefore, although previous derivations of ERPG, EEL and IDLH values (see Table 10) are based on data from secondary sources which can be traced back to Lehmann (1894), the results of this study will not be used for the derivation of AEGL-values.

6.2 Summary of Animal Data Relevant to AEGL-2

As in humans, at non-lethal concentrations, acute effects on the central nervous system were observed. Irritation of eyes and/or mucous membranes occur at concentrations that already have effects on the CNS.

In squirrel monkeys, limited data from one study (Weiss et al. 1979) show behavioral alterations in response to an aversive electric shock during exposure to 600 ppm for 2 h. During the last 30 minutes, the animal responded as often as during the control phase but the aversive threshold was elevated suggesting an anesthetic and/or an analgesic effect. This effect was similarly observed in a second monkey.

In rats, alterations in a neurobehavioral study (inhibition of escape and avoidance response in a pole climbing test) were observed at exposure to 2000 ppm for 4 hours, no such effects were seen after 4-hour exposure to 1000 ppm (Goldberg et al. 1964). It is likely that this inhibition of response is related to the narcotic effects of CS₂ which are described in other studies following acute exposure at similar and lower concentrations (see below).

In rats, 500 ppm for 6 hours reduced activity (Kivisto et al. 1995). A little higher concentration of 600 ppm but a longer exposure period of 10 hours caused narcotic-like stupor (Wilmarth et al. 1993). In single exposure studies, the effect of exposure time is obvious in three studies in rats exposed to 770 – 800 ppm: No visible signs of toxicity were reported after 12 h; ataxia, tremors, occasional convulsions occurred after 15 hours, and severe narcosis was seen after 18 hours (Tarkowski et al. 1980; Tarkowski and Cremer 1972; Tarkowski and Sobczak 1971).

Developmental toxicity effects have been described in some studies with rats and rabbits following repeated exposure during gestation (and in some studies, also additionally pregestational). On the other hand, in other studies at higher concentrations no such effects were observed. No studies were available with single exposure of animals to CS₂. The relevance of an exposure duration of about one third

to full gestation (or even additional pregestational exposure) in rats or rabbits to a less than one day exposure in humans is questionable. Moreover, it has to be considered that carbon disulfide reacts with the NH₂-group of endogenous compounds (e.g., amino acids) forming dithiocarbamates. Since some dithiocarbamate chemicals are reproductive and/or developmental toxins in animals, dithiocarbamates formed could play a role in the occurrence of developmental effects following carbon disulfide exposure. Although this cannot be ruled out, it has to be taken into account that while carbon disulfide itself ("free" CS₂) is rapidly eliminated from the body after ceasing exposure, the so-called "acid-labile" pool of bound carbon disulfide containing thiocarbamates has a long half-life and increases with daily repeated exposures. Therefore, it is unclear whether developmental effects observed after repeated exposure to carbon disulfide are of relevance for single acute exposures. For the reasons noted above, the results from developmental toxicity studies with CS₂ will not be used for the derivation of AEGL.

6.3 Derivation of AEGL-2

The AEGL-2 is based on the NOAEL of 1000 ppm (4-hour exposure) for behavioral alterations (inhibition of escape response) (Goldberg et al. 1964). At the next higher concentration, an inhibition of escape (and of avoidance) response was observed.

A total uncertainty factor of 10 was applied. An interspecies uncertainty factor of 3 was used based on the similarity of acute effects seen in rodents compared to humans produced by agents affecting the CNS. Moreover, use of a default interspecies uncertainty factor of 10 would have resulted in values which are contradicted by experimental human studies in which no serious or escape-impairing effects were reported during or following 6 – 8 hours of exposure to 80 ppm. An intraspecies uncertainty factor of 3 was applied to account for sensitive individuals because the threshold for CNS impairment is not expected to vary much among individuals. Time scaling was performed according to the regression equation $C^n \times t = k$ (Ten Berge et al. 1986). As outlined in NRC (2001), a default of $n = 3$ for shorter exposure periods (30 minutes and 1 hour) and $n = 1$ for longer exposure periods (8 hours) was applied, due to the lack of suitable experimental data for deriving the concentration exponent. For the 10-minute AEGL-2 the 30-minute value was used because the derivation of AEGL-2 values was based on a long experimental exposure period and no supporting studies using short exposure periods were available for characterizing the concentration-time-response relationship.

The calculated values are listed below.

TABLE 7: AEGL VALUES FOR CARBON DISULFIDE					
AEGL Level	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour
AEGL-2	200 ppm (620 mg/m ³)	200 ppm (620 mg/m ³)	160 ppm (490 mg/m ³)	100 ppm (310 mg/m ³)	50 ppm (160 mg/m ³)

The obtained values are supported by data from controlled studies in humans in which 8 hours of exposure up to 80 ppm were well tolerated (Freundt et al. 1976b).

