INTERIM ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

DIMETHYL SULFATE

(CAS Reg. No. 77-78-1)

for NAS/COT-Subcommittee on AEGLs

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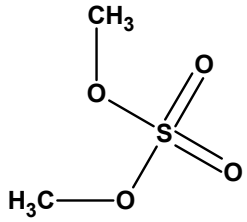

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PREFACE

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/m^3]) 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/m^3) 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/m^3) 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

Dimethyl sulfate ($DMSO_4$) is a colorless, oily liquid with a slight onion-like odor. A minimum odor threshold may not be derived.

Dimethyl sulfate is miscible with organic solvents, and moderately soluble in water. It hydrolyzes readily in contact with water or moist surfaces to form monomethyl sulfate and methanol, and further to form sulfuric acid.

Dimethyl sulfate is used as an alkylating agent in the dye, agricultural, pharmaceutical, surfactant, and perfumery industries and exposure results exclusively from industrial processes, mostly via inhalation pathway.

Inhalation exposure to dimethyl sulfate results in irritation and other adverse effects of upper respiratory tract and eyes as primary effects, followed by lesions in bronchi and lung. Usually a latency period for local effects of 4 to 12 hours between exposure and onset of effects was reported from human case studies. From experimental studies on animals latency periods of few minutes were given after administration of high doses via different pathways. Systemic effects show no such latency period.

Cogent evidences for developmental toxicity are not available. After single intraperitoneal high-dose exposure of 75 mg/kg slight adverse effects on reproduction of mice were reported in a single study, which were not found in studies with inhalation exposure. Sufficient evidence for carcinogenic effects in animals after prolonged inhalation exposure is available. Genotoxicity was observed in vitro and in vivo.

Data on toxic effects in humans are available from case studies without qualified exposure data of dimethyl sulfate concentration.

The AEGL-1 values are based on a 14-day repeated exposure study in rats ((6 h/d, 5 d/w, 10 exposures) (Frame et al. 1993; abstract publication). At 0.1 ppm for altered nasal cell proliferation without histopathological findings was observed. More pronounced effects above AEGL-1 threshold, as breathing difficulties and asthmatic-like breathing, were reported by Schlögel (1972) at 0.5 ppm after first treatment period of a repeated 6-hour exposure. Therefore, 0.1 ppm is selected to derive AEGL-1. Evidence of only modest differences in toxicokinetics and toxicodynamics is available, therefore an interspecies factor of 3 is applied. The interspecies factor is further justified because the critical study used repeated exposure (Frame et al. 1993). No large differences in susceptibility between individuals are expected for unspecific irritating effects, therefore an intraspecies factor of 3 is chosen. An overall uncertainty factor of 10 is applied on the 0.1 ppm concentration. Suitable data to derive a substance specific exponent for time extrapolation in the equation $k = C^n \times t$ are available. Thus, a value of $n = 2$ in the exponential function was used for extrapolation from the 6-hour exposure to all durations except 10 minutes. Because extrapolation from 6 hours to short durations leads to very high uncertainty, the values for 10 minutes are set equal to the values for 30 minutes.

The AEGL-2 values are based on the effect concentration in rats, mice, and golden hamsters following a 6-hour exposure to 0.5 ppm, investigated by Schlögel (1972). This concentration results in breathing problems and asthmatic-like breathing. As reported by Frame et al. (1993) 0.7 ppm already leads to lesions of respiratory and olfactory epithelia in rats after repeated exposure (2 wk, 6 h/d, 10 exposures). Evidence of only modest species differences in toxicokinetics and toxicodynamics is available, therefore an interspecies factor of 3 is applied. No large differences in susceptibility between individuals are expected for unspecific irritating effects, therefore an intraspecies factor of 3 is chosen. An overall uncertainty

factor of 10 is applied on the 0.5 ppm concentration. Suitable data to derive a substance specific exponent for time extrapolation in the equation $k = C^n \times t$ are available. Thus, a value of n = 2 in the exponential function was used for extrapolation from the 6-hour exposure to all durations except 10 minutes. Because extrapolation from 6 hours to short durations leads to very high uncertainty, the values for 10 minutes are set equal to the values for 30 minutes.

The AEGL-3 values are based on an acute toxicity study in rats, mice, guinea pigs and hamsters in which LC_0 and LC_{50} values were derived by Hein (1969). The rat LC_0 of 49 ppm was chosen as derivation basis for AEGL-3, and it was supported by other effect data. At this concentration dyspnea with inspiratory stridor and lacrimation were noticed during exposure and necropsy showed severe inflation of stomach and small intestine and occasionally emphysema and edema of lungs. Because the derived AEGL-values are not based on effect concentrations in the most susceptible species, which would be the guinea pig, an interspecies factor of 10 is applied. No large differences in susceptibility between individuals is expected for unspecific irritating effects, therefore an intraspecies factor of 3 is chosen. An overall uncertainty factor of 30 is applied on the 49 ppm concentration. Suitable data to derive a substance specific exponent for time extrapolation in the equation $k = C^n \times t$ are available. Thus, a value of n = 2 in the exponential function was used for extrapolation from the 1-hour exposure to all durations.

The carcinogenic activity for life span exposure is calculated with I_{conc} = 2.2 mg/m³ (ECB 2002). Concentration of dimethyl sulfate, that would cause a theoretical excess cancer risk of 10^{-4} was calculated with 411 µg/m³.

The calculated values are listed in the Table below.

Summary of Interim AEGL Values for Dimethyl Sulfate [ppm (mg/m³)] *)

Classification	10-min	30-min	1-hour	4-hour	8-hour	Endpoint / Species	Reference
AEGL–1 (Nondisabling)	0.035 (0.18)	0.035 (0.18)	0.024 (0.12)	0.012 (0.062)	0.0087 (0.045)	nasal cell proliferation rat	Frame et al. (1993)
AEGL–2 (Disabling)	0.17 (0.88)	0.17 (0.88)	0.12 (0.62)	0.061 (0.32)	0.043 (0.22)	breathing problems rat, mouse, hamster	Schlögel (1972)
AEGL–3 (Lethal)	4 (21)	2.3 (12)	1.6 (8.3)	0.82 (4.3)	0.58 (3.0)	lethality due to emphysema and edema rat	Hein (1969)

*) Relevant skin uptake and sensitizing properties of dimethyl sulfate can not be excluded. Dimethyl sulfate is a methylating and mutagenic substance, classified as suspected human carcinogen (A2: ACGIH, 1991; 2A: IARC, 1999; Carc. Cat. 2, R45: BAuA, 2001).

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1. INTRODUCTION

Dimethyl sulfate ($DMSO_4^{-2}$) is a colorless, oily liquid with a slight onion-like odor, which however seems not to be perceptible by every individual (WHO 1985). No minimum odor threshold can be indicated.

TABLE 1. Chemical and Physical Properties

Parameter	Value	Reference
Chem. Abstr. Name	Sulfuric acid, dimethyl ester	IARC (1999)
Synonyms	Dimethyl monosulfate; methyl sulfate; sulfuric acid dimethyl ester	ECB (2002)
Chemical formula	$C_2H_6O_4S$	IARC (1999)
Molecular weight	126.13	IARC (1999)
CAS Reg. No.	77-78-1	IARC (1999)
Physical state	oily colorless liquid	IARC (1999)
Solubility in water	29 g/l 28 g/l at 18 °C (with hydrolyses)	Druckrey et al. (1970) Cartlidge et al. (1996)
Vapor pressure	90 Pa at 25 °C 80 Pa at 20 °C (calculated)	NLM (2003) Roßman and Gill (1952)
Vapor density (air =1)	4.35	NLM (2003)
Liquid density (water =1)	1.3322 g/cm3	NLM (2003)
Melting point	- 27 °C	IARC (1999)
Boiling point	188 °C (with decomposition)	IARC (1999)
Hydrolysis half-life	1.2 hours at pH 7 and 25 °C	NLM (2003)
Conversion factors	$mg/m^3 = 5.16 \times ppm$ $mg/m^3 = 5.24 \times ppm$	IARC (1999) Cartlidge et al. (1996)

Dimethyl sulfate is miscible with polar organic solvents and aromatic hydrocarbons, and moderately soluble in water.

In the environment dimethyl sulfate results exclusively from anthropogenic sources. Since the beginning of the last century it is used in the production of methyl esters, ethers, and amines in various substances in the dye, agricultural, pharmaceutical, surfactant, and perfumery industries. In World war I it was tested as a warfare agent as both the liquid and the vapor. Around 55 companies use it as a methylating agent in Great Britain (Cartlidge et al. 1996). WHO (1985) lists 340 tonnes / year for the production of dimethyl sulfate in the USA based on calculations from 1969, however a production of 45,000 tonnes was reported for 1977. Worldwide production in 2000 was about 90,000 tonnes (Kreiling, personal communication 2003).

In industrial processes dimethyl sulfate is used within enclosed plants and employees wear personal protective clothing. The closed systems are run with underpressure to ensure that no dimethyl sulfate leaks out. Nowadays exposure can occur during maintenance, filling, unloading, spillage, or accidental release. Occupational exposure occurs where dimethyl sulfate is produced or added to production process. Inhalation is the most important exposure route for dimethyl sulfate, whereas dermal exposure is considered to occur only accidentally. ECB (2002) lists workplace measurements reported by industry up to 1 ppm for short term levels. Industrial processes producing the highest air concentrations are pumping, connection of transfer lines, and sampling.

Several measurements of airborne dimethyl sulfate in and around reactor sites and storage facilities indicated concentrations below 0.1 ppm (Cartlidge et al. 1996). At leaking points of 2 sites handling dimethyl sulfate in USA air concentrations of 0.2 - 1 ppm were reported by ECB (2002). Lee et al. (1980) demonstrated, that dimethyl sulfate is present also in coal fly ash in the environment and not only limited to the vicinity of industrial plants. The measured concentrations were 0.74 - 0.84 µM/g. From combustion processes it is presented in both particles and in the gas phase (Vyskocil and Viau 1999). However, due to the high reactivity of dimethyl sulfate these values should be regarded cautiously.