7 DATA ANALYSIS OF AEGL-3

AEGL-3 is the airborne concentration (expressed as ppm or mg/m³) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

7.1 Summary of Human Data Relevant to AEGL-3

In the study of Lehmann (1894), very high CS₂ concentrations were applied. The reason for this was that the exposure of occupational workers to CS₂ at that time often was very high and even lethal but safe concentrations were unknown. The authors expressed the opinion that studies with controlled exposure of humans would be helpful in obtaining exposure concentrations which would be safe at least with respect to acute exposure. In view of the chronic toxicity of CS₂ which became better known later, it is not surprising that no further studies were carried out using similarly high concentrations. In the study, exposure to about 2000 ppm for 1 hour caused severe intoxication with difficulty to perform tasks, anxiety, nausea, progressing dizziness, and the beginning of marked central paralysis. After exposure, staggered gait, strong dazed feeling, autonomic nervous system reactions (sudden salivation, increased pulse), vomiting and up to 2 days of feeling ill were recorded. One exposure in one subject to 2180 – 3370 ppm for 1.5 hours caused strong dizziness, nausea, seminarcotic state, and irregular respiration. For the reasons mentioned above (see 6.3), the study of Lehmann (1894) will not be used for the derivation of AEGL-values.

7.2 Summary of Animal Data Relevant to AEGL-3

With respect to lethality, the data for rats indicate a steep concentration-reponse curve: While none of 6 rats survived a 4-hour exposure to 3500 ppm, all 6 rats survived at 3000 ppm (Du Pont 1966). No rats died after a single exposure to 2000 ppm for 4 hours (Goldberg et al. 1964) and to 1500 ppm for 2 hours (Savolainen and Järvisalo 1977).

In rabbits, death occurred in animals after single exposures to 3000 ppm or 3200 ppm for 6 hours (Flury and Zernik 1931; Lehmann 1894; PAI 1991). Individual cats died after exposure to 6450 ppm for 2 ¼ hours or after exposure to 3200 ppm for 4 ¼ h.

For mice, LC₅₀ values were reported of 3210 ppm (2 h) (Izmerov et al. 1982) and of 4500 ppm (30 minutes) (“average lethal concentration”) (Kuljak et al. 1974).

No treatment related deaths were observed in rats and mice following repeated exposures for at least 2 weeks to 800 ppm CS₂ (Toxigenics 1983a; Toxigenics 1983b; Toxigenics 1983c; Lewis et al. 1999; Moser et al. 1998; Wilmarth et al. 1993). In one study with mice, about 30 % of the those animals died which were given a high fat diet after the 1st exposure to 800 ppm CS₂ (Lewis et al. 1999). Necropsy did not reveal the cause of death in these animals. This observation deserves further investigation.

Embryo-/fetotoxic effects were observed in rats (CMA 1993) and rabbits (PAI 1991) following repeated exposure to 500 or 600 ppm, respectively, during gestation. No developmental studies were available with single exposure of animals to CS₂. As outlined above (see 6.2), these results will not be used for the derivation of AEGL for CS₂.

7.3 Derivation of AEGL-3

The derivation of AEGL-3 values is based on a study in rats in which a 4-hour exposure to 3000 ppm was not lethal during exposure or within a two week postobservation period (Du Pont 1966).

A total uncertainty factor of 10 was applied. An interspecies uncertainty factor of 3 was used based on the similarity of acute effects seen in rodents compared to humans produced by agents affecting the CNS. Moreover, use of a default interspecies uncertainty factor of 10 would have resulted in values which are contradicted by experimental human studies in which no serious or escape-impairing effects were reported during or following 6 – 8 hours of exposure to 80 ppm. An intraspecies uncertainty factor of

3 was applied to account for sensitive individuals because the threshold for CNS impairment is not expected to vary much among individuals. Time scaling was performed according to the regression equation $C^n \times t = k$ (Ten Berge et al. 1986). As outlined in NRC (2001), a default of $n = 3$ for shorter exposure periods (30 minutes and 1 hour) and $n = 1$ for longer exposure periods (8 hours) was applied, due to the lack of suitable experimental data for deriving the concentration exponent. For the 10-minute AEGL-3 the 30-minute value was used because the derivation of AEGL-3 values was based on a long experimental exposure period and no supporting studies using short exposure periods were available for characterizing the concentration-time-response relationship.

The calculated values are listed below.

TABLE 8: AEGL VALUES FOR CARBON DISULFIDE					
AEGL Level	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour
AEGL-3	600 ppm (1480 mg/m ³)	600 ppm (1480 mg/m ³)	480 ppm (990 mg/m ³)	300 ppm (930 mg/m ³)	150 ppm (470 mg/m ³)

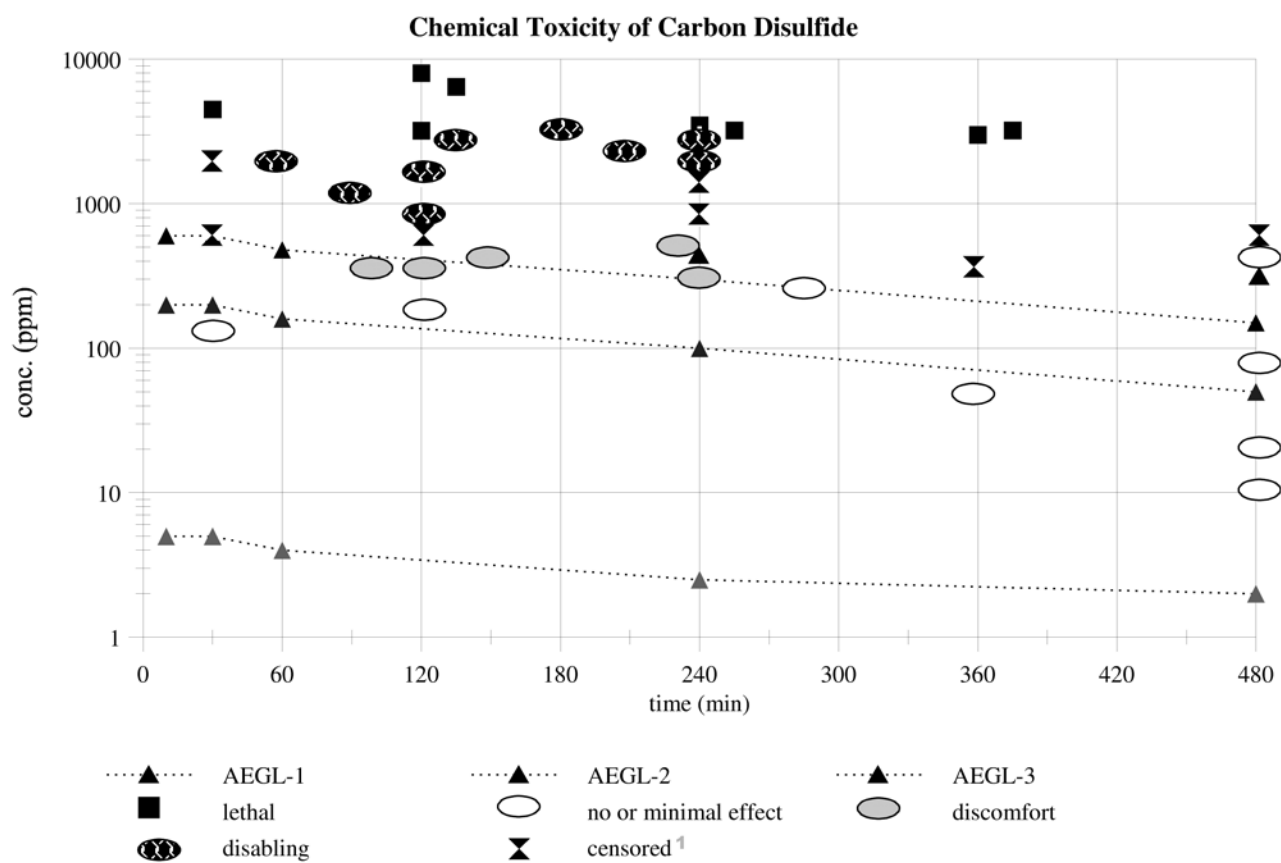
The obtained values are supported by data from a controlled human study in which exposure for a total of 3 hours 50 minutes to a mean concentration of 505 ppm caused intoxication with headaches within 30 minutes and later on lacrymation, cough attacks, dizziness and anxiety, but no life-threatening symptoms (Lehmann 1894, see TABLE 2).

8 SUMMARY OF PROPOSED AEGLS

8.1 AEGL Values and Toxicity Endpoints

TABLE 9: SUMMARY/RELATIONSHIP OF PROPOSED AEGL VALUES ^a					
Classification	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour
AEGL-1 (Nondisabling)	5.0 ppm (16 mg/m ³)	5.0 ppm (16 mg/m ³)	4 ppm (12 mg/m ³)	2.5 ppm (7.8 mg/m ³)	2.0 ppm (6.2 mg/m ³)
AEGL-2 (Disabling)	200 ppm (620 mg/m ³)	200 ppm (620 mg/m ³)	160 ppm (490 mg/m ³)	100 ppm (310 mg/m ³)	50 ppm (160 mg/m ³)
AEGL-3 (Lethality)	600 ppm (1480 mg/m ³)	600 ppm (1480 mg/m ³)	480 ppm (990 mg/m ³)	300 ppm (930 mg/m ³)	150 ppm (470 mg/m ³)

^a: Cutaneous absorption may occur. Liquid CS₂ is a severe skin irritant and vesicant. Direct skin contact with the liquid must be avoided.



1: severity category could not be established

FIGURE 3: CATEGORICAL REPRESENTATION OF ALL CARBON DISULFIDE INHALATION DATA

8.2 Comparison with other Standards and Criteria

Other standard and guidance levels for workplace and community are listed in Table 10.