Due to the vapor pressure routes of exposure are by inhalation and additionally by dermal contact. The saturated vapor concentration in the air at 20 °C is estimated as 3720 mg/m^3 (710 ppm) (WHO 1985) and 3100 mg/m^3 (592 ppm) (ECB 2002), both based on the vapor pressure. However, Cartlidge et al. (1996) indicate 6000 ppm at 20 °C (calculated) as saturated vapor concentration. Flury and Zernik (1931) refer on an inhalation study in guinea pigs, where 2072 ppm (10,700 mg/m^3) were administered. It is not mentioned if that was a saturated atmosphere. For statements concerning saturated vapor a concentration of 592 ppm is used in this document.

For conversion between ppm and mg/m^3 a factor of 5.2 is used in this document.

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

Several cases of lethal intoxication are reported in literature after inhalation, dermal and oral exposure. Unfortunately, no measurement of concentration was conducted in any of the reported cases. Sometimes description of exposure scenario supplies information if intoxication was due to low or high concentration of dimethyl sulfate, at least. Lethal intoxication can occur at low temperatures although due to the vapor pressure dimethyl sulfate is not classified as an extreme volatile substance.

2.1.1 Case Reports

Lethality after inhalation exposure

Weber (1902) reported one case of lethal intoxication after inhalation of dimethyl sulfate at working place. The first symptoms of an intoxication, described as "chest, throat and eye pain" by the worker, occurred shortly after handling a boiler leaking vapor of dimethyl sulfate for 4 hours. When the man was taken to the hospital 48 hours later he suffered from a double-sided pneumonia and died at the same day. The uvula and parts of the pharynx revealed a white discoloration and demucosation. Necropsy showed a spacious destruction of respiratory tract mucosa and petechiae of pericardium, endocardium, duodenum, and renal pelvis, and the liver parenchyma was swollen.

One case of lethal intoxication via inhalation at 4 °C (39.2 °F) was reported by Roßmann and Grill (1952) and Thiess and Goldmann (1967) after workplace exposure for about 3 hours due to a leaky container. 4 hours after onset of exposure the man suffered from irritation of upper respiratory tract and fever. During disease process he developed irritation of conjunctiva and a glottis edema. Death occurred 3 days later due to pulmonary and brain edema. The histopathological examination showed severe corrosion of the respiratory tract (pharynx, larynx, trachea), congestion of organs in the abdominal cavity and a liver swelling. All parts of the respiratory tract up to the bronchial system showed a greyish-yellow coating and demucosation in parts. Based on values gained from lethality studies in monkeys, referred from Flury and Zernik (1931), they assumed following Habers law (n = 1) a lethal concentration of 28 mg/m^3 (5.4 ppm) for this worker based on the exposure duration of 180 minutes. However, the validity of the assumption is not provided and the concentration was not measured.

Lethality after dermal exposure

A second intoxication described by Weber (1902) occurred mistakenly after spilling of about 20 g dimethyl sulfate on the work clothes. Hours later the worker was troubled with diffuse whole-body pain, and additionally burning of trachea. Subsequently he developed inflammation of the whole respiratory tract (pharyngitis, tracheitis, bronchitis, pneumonia) and a severe conjunctivitis with eyelid edema. First to third degree corrosive skin burns were diagnosed locally. Despite of a temporary recovery of conjunctivitis and pharyngitis the man died 4 days after exposure.

Lethality after oral exposure

Von Nida (1947) reported a lethal case of dimethyl sulfate intoxication of a 38-year old man after licking his finger moistened with dimethyl sulfate. Immediately after ingestion, the man felt an intensive nausea followed by an enhanced salivation. 12 hours later he suffered from a sudden dyspnea, and a cyanosis and died of a glottis edema shortly thereafter. The pathological examination revealed acute

inflammations of a major part of the respiratory tract with severe injuries of mucosa (necrosis and demucosation). Additionally, injuries of digestive tract were reported (gastritis, fibrinous adhesion of caecum and lower ileum).

Bartalini et al. (1957) describe a lethal case after mistakenly ingestion of two gulps of dimethyl sulfate. Following oral absorption onset of effects occurred already after 30 minutes and comprised vomiting, nausea and a burning pain in the gastro-intestinal tract. Dyspnea, diarrhea, cyanosis and neurotoxic effects followed these first symptoms. Death occurred 3 hours after ingestion and was due to cardiac failure.

2.2. Nonlethal Toxicity

Some cases with a similar exposure pattern and comparable concentrations as described at lethal intoxication did not lead to mortality. Unfortunately, concentration measurements were not conducted for most of the case studies. All of the described cases showed a severe disease process and recovery was more or less prolonged.

2.2.1. Case Reports

Nonlethal toxicity after inhalation exposure

Strothmann (1929) describes a nonlethal intoxication of a 19-year old man, who was exposed to vapors of dimethyl sulfate and alcohol. Initial symptoms were described as lacrimation, eye pain and burning pain of the pharynx and were perceived immediately. The clinical observations revealed cyanosis, panting breathing, strong coughing reflex, and swollen and reddened conjunctiva. The upper respiratory tract was intensive reddened, and a white, scurfy coating was observed in parts. Hours later, the patient developed severe edema of lung and epiglottis. After 16 days the patient was recovered with exception of a slight conjunctivitis and bronchitis.

In addition to the abovementioned lethal workplace intoxication (Section 2.1.1) described by Roßmann and Grill (1952) a nonlethal case under the same conditions, but for 8-hour exposure was reported. One hour after cessation of exposure signs of poisoning comprised the eyes (conjunctivitis, keratitis) and the respiratory tract (cough, bronchospasm, dyspnea). Except a long lasting dry cough and irritated conjunctiva the patient was fully recovered after 8 days. Following Habers law the authors assumed a concentration of 7 mg/m^3 (1.35 ppm) to induce severe symptoms after 3 hours based on studies in monkeys.

Nebelung (1957) describes the case of a 60-year old worker in the pharmaceutical industry who was exposed to unknown concentrations of vaporized dimethyl sulfate, which leaked from a container overnight (about 10 liter). 6 hours after exposure first discomfort occurred including burning of the eyes and difficulties in breathing and swallowing. About 7 hours after exposure a glottis edema was diagnosed, that worsened fast. Additionally he developed a pulmonary edema that was described as severe 13 hours after exposure The next days he developed pneumonia, a putrid cough and conjunctivitis. During the following year he suffered from a recurrent bronchitis.

Thiess and Goldmann (1967) reported several cases of intoxication due to accidental inhalation of dimethyl sulfate vapors at workplace. 2 of 5 patients suffered from eye troubles (irritation, burning pain, lacrimation) and respiratory troubles (rhinitis, burning pain, feeling of dryness, coughing reflex). One patient recovered within 2 days, the other still revealed dyspnea almost 9 month later. One further patient

felt a slight dyspnea 1 hour after exposure of “one breath” dimethyl sulfate, but developed no health injury. Another patient only developed irritation and redness of eyes and pharynx that lasted for 30 minutes. The 5th case describes the inhalation of a 20 %-solution of dimethyl sulfate with chloroform in an organic base that leads to no subjective troubles at all. At clinical examination slight pathological respiratory sounds were diagnosed. For all these cases it can be assumed from disease process, that exposure concentration has been low, however measurement of concentrations has not been conducted.

Savic (1971) as well as Barral-Chamaillard and Roux (1979) summarize several cases of eye lesion after mistakenly inhalation of low dimethyl sulfate concentrations at workplace, as can be assumed from the long latency period of several hours and the rapid recovery. All toxic effects observed by Savic were restricted to the eyes and comprise slight to severe hyperemia of conjunctiva and lesions of cornea. The observed lesions were reversible after 8 days at the latest. Beyond these effects Barral-Chamaillard and Roux reported irritation of upper respiration tract (pharyngonasal part).

Roux et al. (1977) summarize cases of dimethyl sulfate poisoning in France. The exposures occurred during work and affected 4 men, aged 33 to 58. In three cases the protection standards were ignored. All of the patients showed rhinorrhea, lacrimation, conjunctival inflammation, visual blur and dry cough as first symptoms (phase 1 of poisoning, as described by the authors). The latency of onset of intoxication signs was 3 to 4 hours in 3 cases, and 30 minutes in 1 case. The shorter the latency, the more serious were the toxic effects. The phase 2 of poisoning includes mainly the eyes, the larynx, the pharynx, and the lungs and was due to the corrosive effects of dimethyl sulfate.

Wang et al. (1988) reported 62 cases of inhalation dimethyl sulfate intoxication in China. The duration of exposure ranged from 1 min to 8 hours. The authors describe the same disease process similarly as Roux et al. (1977). The initial symptoms appeared 20 minutes to 12 hours after exposure. Following a moderate intoxication necrosis and desquamation of respiratory mucosa as well as pneumonia and myocardial injuries were observed. The authors categorize it as a severe intoxication when additionally laryngeal and pulmonary edema, toxic shock and encephalopathy occurred. Some cases retained an impairment of vital capacity up to 12 years. No concentrations were reported, but the authors assume, that the exposure to 500 mg/m^3 (97 ppm) for 10 minutes causes lethal injuries. Exposure up to 5 mg/m^3 (about 1 ppm) is assumed by the authors to cause irritative symptoms of the eyes, as concluded from literature data and own investigations. However, these investigations are not described in the publication. In case of the described intoxications exposure concentrations of dimethyl sulfate was estimated to be in excess of 5 mg/m^3. The authors reported, that patients with peribronchitis, pneumonia, and pulmonary edema were exposed for a longer time and to higher concentrations. Clinical chemistry examinations revealed leucocytosis that increased with aggravation of the clinical conditions.

Two cases of dimethyl sulfate intoxication during occupational exposure of a 30 and a 23 years old man were reported by Ip et al. (1989). A few hours after inhalation contact, presumably due to vaporized dimethyl sulfate, soreness of throat and eyes as well as cough were the first perceived symptoms in both cases. The work was stopped after 6 hours. The following disease process was only slight in case 2, but case 1 developed severe lung injuries (hypoxemia, bilateral parenchymal infiltrates, pulmonary edema). Daily cough and sputum as well as anosmia remained for 4 month.