TABLE 10: EXTANT STANDARDS AND GUIDELINES FOR CARBON DISULFIDE							
Guideline	Exposure duration						
	10 minutes	30 minutes	1 hour	4 h	6 h	8 h	24 h
AEGL-1	5 ppm	5 ppm	4 ppm	2.5 ppm		6.2 ppm	
AEGL-2	330 ppm	330 ppm	260 ppm	170 ppm		130 ppm	
AEGL-3	840 ppm	840 ppm	670 ppm	420 ppm		330 ppm	
ERPG-1 (AIHA) ^a			1 ppm				
ERPG-2 (AIHA)			50 ppm				
ERPG3 (AIHA)			500 ppm				
IDLH (NIOSH) ^b		500 ppm					
EEL (NRC) ^c	200 ppm	100 ppm	50 ppm				
Air-MEG (USACHPPM) ^d			Minimal: 1 ppm signif.: 50 ppm severe: 500 ppm			3 ppm	
Acute REL ^e (OEHHA)			144 ppm		2 ppm		
PEL-TWA (OSHA) ^f						20 ppm	
Acceptable peak (OSHA) ^g		30 ppm					
REL-TWA (NIOSH) ^h						1 ppm	
TLV-TWA (ACGIH 1995) ⁱ						10 ppm	
MAK (DFG, Germany) ^j						5 ppm	
MAK (DFG, Germany) Kurzzeitkategorie ^k		10 ppm					
Einsatztoleranzwert ^l				10 ppm			
AQG (WHO 2000) ^m		20 µg/m ³ (.006 ppm)					100 µg/m ³ (.032 ppm)
MRL (ATSDR 1996) ^o							0.30 ppm

a **ERPG** (Emergency Response Planning Guidelines, American Industrial Hygiene Association)

The ERPG-1 is the maximum airborne concentration below which nearly all individuals could be exposed for up to one hour without experiencing or developing effects more serious than mild irritation, other mild transient health effects, or perception of a clearly objectionable odor. The ERPG-1 for CS₂ is based on a

reported odor threshold of 0.21 ppm which is referenced as ASTM (1973), a compilation of odor threshold data. The original source for this odor threshold is Leonardos et al. (1969). The ERPG-1 of 1 ppm is nearly five times greater than the reported odor threshold. In a critical review of odor threshold data, AIHA (1997) rejected the use of the 0.21 ppm threshold because this value represents a 100 % recognition concentration. The ERPG-2 is the maximum airborne concentration below nearly all individuals could be exposed for up to one hour without experiencing or developing irreversible or other serious adverse health effects or symptoms that could impair an individual's ability to take protective action. The ERPG-2 for carbon disulfide is based on findings that although individuals may experience transitory effects such as headache, confusion, and eye irritation, the effects would be reversible, and serious effects are not expected to occur. Additionally, although developmental effects were reported to occur in rats exposed 8 hours/ day to 32 and 64 ppm, exposure at 40 ppm did not result in maternal toxicity or developmental effects.

The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing life-threatening health effects. The ERPG-3 for carbon disulfide is based upon reports of severe poisoning at 1150 ppm for 30 minutes and reports of psychosis and paralysis following acute exposure at 500 ppm.

b: IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)

Basis for original IDLH: The IDLH is based on the statement in Patty [1963] that symptoms occur after 30 minutes of exposure to 420 to 510 ppm [Flury and Zernik 1931]. AIHA [1956] reported that severe symptoms and unconsciousness may occur within 30 minutes at 1,100 ppm [Patty 1963]. Patty [1963] also reported that exposure of humans to 4,800 ppm for 30 minutes causes coma and may be fatal [Flury and Zernik 1931]. Basis for revised IDLH: Based on acute inhalation toxicity data in humans [Bittersohl et al. 1972; Browning 1953; Flury and Zernik 1931; Lefaux 1968], the original IDLH for carbon disulfide (500 ppm) is not being revised at this time (NIOSH 1996).

c: EEL (Emergence Exposure Limit, National Research Council, Committee on Toxicology)

The EEL is defined as a ceiling limit for an unpredictable single exposure, usually lasting 60 minutes or less, and never more than 24 hours – an occurrence expected to be rare in the lifetime of any person. It reflects an acceptance of the statistical likelihood of the occurrence of a nonincapacitating, reversible effect in an exposed population. It is designed to avoid substantial decrements in performance during emergencies and might contain no uncertainty factor. The use of uncertainty factors will depend on the specific compound in question and on the type of effect produced by the compound. The values for carbon disulfide are based on neurotoxic symptoms in humans (NRC 1984).

d: MEG (Military exposure guidelines) (USACHPPM 2001):

MEGs are concentrations of chemicals in air, water, and soil that can be used during deployments to assist in the assessment of the significance of field exposures to occupational and environmental health (OE) chemical hazards. TG 230 MEGs are designed to address a variety of scenarios such as a single catastrophic release of large amounts of a chemical, for temporary exposure conditions lasting hours to days, or for ambient environmental conditions such as regional pollution, use of a continuously contaminated water supply, or persistent soil contamination where there is regular contact. For each media there are slightly different exposure scenarios of concern. Specifically, a MEG is a chemical concentration in air, water or soil that, after a one-time exposure of specified duration, represents an estimate of the level above which certain types of health effects may begin to occur in individuals amongst the exposed population.