Testud et al. (1999) describe one case of dimethyl sulfate poisoning at workplace following accidental spilling from a tank. The duration of exposure to vapors did not exceeded 5 minutes. Itching of eyes, nose and throat were the first symptoms noticed by the patient, followed by eye pain, and rhinorrhea. At hospital fever, conjunctivitis, photophobia, tachycardia, and bronchopneumonitis were diagnosed. Clinical chemistry revealed a leucocytosis with 83 % polymorphonuclear leucocytes, respectively, as a result of an inflammation process.

Goldblatt (1955) lists concentrations of dimethyl sulfate, that produce effects in humans. 15 ppm (78 mg/m^3) cause severe toxic effects in persons exposed for 1 minute. 10 ppm (52 mg/m^3) for more than a short time may lead to symptoms of illness. 5 ppm (26 mg/m^3) is listed as upper limit of concentration to satisfactory conditions in general atmosphere of plants. Patty (1962) evaluates 13 ppm of dimethyl sulfate in atmosphere to cause "effects" in human exposed for 20 minutes. These statements are without sufficient background data and no literature source is reported.

Nonlethal toxicity after dermal exposure

Mohlau (1920) reported 2 cases of workers who were exposed together to both the vapor and the liquid dimethyl sulfate. Shortly after exposure the men noticed slight irritation of their throats and eyes. Several hours later the irritations aggravated and additionally inflammation of the bronchi developed. At hospital severe congestion and edema of the throat and the lung, bronchitis, chemical pneumonia and painful eye injuries (inflammation, scars on the cornea), together with photophobia and dramatically reduced field of vision were diagnosed. All the mucous membrane of respiratory tract had suffered a very decided corrosion. The temperature was moderately elevated and their pulse rates were fairly rapid. Urinary examinations revealed an increase in phosphates and sulfates, with a little trace of albumin, and hyaline casts.

Two cases of accidental dimethyl sulfate intoxication at workplace were reported by Littler and McConnell (1955). One case followed dermal exposure by breaking a 2 l-bottle in a fume cupboard. The liquid was poured over the hands and trousers, where it soaked to genitalia and left thigh. Until two hours later the man showed no symptoms, however beginning three hours after exposure the genitalia were swollen and pink, and he had troubles with his eyes (bloodshot, painful, blurred vision, gross lid edema, extensive excoriation of the corneal epithelium; listed in chronological order). Later on he developed difficulties in breathing, retrosternal pain, slight fever, running nose, and hoarseness. His uvula was swollen and there was a marked bronchospasm. Large blisters on exposed skin parts were noticed 13 hours after the accident. Clinical chemistry revealed a leucocytosis, a slightly elevated erythrocyte count, and a moderate amount of albumin and erythrocytes in urine. About 3 weeks after the accident the man was completely recovered except for the granulating areas on the genitalia.

For the other case a specific intoxication pathway could not be figured out. Regarding the symptoms a dermal exposure can be assumed, too. As first signs of intoxication the man lost the sense of smell and sight. Twelve hours after first symptoms he suffered from edema of eyelids, bulbus, soft palate and uvula, additionally excoriation of corneae, bronchospasm and fever were diagnosed. About 2 weeks after onset of symptoms he was recovered, but still complained of photophobia.

Bartalini et al. (1957) describe 3 cases of dimethyl sulfate poisoning after exposure of splashes on the face and in the eyes. The authors reported skin edema, blisters, conjunctival irritations and alterations of the visual organ (enlargement of the blind spot, permanent limitation of the visual field). The lesions appeared after a latency period of about 12 hours.

Thiess and Goldmann (1967) reported cases of dimethyl sulfate intoxication due to accidental dermal exposure ("a few splashes") at workplace. For all 3 cases redness and swelling of the concerning parts of the skin were reported, as well as corrosive alterations that developed several hours after exposure (between 3 and more than 12 hours). The patients revealed no signs of disease that could have been resulted from an additional inhalation of dimethyl sulfate.

One case of poisoning of face and upper part of the body at the workplace was described by Testud et al. (1999). Immediately after eye contact the patient noticed intensive pain. Later on he developed a severe

rhinorrhea, pain in pharynx, and difficulties in breathing, that got worse rapidly. At hospital burns of 2nd degree were observed at exposed skin parts. Clinical chemistry revealed a leucocytosis with 85 % polymorphonuclear leucocytes. The patient developed edema of uvula and lung, congestion of vocal ligaments, and extensive ulceration of cornea.

Nonlethal toxicity after oral exposure

A nonlethal intoxication after accidentally ingestion of a "few drops at the most" (description by the patient) of dimethyl sulfate was reported by Bodenstein (1921). Immediately after exposure the patient, a 47-year old woman, suffered from a burning pain and enhanced salivation. The following hours, she vomited several times and reddening and edema of the upper respiratory tract were diagnosed at the clinical examination about 12 hours after ingestion. Parts of the respiratory tract revealed a scurfy mucosa that increased the following two days and healed up afterwards. One month later, the patient was cured nearly completely.

2.2.2. Epidemiologic Studies

Clinico-hygienic, immunological and cytogenetic investigations on 23 workers with long-term mixed inhalation and dermal exposure to dimethyl sulfate and 19 unexposed control persons were conducted by Molodkina et al. (1985). The concentration at workplace is indicated of about 19 ppm (100 mg/m^3), where the workers spend between 5 and 30 % of the daily working hours using breathing apparatus. No information was delivered if workplace measurements are based on area sampling or personal monitoring. Measurements of the skin revealed concentrations of 2.09 ± 0.07 mg/dm^2. Half of the workers revealed alterations of upper respiratory tract mucosa with chronic inflammation of pharynx. Clinico-chemical investigations suggest an injury of hepatocyte membrane (increase of transaminase activity, alteration of bilirubin composition, glucuronic acid conjugates). Immunological examinations revealed alterations in the rosette test (decreased adhesion of lymphocytes to sheep erythrocyte; increased auto-rosette formation). Cytogenetic investigations resulted in elevation of altered cells (single-strand fragments and double-strands) in all except 2 workers.

Zhao (1989) investigated workers exposed to vapors of dimethyl sulfate for a longer time in a Petroleum Chemistry Factory in LioNing, China, and unexposed volunteers (abstract publication). The study participants were checked for ophthalmology including history, functional examination and physical examination of the eyes. Many involved workers suffer from eye pains, lacrimation and related troubles. Statistically ($p < 0.05$ and $p < 0.01$) more exposed workers showed blurred vision and conjunctival congestion than the volunteers in the unexposed control group. The workplace concentration of dimethyl sulfate is indicated by the author as 2.89 - 0.07 mg/m^3 (0.56 - 0.014 ppm).

2.3. Carcinogenicity

Druckrey et al. (1966) reported one case of a worker exposed to dimethyl sulfate for 11 years, who developed an oat-cell bronchial carcinoma (upper trachea to bronchi) with metastasis. From the location of carcinoma the authors assume dimethyl sulfate to be responsible. 3 out of 6 to 10 co-workers, that were exposed to dimethyl sulfate and other substances died of bronchial carcinoma. No other details are provided.

Pell (1972) conducted an epidemiological study in dimethyl sulfate exposed workers revealing a tumor incidence (lung cancer) of 4 out of 386 and 257 out of 43 000, respectively. The study shows no excess

incidence of cancer of respiratory tract among the examined workers. All data are of limited quality and no information on other clinical signs or controls is given.

2.4. Summary

Due to the use of dimethyl sulfate as a methylating agent in many different industrial processes several cases of lethal intoxication are reported in literature, mainly after accidental exposure or exposure due to carelessness at workplace. For most of these cases intoxication occurred via inhalation, one lethal intoxication each occurred after dermal exposure and after oral exposure, respectively.

A major cause of lethality after inhalation exposure to dimethyl sulfate results from respiratory failure due to direct dimethyl sulfate effect (lesions of mucosa, demucosation, infectious diseases, e.g. chemical induced bronchitis and pneumonia) (Weber 1902; Roßmann and Grill 1952; Thiess and Goldmann 1967). Beside glottis and pulmonary edema, alterations of other organs are reported, e.g. congestion and petechiae of organs, swollen liver, or brain edema. Additionally, coating of parts of respiratory tract was reported by several authors (Strothmann 1929; Roßmann and Grill 1952; Thiess and Goldmann 1967). There exist no sharp line between lethal and nonlethal intoxication, therefore some or all of these symptoms are also described in case studies without exposure to a deadly dose. Based on data from animal experiments, Roßmann and Grill (1952) assume a lethal intoxication at 5.4 ppm for one exposed worker for a 3-hour duration. An estimated lethal intoxication after 10 minutes exposure to 97 ppm was reported by Wang et al. (1988). If intoxication is not lethal, recovery is usually complete, independently of pathway of intoxication, but slight to moderate symptoms can persist for month or years (Ip et al. 1989; Bodenstein 1921; Nebelung 1957; Strothmann 1929; Wang et al. 1988).

Exposure to dimethyl sulfate via inhalation and dermal pathway leads to very similar effects, as, for example, demonstrated by Testud et al. (1999). It can be assumed, that effects following dermal exposure are due to the vaporized dimethyl sulfate from skin to a large part. Following inhalation and dermal contact to dimethyl sulfate problems with the eyes, e.g. swollen eyelids, conjunctivitis, photophobia, were reported by all authors. According to Barral-Chamaillard and Roux (1979) as well as Savic (1971) irritation of eyes and upper respiration tract are the only signs of a slight intoxication. This agrees with the investigations, Zhao (1989) conducted on exposed workers and unexposed volunteers, where effects were restricted to disturbances of vision. As observed by Savic and Zhao the eyes seem to be a highly sensitive organ, presumably due to the reaction of dimethyl sulfate with lacrimal fluid. After exposure to higher concentrations, glottis edema was observed after inhalation (Strothmann 1929; Nebelung 1957; Roßmann and Grill 1952; Thiess and Goldmann 1967) as well as after oral exposure (von Nida 1947). The typical symptoms of a glottis edema are stridor at inhalation, hoarseness, pain in swallowing, and fever. All or some of these symptoms were also described by several other authors (Littler and McConnell 1955; Roux et al. 1977; Wang et al. 1988; Ip 1989). Chemical induced glottis edema seems to be a typical effect of dimethyl sulfate. Beside glottis, other parts of the respiratory tract can develop inflammations as well as edema (lung, uvula, larynx).

Wang et al. (1988) categorized the degree of dimethyl sulfate intoxication criteria for grading, what corresponds in general to the observations reported by other authors (see Table 2). Additional, some authors describe further effects on eyes as signs of a slight to moderate intoxication (lacrimation, burning, itching, lid and conjunctival edema) (Strothmann 1929; Roßmann and Grill 1952; Nebelung 1957; Thiess and Goldmann 1967; Savic 1971; Roux et al. 1977).

Ingestion of dimethyl sulfate leads to vomititing, nausea, burning pain, and enhanced salivation immediately or soon after exposure (Bodenstein 1921; von Nida 1947; Bartalini et al. 1957). The life-endangering factors after oral exposure result from troubles with respiratory and gastrointestinal tract, and

disease process can be different from inhalation and dermal contact (Bodenstein 1921; von Nida 1947; Bartalini 1957). However, lethal glottis edema, dyspnea and demucosation were also observed after oral exposure (von Nida 1947).

TABLE 2. Criteria for Grading the Degree of Dimethyl Sulfate Intoxication by Wang et al. 1988

Grading	Symptoms
Irritative reactions	mucosal irritation in eyes, nose, and pharynx; no leucocytosis; no systemic signs of intoxication
Mild intoxication	additionally irritative and erosive actions on the respiratory tract; congestion of pharynx, larynx, uvula, abnormal breath sounds; peribronchitis and / or leucocytosis
Moderate intoxication	additionally necrosis and desquamation of respiratory mucosa; pneumonia or interstitial pneumonia; leucocytosis; myocardial damage
Severe intoxication	additionally laryngeal edema; pulmonary edema; and/or toxic shock; and/or toxic encephalopathy; and/or toxic myocardial damage

Human data concerning the carcinogenic potential of dimethyl sulfate reported by Druckrey et al. (1966) are of limited quality, however give indications of carcinogenic effects.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Non-human Primates

Lethality after inhalation exposure

Flury and Zernik (1931) conducted studies in monkey(s) (number of animals not given) that were inhalatively exposed to 25.5 ppm (132 mg/m^3) for 40 minutes. The animal(s) died 3 days after inhalation. Following Habers law Roßmann and Grill (1952) calculated a (C^1)(t) product of about 5200 (mg/m^3 x minutes) for lethality. However, they did not show the validity to use Habers law with n = 1. No further details are reported.

3.1.2. Dogs

Lethality after inhalation exposure

Weber (1902) exposed one dog to unknown concentrations ("dimethyl sulfate mixed with very much air", according to the author) in an approx. 100 liter glass cover for several minutes. Dimethyl sulfate vapors were produced by heating in a flask connected to the glass cover. 6 minutes exposure led to lethal effects within 24 hours due to pulmonary edema after the dog suffered from cough with vomiting of mucoid pulp for several hours. Necropsy revealed additional inflammations of upper parts of the respiratory tract.

3.1.3. Cats

Lethality after inhalation exposure

Whole-body inhalation studies with exposure to 3 different concentrations (19, 78, and 174 ppm) for 11 minutes were conducted by Flury and Zernik (1931) in cats (number of animals not reported). Determination of dimethyl sulfate concentration was conducted with alkali and subsequent measurement of the precipitated barium sulfate. At all concentrations lethality due to respiratory failure was observed after a few days up to 1 1/2 weeks following exposure, depending on concentration. Presumably all animals died at 78 and 174 ppm, respectively. The lowest concentration was not lethal for all animals, however no mortality rate was reported for any concentration. Flury describe the lethality observed at 19 ppm as "animals died at certain circumstances". No details are reported.

3.1.4. Rats

Lethality after inhalation exposure

Hein (1969) investigated the acute toxicity of dimethyl sulfate after whole-body inhalation exposure of one hour to various concentrations in rats, mice, guinea pigs and hamsters (see following Sections) and determined the average period of survival. The animals were exposed in a steel-glass chamber of about 224 l volume (whole body). Exposure concentrations were given as analytical concentrations, measured by gas chromatography. A period of more than 3 weeks survival was determined as "no mortality". Groups of 5 female Wistar rats each (100 - 300 g body weight) were exposed to 10, 49, 64, 71, and 127 ppm. From 49 ppm onwards lacrimation, and dyspnea with inspiratory stridor were observed. After exposure to 127

ppm all animals developed a severe conjunctivitis and died about 26 hours after exposure. The stomach and small intestine were severe inflated even at 49 ppm. At necropsy surviving animals revealed emphysema and edema of lungs. The period of survival was more than 3 weeks for animals of the 10 and 49 ppm group. The animals survived 97 hours on average after exposure to 64 ppm, 84 hours at 71 ppm, and 26 hours at 127 ppm. The LC_{50} for 1 hour was calculated as 64 ppm (57 - 71.6 ppm confidential interval) using the Litchfield and Wilcoxon-method (Litchfield and Wilcoxon 1949). Schlögel (1972) concludes that 34 ppm were a sublethal concentration (LC_0). Further results are provided in Table 3. Benchmark recalculation largely confirmed the LC_0 and the LC_{50} with a $BMCL_{05}$ (lower confidence limit of benchmark concentration for 5 % lethality) of 32 ppm and a LC_{50} of 65 ppm.

Smyth et al. (1951) reported a maximum time for no death after inhalation of dimethyl sulfate in saturated vapor of 2 minutes in albino rats. A 4-hour inhalation to 30 ppm (nominal concentration) caused lethality in 5 of 6 animals after 14 days. No lethality was observed after a 4-hour exposure to 15 ppm (number of animals not given). No further details concerning exposure scenario and observed effects were reported.

Ghiringhelli et al. (1957) exposed 16 rats to 75 ppm dimethyl sulfate (390 mg/m^3) in an inhalation chamber and measured the time to death of 50 % of the animals (LT_{50}). Determination of dimethyl sulfate concentration was conducted with sodium hydroxide and subsequent measurement of the precipitated barium salt. The exposition chamber contained 8 l and was charged with a dimethyl sulfate-air mixture of controlled concentration at 25 °C. After exposure for 26.1 minutes (calculated) 50 % of the rats died a few days later. All animals developed irritation of conjunctiva and of the respiratory tract as well as signs of impairment of the nervous system. The lesions observed at necropsy covered congestion of kidneys, spleen, liver and lungs. On histopathological examination, additional injuries were observed within the lungs (emphysema, peribronchitis) and the liver (cloudy swelling, fatty degeneration and necrosis).

Batsura et al. (1980) exposed rats to 45 mg/m^3 (8.65 ppm) dimethyl sulfate for 4 hours. In secondary literature this concentration is cited as lethal ("LC_{50}"). However, in this study the animals were sacrificed and no lethality concentration was determined.

BASF (1968) conducted an IRT (inhalation-risk test) in dimethyl sulfate saturated atmosphere (5 cm dimethyl sulfate layer, air flow through) at 20 °C (according to the rapporteur 592 ppm = 3100 mg/m^3) and reported 100 per cent mortality after exposure of 30 minutes (12 of 12 animals) and one hour (6 of 6 animals). All animals (6 and 12, respectively) survived 3 and 10 minutes, respectively, in saturated atmosphere, however period of survival is unknown due to subsequent sacrifice. In the beginning of exposure, attempts to escape were observed. The clinical symptoms comprised irritations of mucosa and labored breathing. At necropsy, pulmonary edema was observed occasionally. No strain and no data on exposure measurement were provided.

DuPont (1971) conducted an inhalation study on young adult male CHR-CD rats (initial body weight 250 - 285 g). 6 rats were whole-body exposed for one hour in a chamber delivered with dimethyl sulfate vapors by a metered stream of air passing through a stainless steel T-tube. The animals were exposed to analytical concentrations of 58 ppm, 90 ppm (2 groups), 105 ppm, and 120 ppm. The surviving animals were held 14 days after exposure for observation. At exposure difficulties in breathing, face washing, and gasping were observed at all groups, additionally lacrimation and face pawing were observed from 90 ppm onwards, and gasping aggravated. No mortality was observed at 58 ppm, one animal each died at 90 ppm (second testing) and 105 ppm, 2 animals died at 90 ppm at first testing, and a LC_{100} was reported for 120 ppm. From these results a LC_{50} of 100 ppm was calculated based on the method by Litchfield and Wilcoxon (Litchfield and Wilcoxon 1949). Animals of all groups showed nasal discharge and persisting

corneal opacity. Death occurred within 2 days. Histology suggested acidic type of attack to lungs and eyes at lower concentration.

Lethality after exposure to other routes

Druckrey et al. (1966) determined LD_{50}-values for oral administration (gavage) of 440 mg/kg, for subcutaneous application (oily solution) of 100 mg/kg and for intravenous injection (aqueous solution) of 40 mg/kg.

A twice lower oral LD_{50} of 205 mg/kg was reported by Molodkina et al. (1979). A LD_{50} of 107 mg/kg was reported by BASF (1968) following oral exposure to a 0.5 - 1 % solution of water with traganth after 7 days observation.