1-hr SEVERE: The airborne concentration above which continuous exposure for 1 hour could begin to produce life-threatening or lethal effects in a small portion of individuals. Increasing concentrations and/or duration of exposure will increase incidence of lethality and severity of non-lethal severe effects.

1-hr SIGNIFICANT: The airborne concentration above which continuous exposure for 1 hour could begin to produce irreversible, permanent, or serious health effects that may result in performance degradation and incapacitate a small portion of individuals. Increasing concentrations and/or duration of exposure will increase incidence and severity of effects.

1-hr MINIMAL TO NONSIGNIFICANT: The airborne concentration above which continuous exposure for 1 hour could begin to produce mild, non-disabling, transient, reversible effects, if any. Such effects should not impair performance. Increasing concentration and/or duration could result in performance degradation, especially for tasks requiring extreme mental/visual acuity or physical dexterity/strength.

8-hr and 24-hr MINIMAL TO NONSIGNIFICANT: The airborne concentration above which continuous exposure for 8 or 24 hours could begin to produce mild, non-disabling, transient, reversible effects, if any. Such effects should not impair performance. Increasing concentration and/or duration could result in performance degradation, especially for tasks requiring extreme mental/visual acuity or physical dexterity/strength.

e: **Acute REL** (Acute Reference Exposure Levels for Airborne Toxicants) (OEHHA 1999b).

The concentration level at or below which no adverse health effects are anticipated for a specified exposure duration is termed the reference exposure level (REL). The REL for a 6-h exposure protective against severe adverse effects of CS₂ is based on a developmental toxicity study in rats (Saillenfait et al. 1989). The 1-h level protective against life-threatening effects is based on CNS effects in occupationally exposed workers (Vigliani 1954) OEHHA 1999a.

f: **OSHA PEL-TWA** (Occupational Health and Safety Administration, Permissible Exposure Limits - Time Weighted Average) for 8 hours (OSHA 2000).

g: **Acceptable Peak OSHA** (Occupational Health and Safety Administration, Permissible Exposure Limits) (OSHA 2000). The maximum peak is 100 ppm.

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

i: **ACGIH TLV-TWA** (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average) (ACGIH 1996):

The time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

j: **MAK** (Maximale Arbeitsplatzkonzentration [Maximum Workplace Concentration], Deutsche Forschungsgemeinschaft [German Research Association], Germany) (Greim 1998) is defined analogous to the ACGIH-TLV-TWA.

k: **MAK Kurzzeitkategorie** (Kategorie II, 2) (Short term Category II, 2) (DFG 2001)

constitutes the maximum average concentration to which workers can be exposed for a period up to 30 minutes (mean value) no more than 2 times per workshift.

l: **Einsatztoleranzwert** (Buff and Greim 2000)

Einsatztoleranzwert (Action Tolerance Levels), Vereinigung zur Förderung des deutschen Brandschutzes e. V. (Federation for the Advancement of German Fire Prevention) constitutes a concentration to which unprotected firemen and the general population can be exposed to for up to 4 hours without any health risk.

m: **Air Quality Guidelines for Europe** (WHO 2000).

The guideline value provides a concentration below which no adverse effects or (in the case of odorous compounds), no nuisance or indirect health significance are expected, although it does not guarantee the absolute exclusion of effects at concentrations below the given value. The guideline value was derived from epidemiological studies indicating an adverse effect at about 10 mg/m³, which may be equivalent to a concentration in the general environment of 1 mg/m³. The 30 minutes value is based on the sensory effects (odor) of carbon disulfide.

o: **Minimal Risk Level** (ATSDR 1996)

An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. The MRL for CS₂ is based on effects on the peripheral nervous system observed in a study on workers chronically exposed to carbon disulfide (Johnson et al. 1983).

8.3 Data adequacy and research needs

Because CS₂ is a solvent which has been used in large quantities in industry for more than a century, its chronic effects have been extensively studied and the data base is large. Effects of acute intoxication in occupational workers who were also chronically exposed have also been described. In these reports, appropriate exposure concentrations are lacking. Very few controlled studies with humans are available which could be used for the derivation of AEGL. These studies focussed on toxicokinetics, inhibition of biotransformation, and other alterations of liver functions. The AEGL-1 was derived from a controlled metabolism study in weakly alcoholized subjects. Studies on odor perception are also available, but the detection threshold has not been characterized. It is likely that odor perception will be markedly affected by the impurities which form in carbon disulfide under the influence of air and light. Only one older study with controlled exposure of two students described acute effects over a wide range of concentrations. The data from this study were used to derive AEGL-2 and AEGL-3. In view of the severe acute effects of CS₂ observed in this study and of the chronic effects at continued exposure which have become known since that study was performed, any further studies with controlled exposure to high concentrations must be considered risky and under ethical points of view cannot be justified. Animals studies, largely conducted with rats, indicate a steep concentration-response curve for lethality. In animals, there is a broad data base from studies on acute non-lethal effects, mostly on the nervous system and the liver. These data are in agreement with the limited data from controlled human studies and support the AEGL values derived from human studies.