3.1.5. Mice

Lethality after inhalation exposure

Hein (1969) exposed female NMRI mice (17 - 24 g body-weight) to 10 ppm, 42 ppm and 49 ppm (20 animals each) as well as to 64 ppm, 71 ppm, and 127 ppm (10 animals each) (whole-body). For detailed study design see Section 3.1.4. At 10 ppm the first symptoms were observed after 30 minutes and comprise retardation of movement, cleaning reflexes, and closed eyes. Additionally thoracic respiration was observed in some animals, which is possibly due to difficulties of breathing. The higher the concentrations, the earlier these symptoms set on. 2 hours after exposure period most of the animals revealed a slight stridor at inhalation. At the highest concentration of 127 ppm animals already were dyspneic during exposure, which got worse up to 2 hours after exposure and came along with a slight irritation of conjunctiva. 8 out of 10 animals died at this concentration. The surviving animals recovered from these symptoms within 24 hours. The period of survival was more than 3 weeks for all animals of the 49 ppm group. 1 animal each of the 10 ppm and the 42 ppm group died after 16, and 96 hours, respectively. The animals of the 64 ppm, 71 ppm, and 127 ppm group survived on average 192, 108, and 103 hours, respectively. At necropsy pulmonary emphysema, dilatation of capillaries and of trachea were diagnosed beginning at 42 ppm. Within the 10 ppm group no pathological findings were observed except lung dissection revealing a hemorrhagic spot in one animal. It is not mentioned if this finding was in the one deceased animal at this concentration. At the highest concentration of 127 ppm, stomach and small intestine were inflated. A LC_{50} (1 h) of 98 ppm (76.6 - 120 ppm confidential interval) was calculated using the Litchfield and Wilcoxon-method (Litchfield and Wilcoxon 1949). Schlögel (1972) concludes that 48 ppm were a sublethal concentration (LC_0). Further results are provided in Table 3. Benchmark recalculation largely confirmed the LC_0 and the LC_{50} with a $BMCL_{05}$ (lower confidence limit of benchmark concentration for 5 % lethality) of 44 ppm and a LC_{50} of 96 ppm, however no clear dose-response relationship has been observed at low doses.

Ghiringhelli et al. (1957) exposed 40 mice to 75 ppm dimethyl sulfate (390 mg/m^3) in an inhalation chamber and measured the time to death of 50 % of the animals (LT_{50}) (for study details see Section 3.1.4.). After exposure of 17.6 minutes (calculated) 50 % of the mice died. All animals developed irritation of conjunctiva and of the respiratory tract as well as signs of impairment of the nervous system. The lesions observed at necropsy covered congestion of kidneys, spleen, liver and lungs. On histopathological examination, additional injuries were observed within the lungs (emphysema, peribronchitis) and the liver (cloudy swelling, fatty degeneration and necrosis).

Lethality after oral exposure

Schmezer and Schmähl (1987) reported a LD_{50} of 140 mg/kg after oral exposure in mice.

3.1.6. Hamsters

Lethality after inhalation exposure

Hein (1969) exposed 5 female golden hamsters each (25 - 50 g body weight) to 33 ppm, 40 ppm, 49 ppm, 64 ppm, 71 ppm, and 127 ppm (whole-body). For detailed study design see Section 3.1.4. 33 ppm and 40 ppm caused only slight effects similar to that observed in mice, and 1 animal each died at these concentrations. At 64 ppm, 71 ppm, and 127 ppm behavioral changes were observed. The animals showed a staggering gait and affected righting and postural reflexes. The author does not preclude, that these effects are due to nervous injury. 1 animal each of the 33 ppm and the 40 ppm group died after 72.5, and 80 hours, respectively. The animals of the 49 ppm, 64 ppm, 71 ppm and 127 ppm group survived on average 123, 204, 136 and 71 hours, respectively. No pathological abnormities were observed up to 40 ppm. At higher concentrations pulmonary emphysema and inflation of gastrointestinal tract were observed and 1 animal of the 49 ppm-group revealed an enlarged liver. A LC_{50} (1 h) of 56 ppm (37.4 - 84 ppm confidential interval) was calculated using the Litchfield and Wilcoxon-method (Litchfield and Wilcoxon 1949). Further results are provided in Table 3. Benchmark recalculation largely confirmed the LC_0 and the LC_{50} with a $BMCL_{01}$ (lower confidence limit of benchmark concentration for 1 % lethality) of 12.6 ppm and a LC_{50} of 52 ppm. This is in accordance to relevant lethality already after first or second exposure in the chronic study with "sublethal" concentration in this species (Schlögel, 1972). Hence, 10 ppm are used as lethality threshold in the study conducted by Schlögel.

3.1.7. Guinea Pigs

Lethality after inhalation exposure

Hein (1969) exposed 5 guinea pigs each (250 - 350 g body weight; sex not indicated) to 10 ppm, 33 ppm, 40 ppm, and 71 ppm. For detailed study design see Section 3.1.4. The most sensitive endpoint in guinea pigs seems to be the eye. No lethality was observed at 10 ppm. At 33 ppm all animals were presumably blind and died one week after exposure. The period of survival was more than 3 weeks for all animals of the 10 group. The animals survived 33.5 hours on average after exposure to 33 ppm, 45 hours at 40 ppm, and 19 hours at 71 ppm. Red infiltrations, partly hemorrhagic, were observed in lung tissues, however emphysema were seen at 71 ppm only. Among all species (see previous Sections) examined by Hein, guinea pigs developed the most severe inflation of gastrointestinal tract. A LC_{50} (1 h) of 32 ppm (23.4 - 43.8 ppm confidential interval) was calculated using the Litchfield and Wilcoxon-method (Litchfield and Wilcoxon 1949). Further results are provided in Table 3. Benchmark recalculation largely confirmed the LC_0 and the LC_{50} with a $BMCL_{05}$ (lower confidence limit of benchmark concentration for 5 % lethality) of 5.8 ppm and a LC_{50} of 28 ppm.. 10 ppm could be taken as LC_0 if the incidence in the experiment is used. However, if confidence limits are included the marginally lower $BMCL_{05}$ of 5.8 ppm is derived.

Flury and Zernik (1931) reported a lethal concentration of 2073 ppm (10.7 g/m^3) for guinea pigs after unreported duration. No further details are described.

DuPont (1943) conducted an inhalation study on one guinea pig exposed to undiluted dimethyl sulfate mist in a bell jar of 7 l capacity for one hour. Within the first minute of exposure severe tearing and signs

of irritation to the nose were observed and aggravated after cessation of exposure, accompanied by respiratory embarrassment (coughing, gasping, cyanosis). At necropsy and histopathology lung appear pale, inflated and albuminous material was noted in the large bronchi, that occasionally plugged their lumen, as well as desquamation of bronchial epithelium. Portions of the lung were emphysematous.

Ghiringhelli et al. (1957) exposed 16 guinea pigs to 75 ppm dimethyl sulfate (390 mg/m^3) in an inhalation chamber and measured the time to death of 50 % of the animals (LT_{50}) (for study details see 3.1.4.). After exposure of 23.9 minutes (calculated) 50 % of the guinea pigs died. All animals developed irritation of conjunctiva and of the respiratory tract as well as signs of impairment of the nervous system. The lesions observed at necropsy covered congestion of kidneys, spleen, liver and lungs. On histopathological examination, additional injuries were observed within the lungs (emphysema, peribronchitis) and the liver (cloudy swelling, fatty degeneration and necrosis).

3.1.8. Rabbits

Lethality after inhalation exposure

Mohlau (1920) investigated a rabbit that was poisoned with dimethyl sulfate through a saturated cotton swab, placed together under a bell jar. The animal died shortly afterward. The autopsy and histopathology revealed intensive congestion and parenchymatous degeneration of all organs, most marked changes being in the liver. Smears of blood showed a normal count, however a relative lymphocytosis was present. The author assume, that the degenerative effects on tissues is due to distribution of dimethyl sulfate by the blood stream to the various organs.

Lethality after exposure to other routes

Weber (1902) conducted several studies on application of dimethyl sulfate via different pathways (dermal, oral, subcutaneous) in rabbits. One rabbit was dermally exposed to 5 ml dimethyl sulfate rubbed on the shaved back. The animal developed a dyspnea and irritation of conjunctiva after 3 hours and died after 22 hours. Necropsy showed inflammation and submucosal hemorrhage of larynx, trachea, and pharynx. Oral application of dimethyl sulfate via gavage to several rabbits revealed indigestion and local lesions of stomach at both dosages (single application of 50 mg/kg and of 260 mg/kg). The high-dose animal stayed comatose until death occurred after 2 hours. At necropsy hyperemia and hemorrhage of stomach and hemorrhage of pia mater were observed. Convulsions, additionally alteration in respiration as systemic effect of dimethyl sulfate were also reported after subcutaneous application of 53 mg/kg and 290 mg/kg within a few minutes. Within both dose groups death occurred after 2 hours and 45 minutes respectively.

Several experiments on intravenous application of dimethyl sulfate were conducted in one rabbit each (Wachtel 1920). 5 ml of a 4 % dimethyl sulfate solution with a sodium chloride solution (0.2 ml dimethyl sulfate at final volume) led to a decreased respiratory rate and breathing irregularities immediately after injection. 11 minutes later death occurred due to respiratory arrest. At lower dose of 0.02 ml dimethyl sulfate at a final concentration no alterations of pulse and respiratory rate were observed and death was due to cachexia following continuous weight reduction.

TABLE 3. Summary of Acute Lethal Inhalation Data in Laboratory Animals					
Species	**Conc. (ppm)**	**Exposure**	**Result**	**Number of animals Most important effects**	**Reference**
monkey	12.8	20 min	LC_0	number of animals not given severe disease process	Flury and Zernik (1931)
monkey	25.5	40 min	LC	number of animals not given death after 3 days	Flury and Zernik (1931)
dog	unknown	6 min	LC	1 animal death after 24 h of pulmonary edema	Weber (1902)
cat	19	11 min	LC	number of animals not given respiratory failure; not all animals died	Flury and Zernik (1931)
cat	78	11 min	LC	number of animals not given respiratory failure; presumably all animals died	Flury and Zernik (1931)
rat	saturated	10 min	LC_0	no strain and no data on exposure measurement provided	BASF (1968)
rat	saturated	30 min	LC_{100}	6 animals pulmonary edema	BASF (1968)
rat	75	26.1 min	LT_{50}	16 animals congestion of organs	Ghiringhelli et al. (1957)
rat	58	1 h	LC_0	6 animals nasal discharge; persisting corneal opacity	DuPont (1971)
rat	100	1 h	LC_{50}	calculated by Litchfield and Wilcoxon-method	DuPont (1971)
rat	12010590 9058.00	1 h	LC	6/6 animals 1/6 1/6 2/6 0/6 respiratory failure analytical concentration	DuPont (1971)
rat	12771644 910.00	1 h	LC	5/5 animals 3/5 3/5 0/5 0/5 pulmonary congestion, hemorrhage	Hein (1969)

Species	Conc. (ppm)	Exposure	Result	Number of animals Most important effects	Reference
rat	64	1 h	LC_{50}	analytical concentration calculated by Litchfield and Wilcoxon-method	Hein (1969)
rat	32	1 h	$BMCL_{05}$	calculated, based on data from Hein (1969)	This TSD (see Section 3.1.4)
rat	15	4 h	LC_0	number of animals not given no further details available	Smyth (1956)
rat	30	4 h	LC	5 of 6 animals died no further details available	Smyth (1951)
rat	32	4 h	LC_{50}	number of animals not given no further information available	Kennedy and Graepel (1991)
mouse	75	17.6 min	LT_{50}	40 animals congestion of organs	Ghiringhelli et al. (1957)
mouse	98	1 h	LC_{50}	calculated by Litchfield and Wilcoxon-method	Hein (1969)
mouse	12771644 94210.00	1 h	LC	8/10 animals 2/10 2/10 0/20 1/20 1/20 pulmonary emphysema analytical concentration	Hein (1969)
mouse	44	1 h	$BMCL_{05}$	calculated, based on data from Hein (1969)	This TSD (see Section 3.1.5)
mouse	54	4 h	LC_{50}	number of animals not given no further data available	Molodkina et al. (1986)
hamster	56	1 h	LC_{50}	calculated by Litchfield and Wilcoxon-method	Hein (1969)
hamster	1,277,164 ,494,033. 00	1 h	LC	5/5 animals 5/5 3/5 2/5 1/5 1/5 pulmonary emphysema analytical concentration	Hein (1969)
hamster	12.6	1 h	$BMCL_{01}$	calculated, based on data from Hein (1969)	This TSD (see Section 3.1.6)

Species	Conc. (ppm)	Exposure	Result	Number of animals Most important effects	Reference
guinea pig	75	23.9 min	LT_{50}	16 animals congestion of organs	Ghiringhelli et al. (1957)
guinea pig	32	1 h	LC_{50}	calculated by Litchfield and Wilcoxon-method	Hein (1969)
guinea pig	71403310	1 h	LC	5/5 animals 3/5 4/5 0/5 pulmonary emphysema analytical concentration	Hein (1969)
guinea pig	5.8	1 h	$BMCL_{05}$	calculated, based on data from Hein (1969)	This TSD (see Section 3.1.7)
guinea pig	mist	1 h	LC	1 animal pale, inflated lung; bronchi plugged by albuminous material	DuPont (1943)
guinea pig	2073	unknown	LC	number of animals not given lethal intoxication	Flury and Zernik (1931)
rabbit	unknown	10 and 12 min	LC	1 animal each hemorrhage of larynx and brain	Weber (1902)
rabbit	unknown	unknown	LC	1 animal congestion and parenchymatous degeneration of organs	Mohlau (1920)

3.2. Nonlethal Toxicity

3.2.1. Nonhuman Primates

Nonlethal toxicity after inhalation exposure

Flury and Zernik (1931) conducted studies in monkey(s) (number of animals not given) that were inhalatively exposed to 12.8 ppm (67 mg/m^3) for 20 minutes. The animal(s) developed "extreme illness" after inhalation with a recovery after 4 weeks. Following Habers law Roßmann and Grill (1952) calculated a $(C^1)(t)$ product of about 1320 (mg/m^3 x minutes) for severe effects observed in this study. However, they did not show the validity to use Habers law with n = 1. No further details are reported.

3.2.2. Cats

Nonlethal toxicity after inhalation exposure

Cats exposed to 19 ppm (whole-body inhalation) for 11 minutes revealed salivation and lacrimation, as well as reddening of visible mucosal membranes (Flury and Zernik 1931). After a latency period of one

to several hours severe inflammations, that were putrid in most cases, developed. The eye lids were closed and eyes and nose secreted a suppurative fluid. Soon after, breathing difficulties, respiratory sounds and cough were observed.

3.2.3. Rats

Nonlethal toxicity after inhalation exposure

Schlögel (1972) investigated the carcinogenic effect and chronic toxicity of dimethyl sulfate in Wistar rats, NMRI mice, and Syrian golden hamster, all of both sexes (for detailed information see Section 3.3). Observations were made after the first exposure period, as confirmed in personal communication (Schlögel 2003), including alterations in behavior and clinical examinations observed within some animals of all species already after 20 minutes (closed or half-closed eyes; ruffled fur) at 0.5 ppm. After exposure, the animals were apathic, eyes were half-open or closed, and breathing problems were apparent. They coughed and sneezed occasionally and breathed sometimes with a loud expiration similar to asthma bronchiale. These effects revealed a dose-relationship in severity, duration, and time of onset. At 2 ppm all effects observed at 0.5 ppm proceeded more severe and with a higher incidence, additionally conjunctivitis and sensitivity to light exposure was diagnosed. Recovery from respiratory problems occurred soon after cessation of exposure, however conjunctivitis remained for several days.

Further investigations by Schlögel were conducted with sublethal concentrations calculated from Hein (1969) (mice 48 ppm, rats 34 ppm, golden hamsters 20 ppm). 4 hours after exposure, a severe dyspnea was observed within all animals. The eyes were kept closed or half-closed. The symptoms aggravated the following 2 days, and a recovery was not observed until 7^{th} days after exposure. For animals that died early after exposure presumably a glottis edema was responsible for lethality, as was assumed by the author due to inspiration sounds and severe edema at the glottis area. No incidences of lethality were reported.

Smyth et al. (1951) reported a maximum time of 2 minutes for an inhalation exposure to saturated vapor of dimethyl sulfate not leading to death in albino rats. A 4-hour inhalation to 15 ppm did not produce lethal effects. No information on time-to-death or follow-up observations are given. Later, based on his investigations and other available data, he judged the threshold limit of 1 ppm that was valid at that time, as low enough to protect against lung injury, but presumably not to prevent from bronchial irritation (Smyth 1956).

Hein (1969) exposed female Wistar rats (100 - 300 g) to 10 ppm, and 49 ppm, respectively, and reported no lethality at these concentrations (for study description see Section 3.1.4.). All animals showed dyspnea with stridor after 45 minutes at 49 ppm, but not at 10 ppm. Flatulence of stomach and small intestine was observed at 49 ppm. In 2 animals of the 10 ppm-group the lung revealed hyperemic zones and enlarged liver at necropsy and the frequency of these observation increased with increasing concentration.

Batsura et al. (1980) exposed rats to 45 mg/m^3 (8.65 ppm) dimethyl sulfate for 4 hours. It is not explicitely stated, whether this was a nominal or analytical concentration. The animals were sacrificed immediately after exposure and at intervals thereafter. After exposure the animals were dyspneic and some of them had nasal discharge. The first section group revealed cyanosis of the mucosa, hyperemia of the lungs, and hemorrhages in the internal organs. At histopathological examination the lung presented hemorrhage and coagulated proteins in the alveoli. The subsequently sacrificed animals developed an accumulation of edematous fluid in the airspaces after a latency period of 5 - 6 hours, which progressed

worse over 24 to 48 hours (acute progressive respiratory failure). The authors observed further a thickening of the blood-brain barrier and disturbances of microcirculation.

Mathison et al. (1995) conducted an inhalation study with plethysmographic measurements to determine if dimethyl sulfate exposure resulted in changes of ventilation. Male CrlCD:BR rats in pairs were nose/head-only exposed to 0, 1, 3, 8, and 22 ppm for 20 minutes. The measurements of respirations per minute, inspiratory time, expiratory time, and tidal volume were conducted at 15-sec intervals. A transient increase in respirations per minute was observed in rats exposed to 8 ppm, but not to 1 or 3 ppm, compared with the unexposed control group. With further exposure a decreased respiratory rate of 78 % compared with control group (100 %) was observed at 22 ppm, which correlated with an increased inspiratory time. However, the animals revealed no signs of discomfort or stress in response to the dimethyl sulfate exposure at any concentration. The authors assume from these results, that the increased inspiration time is due to inspiratory resistance caused by nasal constriction.

Nonlethal toxicity after exposure to other routes

For investigations on carcinogenic potency of dimethyl sulfate in albino BD II rats Druckrey et al. (1966) determined concentrations of no acute effects in a dose finding study. 16 mg/kg (8 animals used) following a subcutaneous application caused local necrosis that healed up quickly. At 8 mg/kg (12 animals used) no necrosis was observed. Assuming a body weight of 350 g a dose of 2.8 mg (2.1 µl) dermally applied would resulted in no local effects and 5.6 mg (4.2 µl) in slight effects.

3.2.4. Guinea Pigs

Nonlethal toxicity after inhalation exposure

Hein (1969) exposed 5 guinea pigs to 10 ppm (whole-body). For detailed study design see Section 3.1.4. All animals revealed closed eyes, cleaning reflexes and intensive lacrimation and salivation after cessation of exposure. Additionally, a macerated cornea after 3 hours, that became cloudy 5 hours later, and a rhinitis with frothy secretion was diagnosed within all animals. At necropsy red infiltrations, partly hemorrhagic, were observed in lung tissues. The animals recovered 3 days after exposure.

3.2.5. Rabbits

Nonlethal toxicity after inhalation exposure

Weber (1902) conducted inhalation studies with 2 rabbits to unknown concentrations ("dimethyl sulfate mixed with very much air", according to the author) in a glass cover for several minutes. Dimethyl sulfate vapors were produced by heating in a flask connected to the glass cover. One animal each was exposed to dimethyl sulfate for 10 and 12 minutes, respectively. Compared to dogs rabbits seem to be less sensitive against vapors of dimethyl sulfate, since effects on respiratory tract, except rubbing of nose and eyes, were not observed during exposure. Rabbits showed salivation and intensified lacrimation after exposure and conjunctivitis, opacity of cornea, and dyspnea 1 1/2 hours later. After 24 hours condition was unchanged, additionally eyes were closed due to lid edema. One animal was sacrificed 3 days later, the other one after unknown time. Autopsy revealed hemorrhage of larynx, irritation and corrosion of trachea, pneumonia, tracheitis, bronchitis, and bronchiolitis. The renal cortex and the region between cortex and medulla, respectively, showed punctate hemorrhage.