Epidemiological studies on occupational cohorts chronically exposed CS₂ cause suspicion of developmental/reproductive effects. The lowest level at which such effects may occur is not known. In animal experiments, embryo/fetotoxic effects, malformations, and alterations of postnatal behavior in offsprings have been described when dams were repeatedly exposed over a number of days in different periods reaching from pregestation to the end of gestation. Some of these studies report that effects could be seen down to very low concentrations but these studies are not properly described. Studies with single exposure are lacking. Thus, additional studies devoted to developmental/ reproductive toxicity would be beneficial. Further studies on metabolism, toxicokinetics, and mechanism of action also would be useful.

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APPENDIX A: DERIVATION OF AEGL VALUES

Derivation of AEGL-1

Key study:	Freundt et al. (1976b)
Toxicity endpoint:	Exposure to 20 ppm for 8 hours in volunteers with a blood alcohol concentration of 0.75 ‰ caused a 50 % increase in blood acetaldehyde level. This effect is explained by an inhibition of acetaldehyde dehydrogenase (ALDH) by CS ₂ which is similarly caused by dithiocarbamates and disulfiram (“antabuse”). The increase of blood acetaldehyde in the key study was asymptomatic, i.e. no “antabuse syndrome” was observed. However, ALDH is a polymorphic enzyme and individuals with low ALDH-activity (as frequently observed in asians) may experience discomfort under conditions as in the experiment described.
Scaling:	$C^3 \times t = k$ for extrapolation to 8 hours, 4 hours, 1 hour, and 30 minutes The 10-minutes AEGL-1 was set at the same concentration as the 30-minutes AEGL-1. $k = 20^3 \text{ ppm}^3 \times 8 \text{ h} = 64000 \text{ ppm}^3 \text{ h}$
Uncertainty/ modifying factors	10 for intraspecies variability
Calculations	
<u>10-minute AEGL-1</u>	10-minutes AEGL-1 = 30-minutes AEGL-1 = 5.0 ppm (16 mg/m ³)
<u>30-minute AEGL-1</u>	$C^3 \times 0.5 \text{ h} = 64000 \text{ ppm}^3 \text{ h}$ $C = 50 \text{ ppm}$ 30-minutes AEGL-1 = 50 ppm/10 = 5.0 ppm (16 mg/m³)
<u>1-hour AEGL-1</u>	$C^3 \times 1 \text{ h} = 64000 \text{ ppm}^3 \text{ h}$ $C = 40 \text{ ppm}$ 1-hour AEGL-1 = 40 ppm/10 = 4 ppm (12 mg/m³)
<u>4-hour AEGL-1</u>	$C^3 \times 4 \text{ h} = 64000 \text{ ppm}^3 \text{ h}$ $C = 25 \text{ ppm}$ 4-hour AEGL-1 = 25 ppm/10 = 2.5 ppm (7.8 mg/m³)
<u>8-hour AEGL-1</u>	$C^3 \times 8 \text{ h} = 64000 \text{ ppm}^3 \text{ h}$ $C = 20 \text{ ppm}$ 8-hour AEGL-1 = 20 ppm/10 = 2.0 ppm (6.2 mg/m³)

Derivation of AEGL-2

Key study:	Goldberg et al. (1964)
Toxicity endpoint:	Behavioral alterations (Inhibition of escape response) in rats exposed to 2000 ppm for 4 hours; NOEL: 1000 ppm, 4 hours
Scaling:	$C^3 \times t = k$ for extrapolation to 30 minutes, 1 hour The 10-minutes AEGL-2 was set at the same concentration as the 30-minutes AEGL-2. $k = 1000^3 \text{ ppm}^3 \times 4 \text{ h} = 4 \times 10^9 \text{ ppm}^3 \text{ h}$ $C^1 \times t = k$ for extrapolation to 4 hours and 8 hours $k = 1000 \text{ ppm} \times 4 \text{ h} = 4000 \text{ ppm h}$
Uncertainty/ modifying factors	3 for interspecies variability 3 for intraspecies variability Combined uncertainty factor of 10
Calculations	
<u>10-minute AEGL-2</u>	10-minutes AEGL-2 = 30-minutes AEGL-2 = 200 ppm (620 mg/m ³)
<u>30-minute AEGL-2</u>	$C^3 \times 0.5 \text{ h} = 4 \times 10^9 \text{ ppm}^3 \text{ h}$ $C = 2000 \text{ ppm}$ 30-minutes AEGL-2 = 2000 ppm/10 = 200 ppm (620 mg/m ³)
<u>1-hour AEGL-2</u>	$C^3 \times 1 \text{ h} = 4 \times 10^9 \text{ ppm}^3 \text{ h}$ $C = 1587 \text{ ppm}$ 1-hour AEGL-2 = 1587 ppm/10 = 160 ppm (490 mg/m ³)
<u>4-hour AEGL-2</u>	$C \times 4 \text{ h} = 4000 \text{ ppm h}$ $C = 1000 \text{ ppm}$ 4-hour AEGL-2 = 1000 ppm/10 = 100 ppm (310 mg/m ³)
<u>8-hour AEGL-2</u>	$C \times 8 \text{ h} = 4000 \text{ ppm h}$ $C = 500 \text{ ppm}$ 8-hour AEGL-2 = 500 ppm/10 = 50 ppm (160 mg/m ³)