Nonlethal toxicity after exposure to other routes

Dimethyl sulfate was estimated by Smyth et al. (1951) as substance with a marked eye injury potency after application of 5 µl directly into the eyes of albino rabbits. In an injury scale of 1 (least damage) to 10 (most damage) dimethyl sulfate was ranked with 8. Correspondingly, dimethyl sulfate was ranked with 6 for primary skin irritation grading on rabbits, with 10 classified as most damage.

Irritation and corrosive injury were investigated by BASF (1968) on back skin and eyes of rabbits (number of animals not reported). Skin corrosion was observed after application of undiluted dimethyl sulfate to rabbits. Application for 1 minute caused no effects at observation after 1 and 8 days. Five minutes treatment caused slight erythema after 24 hours that had disappeared after 8 days. Slight erythema after 24 hours that worsened up to 8 days was observed following treatment for 15 minutes.

Guillot et al. (1982) investigated the ocular-irritating potential of dimethyl sulfate on male albino rabbits (New Zealand strain, 6 animals each) after instilling 0,1 ml (undiluted) (= 133 mg = 25.6 ppm) into the lower lid of one eye. The authors classified dimethyl sulfate as an extreme ocular-irritant.

TABLE 4. Summary of Nonlethal Inhalation Data in Laboratory Animals

Species	Conc. (ppm)	Exposure Time	Number of animals Most important effects	Reference
monkey	12.8	20 min	number of animals not given severe disease process	Flury and Zernik (1931)
rat	saturated	3 min and 10 min	6 and 12 animals no lethality	BASF (1968)
rat	8	20 min	2 animals increased respiratory rate	Mathison et al. (1995)
rat	22	20 min	2 animals decreased respiratory rate	Mathison et al. (1995)
rat	10	1 h	5 animals hyperemic lung parts	Hein (1969)
rat	49	1 h	5 animals dyspnea, hyperemic lung parts	Hein (1969)
rat	15	4 h	6 animals survived	Smyth (1951)
rat	0.5	6 h/d 2x per wk; 15 month	10 animals per sex eye troubles; animals coughed and sneezed; seen after first exposure	Schlögel (1972) see Section 3.3.
rat	2	6 h/d 1x per 2 wk 15 month	15 animals per sex eye troubles; animals coughed and sneezed; conjunctivitis; seen after first exposure	Schlögel (1972) see Section 3.3.
rat	34	1 h/d 4 times / year	15 animals per sex same symptoms as at 2 ppm, additionally severe dyspnea	Schlögel (1972) see Section 3.3.

Species	Conc. (ppm)	Exposure Time	Number of animals Most important effects	Reference
rat	0.1	6 h/d 5 d/wk 2 wk	number of animals not given nasal epithelial cell proliferation	Frame et al. (1993) see Section 3.3.
rat	0.7	6 h/d 5 d/wk 2 wk	number of animals not given lesions of nasal and respiratory epithelium	Frame et al. (1993) see Section 3.3.
rat	0.7	6 h/d 5 d/wk 2 wk	25 animals reduced weight gain	Alvarez et al. (1997) see Section 3.3.
mouse	0.5	6 h/d 2x per wk 15 month	10 animals per sex eye troubles; animals coughed and sneezed	Schlögel (1972) see Section 3.3.
mouse	2	6 h 1x per 2 wk 15 month	15 animals per sex eye troubles; animals coughed and sneezed; conjunctivitis	Schlögel (1972) see Section 3.3.
mouse	48	1 h 4 times / year	15 animals per sex same symptoms as at 2 ppm, additionally severe dyspnea	Schlögel (1972) see Section 3.3.
hamster	0.5	6 h 2x per wk 15 month	10 animals per sex eye troubles; animals coughed and sneezed	Schlögel (1972) see Section 3.3.
hamster	2	6 h 1x per 2 wk 15 month	15 animals per sex eye troubles; animals coughed and sneezed; conjunctivitis	Schlögel (1972) see Section 3.3.
hamster	20	1 h 4 times / year	15 animals per sex same symptoms as at 2 ppm, additionally severe dyspnea	Schlögel (1972) see Section 3.3.
guinea pig	10	1 h	5 animals lacrimation, salivation, macerated cornea, hemorrhagic infiltrations	Hein (1969)

3.3. Toxicity after Repeated Exposure

Schlögel (1972) investigated the carcinogenic effect and chronic toxicity of dimethyl sulfate in Wistar rats (196.2 g body weight in average), NMRI mice (29.2 g body weight in average) and Syrian golden hamster (71.1 g body weight in average) all of both sexes. An unexposed control group was used. The steel-glass exposure chamber had a volume of 940.5 l (110 x 90 x 95 cm) and was charged with a dimethyl sulfate-air mixture with controlled concentration at several positions in different height. Quantification of the dimethyl sulfate was conducted using gas chromatography every 30 minutes. Exposure was conducted via inhalation (whole-body) for 6 hours at 0.5 ppm (10 animals of each sex, twice a week on Tuesday and Friday) and 2 ppm (15 animals of each sex, once every 14 days). The entire duration of exposure was about 15 month for all animals. The animals were observed during each exposure. The following

observations were made already after first exposure, as verified in personal communication (Schlögel 2003): At 0.5 ppm alterations in behavior and clinical examinations were observed within some animals of all species already after 20 minutes (closed or half-closed eyes; ruffled fur). It was not able to find out, how many animals have been affected at first exposure and if a worsening of effects happened during exposure. The author could not exclude some experimental influence caused by air circulation (personal communication, Schlögel 2003). After exposure, the animals were apathic, eyes were half-open or closed, and breathing problems were apparent. They coughed and sneezed occasionally and breathed sometimes with a loud expiration similar to asthma bronchiale. These effects revealed a dose-relationship with regard to severity, duration, and time of onset. Dimethyl sulfate-exposed animals of all species revealed a higher incidence of inflammation of the lungs during overall exposure duration. At 2 ppm all effects observed at 0.5 ppm proceeded more severe, additionally conjunctivitis and sensitivity to light exposure was diagnosed. Recovery from respiratory problems occurred soon after cessation of exposure, however conjunctivitis remained for several days.

Further investigations by Schlögel (1972) were conducted with sublethal concentrations calculated from Hein (1969) (mice 48 ppm, rats 34 ppm, golden hamsters 20 ppm) in an exposure chamber with 223.9 l volume (74 x 55 x 55 cm). Quantification of the dimethyl sulfate concentration was conducted every 10 minutes. 15 animals of each sex were exposed every 4th month for 1 hour and revealed similar behavioral alterations observed at 2 ppm. However, 4 hours after exposure a severe dyspnea was observed within all animals. The eyes were kept closed or half-closed. The symptoms aggravated the following 2 days, and a recovery was observed not until 7th days after exposure. The significantly reduced life span in animals exposed to the sublethal concentration indicate that these concentrations are above sublethality. 13.9 % of all animals survived exposure duration. For animals that died early after exposure presumably a glottis edema was responsible for lethality, as was assumed by the author due to inspiration sounds and severe edema at glottis area. No incidences of lethality were reported, however it was stated that lethality was high, especially in the hamster-group, therefore the study was terminated after the 4th exposure. Due to the high mortality, 16 additional hamsters of each sex had been taken into the study group already after the second exposure. From the supplied figures, it can be derived, that isolated lethality occurred already after first exposure in hamsters and rats with a latency period of several days. However, no such figures were given for the unexposed control group, therefore the statements concerning lethality are uncertain.

Frame et al. (1993, abstract publication) reported injuries due to dimethyl sulfate exposure on the respiratory tract in rats (abstract publication). The animals (number not indicated) were exposed nose-only to 0, 0.5, 3.7, and 6.3 mg/m^3 (0, 0.1, 0.7, and 1.5 ppm) for 2 weeks (6 h/day, 5 d/week, excluding the weekend). Dose-dependent lesions of respiratory and olfactory epithelium (erosion, ulceration, atrophy) were observed at the two highest concentrations. At all concentrations tested nasal epithelial cell proliferation was observed, which was measured by means of 5-bromo-2-deoxyuridine (BrdU) incorporation. Labeling indexes (rate of DNA producing cells in the S-phase) for respiratory epithelium were slightly depressed at 0.1 ppm (not significant), equal to control at 0.7 ppm, and elevated in the 1.5 ppm group. For olfactory epithelium, labeling indexes were dose-dependent elevated in all groups. Severity of this lesions decreased from anterior to posterior regions. Hypertrophy, hyperplasia, and squamous metaplasia were restricted to respiratory epithelium. For 0.1 ppm these effects were described as "slight".

Alvarez et al. (1997) conducted a nose-only study on pregnant Crl:CD®BR rats (see study description in Section 3.4). All female rats survived the testing period. Sacrifice was conducted on day 22 of gestation and dams were examined extensively. Maternal rats exposed to either 0.7 or 1.5 ppm dimethyl sulfate, but not to 0.1 ppm revealed a significant reduced body weight gain between day 7 and day 16 of gestation (72 % of the controls at 0.7 ppm, 30 % at 1.5 ppm). Beside of signs related to stress of restraint observed in exposed and control animals (alopecia, as well as facial, periocular and perinasal staining) no compound

related effects on the incidence of clinical observations were seen. No irritation or eye effects were reported at any concentration. However, they were not explicitly excluded.

Repeated inhalation exposure to 2.64 ± 0.043 mg/m^3 (0.5 ± 0.008 ppm) for 4 month conducted by Molodkina et al. (1986) in rats and guinea pigs, induced changes in the nervous system function, liver (fatty degeneration of single hepatocytes), kidney (degeneration of single renal tubuli), respiratory organs (bronchitis), and peripheral blood parameters. A 4-month exposure to 0.29 ± 0.02 mg/m^3 (0.056 ± 0.004 ppm) led to marginal changes (increased body weight, decreased hippuric acid elimination) without morphological alterations. No further information concerning number of animals, exposure duration per day, and examined parameters is given.