Derivation of AEGL-3

Key study:	Du Pont (1966)
Toxicity endpoint:	Acute lethality in rats following 4-hour exposure: 6/6 rats died at 3500 ppm, 0/6 rats died at 3000 ppm
Scaling:	$C^3 \times t = k$ for extrapolation to 30 minutes, 1 hour. The 10-minutes AEGL-1 was set at the same concentration as the 30-minutes AEGL-1. $k = 3000^3 \text{ ppm}^3 \times 4 \text{ h} = 1.08 \times 10^{11} \text{ ppm}^3 \text{ h}$ $C^1 \times t = k$ for extrapolation to 4 hours and 8 hours $k = 3000 \text{ ppm} \times 4 \text{ h} = 12000 \text{ ppm h}$
Uncertainty/ modifying factors	3 for interspecies variability 3 for intraspecies variability Combined uncertainty factor of 10
Calculations	
<u>10-minute AEGL-3</u>	10-minutes AEGL-3 = 30-minutes AEGL-3 = 600 ppm (1870 mg/m ³)
<u>30-minute AEGL-3</u>	$C^3 \times 0.5 \text{ h} = 1.08 \times 10^{11} \text{ ppm}^3 \text{ h}$ $C = 6000 \text{ ppm}$ 30-minutes AEGL-3 = 6000 ppm/10 = 600 ppm (1870 mg/m ³)
<u>1-hour AEGL-3</u>	$C^3 \times 1 \text{ h} = 1.08 \times 10^{11} \text{ ppm}^3 \text{ h}$ $C = 4762 \text{ ppm}$ 1-hour AEGL-3 = 4762 ppm/10 = 480 ppm (1500 mg/m ³)
<u>4-hour AEGL-3</u>	$C \times 4 \text{ h} = 12000 \text{ ppm h}$ $C = 3000 \text{ ppm}$ 4-hour AEGL-3 = 3000 ppm/10 = 300 ppm (930 mg/m ³)
<u>8-hour AEGL-3</u>	$C \times 8 \text{ h} = 12000 \text{ ppm h}$ $C = 1500 \text{ ppm}$ 8-hour AEGL-3 = 1500 ppm/10 = 150 ppm (470 mg/m ³)

APPENDIX B: DERIVATION SUMMARY FOR CARBON DISULFIDE AEGLS

**ACUTE EXPOSURE GUIDELINE LEVELS FOR
CARBON DISULFIDE (CAS Reg. No. 75-15-0)
DERIVATION SUMMARY**

AEGL-1 VALUES				
10 minutes	30 minutes	1 hour	4 hr.	8 hr.
5.0 ppm	5.0 ppm	4 ppm	2.5 ppm	2.0 ppm
Key Reference: Freundt et al. 1976b. Blood acetaldehyde in alcoholized rats and humans during inhalation of carbon disulphide vapor. Int. Arch. Occup. Environ. Health 37, 35 - 46.				
Test Species/Strain/Number: Human/ Healthy young males/12				
Exposure Route/Concentrations/Durations: Inhalation/0, 20, 40, 80 ppm, 8 h				
Effects: At 20 ppm, increase in blood acetaldehyde concentration (ca. 50 % above control level) in healthy human subjects with moderate intake of alcohol (blood alcohol ca. 0.7 ‰). The effect can be explained by an inhibition of the acetaldehyde dehydrogenase (ALDH). The rise in acetaldehyde was not accompanied by signs of an “antabuse” effect. However, alcohol intolerance has been reported in workers occupationally exposed to unknown concentrations of CS₂. In further controlled human studies, exposure to 10 – 80 ppm CS₂ caused a temporary reversible inhibition of xenobiotic biotransformation, but no signs of liver damage were observed.				
Endpoint/Concentration/Rationale: Increase in blood acetaldehyde concentration at 20 ppm, 8 h				
Uncertainty Factors/Rationale: Interspecies: 1, test subjects were humans Intraspecies: 10; subjects were healthy male volunteers. An uncertainty factor of 10 was applied to account for the protection of sensitive population subgroups with an acetaldehyde dehydrogenase (ALDH2(2)) less active than the typical form ALDH2. The presence of the ALDH2(2) allele (which is especially common in Asians but rare or absent in Caucasians) results in low enzyme activity and higher levels of acetaldehyde after ingestion of alcohol compared to persons in which the normal enzyme is present. An additional increase of the acetaldehyde concentration due to exposure to CS₂ may thus lead to an “antabuse syndrome” or aggravate otherwise mild symptoms.				
Modifying factor: NA				
Animal to Human Dosimetric Adjustment: NA				
Time Scaling: Extrapolation was made to the relevant AEGL time points using the relationship $C^n \times t = k$ with the default of $n = 3$ (ten Berge et al. 1986) for shorter exposure periods, due to the lack of experimental data for deriving the concentration exponent. For the AEGL-1 for 10 minutes, the AEGL-1 for 30 minutes was adopted because the derivation of AEGL values was based on a study with a long experimental exposure period of 8 h, no supporting studies using short exposure periods were available characterizing the concentration time-response relationship, and it is considered inappropriate to extrapolate back to 10 minutes. The derived AEGL-1 values are above the reported odor thresholds but below concentrations reported to cause moderate odor annoyance.				
Confidence and Support for AEGL values: A well-conducted study with a sufficient number of human volunteers and an appropriate endpoint for AEGL-1 was available.				

AEGL-2 VALUES				
10 minutes	30 minutes	1 hour	4 hr.	8 hr.
200 ppm	200 ppm	160 ppm	100 ppm	50 ppm
Key Reference: Goldberg, M.E., H.E. Johnson, D.C. Pozzani, and H.F.Jr. Smyth. 1964. Effect of repeated inhalation of vapors of industrial solvents on animal behavior. I. Evaluation of nine solvents vapors on pole-climb performance in rats. Am. Ind. Hyg. Assoc. J. 25: 369-375.				
Test Species/Strain/Number: Rats/ Carworth Farms Elias/ Groups of 8-10 females				
Exposure Route/Concentrations/Durations: Inhalation 0, 250, 500, 1000, 2500 ppm, 4 hours				
Effects: At 2000 ppm, inhibition of escape response in 12 % (and of avoidance response in 50 %) of the animals was observed. No inhibition of escape (and avoidance) response was observed at 1000 ppm.				
Endpoint/Concentration/Rationale: Exposure to 1,000 ppm for 4 hours was a NOAEL for inhibition of escape response.				
Uncertainty Factors/Rationale: Total uncertainty factor: 10 Interspecies: 3 – based on the similarity of acute effects seen in rodents compared to humans produced by agents affecting the CNS Intraspecies: 3 – human data suggest that acute effects of volatile anesthetics and gases on the CNS show little intraspecies variability (about 2-3 fold).				
Modifying factor: NA				
Animal to Human Dosimetric Adjustment: NA				
Time Scaling: Extrapolation was made to the relevant AEGL time points using the relationship $C^n \times t = k$ with the default of $n = 3$ for shorter exposure periods of 1 hour and of 30 minutes and of $n = 1$ for longer exposure periods of 4 and 8 hours (ten Berge et al. 1986; NRC 2001). The 10-minutes AEGL-2 was assigned the same value as that for the 30-minutes AEGL-2 as it was considered inappropriate to extrapolate from an experimental period of 4 hours to 10 minutes.				
Confidence and Support for AEGL values: AEGL-2 values are protective of human health. The level is based on a NOEL for inhibition of escape response in a behavioral study with rats in which concentrations in the exposure chamber were monitored. Additionally, the AEGL-values are supported by data from human studies in which no effects meeting the AEGL-2 definition were observed at similar concentrations.				

AEGL-3 VALUES				
10 minutes	30 minutes	1 hour	4 hr.	8 hr.
600 ppm	600 ppm	480 ppm	300 ppm	150 ppm
Key Reference: Du Pont 1966. Acute inhalation toxicity - progress report. Haskell Laboratory Report No. 161-66. EI Du Pont De Nemours and Company. Haskell Laboratory, Newark, De.				
Test Species/Strain/Number: Rats/ CD/ 6 males				
Exposure Route/Concentrations/Durations: Inhalation/ 3500 ppm, 3000 ppm/ 4 hours				
Effects: 6/6 rats died at 3500 ppm, none of 6 rats died at 3000 ppm				
Endpoint/Concentration/Rationale: No lethality following 4 hours of exposure to 3000 ppm.				
Uncertainty Factors/Rationale: Total uncertainty factor: 10 Interspecies: 3 – based on the similarity of acute effects seen in rodents compared to humans produced by agents affecting the CNS Intraspecies: 3 – human data suggest that acute effects of volatile anesthetics and gases on the CNS show little intraspecies variability (about 2-3 fold).				
Modifying factor: NA				
Animal to Human Dosimetric Adjustment: NA				
Time Scaling: Extrapolation was made to the relevant AEGL time points using the relationship $C^n \times t = k$ with the default of $n = 3$ for shorter exposure periods of 1 hour and of 30 minutes and of $n = 1$ for longer exposure periods of 4 and 8 hours (ten Berge et al. 1986; NRC 2001). The 10-minutes AEGL-2 was assigned the same value as that for the 30-minutes AEGL-2 as it was considered inappropriate to extrapolate from an experimental period of 4 hours to 10 minutes.				
Confidence and Support for AEGL values: AEGL-3 values are protective of human health. The available indicate a very steep concentration-lethality response curve and the values are based on a no observed lethality concentration in rats. Additionally, the AEGL-3 values are supported by data from a human study in which the effects noted were milder than those defined by the AEGL-3 definition.				