3.4. Developmental/Reproductive Toxicity

Developmental / reproductive toxicity after inhalation exposure

Alvarez et al. (1997) investigated the developmental toxicity of dimethyl sulfate in pregnant Crl:CD®BR rats after inhalation exposure. Groups of 25 animals were nose-only exposed to 0, 0.1, 0.7 or 1.5 ppm dimethyl sulfate (purity greater than 99.5 %) by inhalation for 6 hr/day from day 7 through 16 of gestation (10 exposures). Each rat was individually kept in a glass and stainless steel 150-liter chamber so that only the nose protruded into the chamber. The chambers were operated with 16 air changes per hour in a flow-through mode. Weight, feed consumption and clinical signs were recorded regularly. Individual clinical signs were recorded each morning and afternoon throughout the exposure period. None of the reproductive parameters was altered in any of the treatment groups compared to control group. No significant differences in the incidence of malformation or clinical observations were reported. Dimethyl sulfate revealed no embryo toxicity in the rat following inhalation exposure up to 1.5 ppm (7.8 mg/m^3) during period the of major organogenesis.

Molodkina et al. (1986) observed no effects of dimethyl sulfate on reproductive organs, spermatogenesis and sperm morphology in rats and guinea pigs exposed to 2.64 ± 0.043 mg/m^3 (0.5 ± 0.008 ppm) and to 0.29 ± 0.02 mg/m^3 (0.056 ± 0.004 ppm). No further information concerning number of animals, exposure duration per day, and examined parameters is given.

In ACGIH (1991) a reproduction / developmental toxicity study is reported. In mice and rats, inhalation of 0.1 - 4 ppm dimethyl sulfate throughout pregnancy caused preimplantation losses and embryotoxic effects, including anomalies of the cardiovascular system. No further information is available.

Developmental / reproductive toxicity after intraperitoneal injection

Bishop et al. (1997) reported slight, but significantly reduced litter size of dimethyl sulfate-treated females compared to control group. To 10 - 12 weeks old female mice a single intraperitoneal injection of 75 mg/kg was administered. In the morning following the day of injection, females were caged individually with untreated males. For ovarian histology females were sacrificed 15 days after injection and examined for small follicles as this represent the most reliable data because they were the easiest to count accurately. The lower litter size was associated with a slight but significant reduction in small follicles.

3.5. Sensitization

Dimethyl sulfate was active in the local lymph node assay (LLNA) after application of 0.25, 0.5, or 1.0 % dimethyl sulfate in acetone/olive oil 80/20 (3 consecutive days) on the dorsum of both ears of mice (number of animals not given), conducted by Ashby et al. (1995). For determination of cell proliferation in the lymph nodes animals were injected intravenously with [3H]thymidine and radio activity was measured as a function of isotope incorporation in draining auricular lymph nodes. Stimulation indices (T/C ratios) of 0.8, 1.9, and 12.0 were measured.

3.6. Methylating Properties and Mutagenicity

As a directly alkylating agent dimethyl sulfate can cause changes in nucleic acids (WHO 1985). Because of the S_N2-type alkylating mechanism dimethyl sulfate reacts predominantly with the N-7 of guanine and forms small amounts of other DNA adducts as could be demonstrated from in vivo and in vitro tests (ECB 2002). Swann and McGee (1968) reported that a single dose of 80 mg/kg to rats increased formation of N7-methylguanine, however concentrations are low compared to other alkylating agents, such as dimethylnitrosamine. A much higher degree of DNA alkylation in lung and brain than in liver and kidney was observed. The authors conclude that dimethyl sulfate reveals an early breakdown in organs that are reached at first.

Methylating properties in vivo

Löfroth et al. (1974) detected N7-methylguanine (N7-MeGua), N3-methyladenine (N3-MeAd) and N1-methyladenine (N1-MeAd) after exposure of male NMRI mice to an average concentration of 0.32 mg dimethyl sulfate /m^3 (0.062 ppm) (for 60 minutes) or 16.3 mg/m^3 (3.16 ppm) (for 135 minutes). 4 animals each were exposed to dimethyl sulfate labeled with ^3H in a 6-l-glass flask. After exposure animals were placed in a metabolic cage. Urine was collected in two periods from 0 to 24 hours and 24 to 48 hours, respectively, and labeled methylated purines were determined. The excretion rate of N7-MeGua was estimated with a $t_{1/2}$ of about 1 day. The ratio of N7-MeGua, N3-MeAd and N1-MeAd was about 88:7:4 for the higher dimethyl sulfate concentration, and 10:10:1 for the lower concentration.

For DNA adduct measurements, Mathison et al. (1995) exposed adult male CrlCD:BR rats for 20 minutes to dimethyl sulfate concentrations of 0, 1, 3, 8, and 22 ppm in a closed-chamber nose/head-only apparatus. At various time points following exposure, animals were sacrificed and respiratory and olfactory mucosa and lung were collected for DNA adduct data. A dose-response relationship up to 22 ppm for N7-MeGua and N3-MeAd was measured in respiratory and olfactory mucosa with a ratio of N7-MeGua and N3-MeAd of approximately 5:1. Methylation of lung DNA was low, possibly due to poor DNA isolation and contamination of DNA with RNA, as concluded by the authors. However, reduced DNA alkylation of lung tissue can also be a result of early breakdown of dimethyl sulfate, as illustrated by Swann and McGee (1968). *Methylating properties in vitro*

Investigations on in vitro DNA-methylation were conducted in several cell-line systems, as in hamster dermal fibroblasts, V-79 cells, and calf thymus cells (ECB 2002). Methylation of the N7 position (MeAd and MeGua) were mainly produced, besides this the methylating products N3-MeAd and O^6-MeGua were detected.

Mutagenicity

Genotoxic effects of dimethyl sulfate have been investigated in bacterial, fungal, and mammalian (animals and cell lines) test systems (ECB 2002). Positive results were obtained from Ames tests with the strains TA98, TA100, TA1535, TA1537, TA1538, TS1121, and TS1157 (reverse mutation test) (Skopek et al. 1978; Braun et al. 1977; Quillardet et al. 1985; Hoffmann et al. 1988). The forward mutation assay to 8-azaguanine resistance revealed also positive results in TM35 and TM677 (Skopek et al. 1978). Also positive tests were conducted in a SOS test in Salmonella typhimurium (Nakamura et al. 1987) and in E. coli PQ37 (Quillardet et al. 1985).

HGPRT gene mutation assay, chromosomal aberration test, and sister chromatid exchange (SCE) test in Chinese hamster cells (ovary (CHO) and V-79) cells gave positive results with dose dependent increase (Connell and Medcalf 1982; Couch et al. 1978; Newbold et al. 1980; Tan et al. 1983). Unscheduled DNA synthesis (UDS) tests and SCE tests with human fibroblasts and UDS in primary rat hepatocytes delivered also positive results (Wolff et al. 1977; Cleaver 1977; Probst et al. 1981).

Two tests in mammals (dominant lethal test assay and mouse spot test) revealed no evidence for in vivo mutagenicity (Epstein and Shafner 1968; Braun et al. 1984). Enhanced DNA fragmentation was observed following alkaline elution test in 6 dimethyl sulfate treated male albino Sprague-Dawley rats (0.25 mmol/kg in 0.01 ml vehicle/g body weight; i.v.) (Robbiano and Brambilla 1987). Rats were sacrificed 1 hour after treatment and brain tissue was used for the assay. Brain DNA fragmentation was significantly ($p < 0.01$) enhanced compared with vehicle-treated control group. The authors conclude, that dimethyl sulfate shows a potential to induce tumors of the central nervous system.

Repeated inhalation exposure to 0.29 ± 0.02 mg/m^3 (0.056 ± 0.004 ppm), 2.64 ± 0.043 mg/m^3 (0.5 ± 0.008 ppm) and 20.26 ± 1.34 mg/m^3 (3.93 ± 0.26 ppm) for 4 month did not induce dominant lethal mutations in germ cells of rats (Molodkina et al. 1986). Dose-dependent increase in chromosomal aberrations in bone-marrow cells of mice and rats were reported after inhalation exposure to 0.5 ± 0.008 ppm and 0.056 ± 0.004 ppm (rats) respectively to 0.24 ± 0.2 mg/m^3 (0.047 ± 0.004 ppm), 4.32 ± 0.75 mg/m^3 (0.84 ± 0.15 ppm), and 22.1 ± 2.35 mg/m^3 (4.28 ± 0.46 ppm) (mice). No further information concerning number of animals, exposure duration per day, and examined parameters is given.

TABLE 5. Summary of Mutagenicity Test Results			
Test	**Result**	**Comments**	**Reference**
Ames test S. typhimurium	pos.	positive in reverse mutations: TA98, TA100, TA1535, TA1537, TA1538, TS1121, TS1157 positive in forward mutations: TM35, TS1157	Skopek et al. (1978) Braun et al. (1977) Quillardet et al. (1985) Hoffmann et al. (1988)
SOS chromotest	pos.	Forward mutation assay without activation E. coli PQ37	Quillardet et al. (1985)
SOS chromotest	pos.	Forward mutation assay without activation S. typhimurium	Nakamura et al. (1987)
UDS test SCE test	pos.	mammalian cells human fibroblasts primary rat hepatocytes	Wolff et al. 1977) Cleaver (1977) Probst et al. (1981)
chromosomal aberration test SCE test HGPRT assay	pos.	positive test results in mammalian cells V 79-cells / CHO-cells	Connell and Medcalf (1982) Couch et al. (1978) Newbold et al. (1980) Tan et al. (1983)
dominant lethal assay mouse spot test	neg.	mammals	Epstein and Shafner (1968) Braun et al. (1984)
dominant lethal mutations	neg.	rats, germ cells	Molodkina et al. (1986)
chromosomal aberrations	pos.	mice and rats, bone-marrow cells	Molodkina et al. (1986)
alkaline elution assay	pos.	enhanced brain DNS fragmentation in rats treated with 0.25 mmol/kg	Robbiano and Brambilla (1987)

3.7. Carcinogenicity

Druckrey et al. (1970) investigated the tumorigenic effect of dimethyl sulfate after inhalation exposure of 27 rats to 10 ppm (55 mg/m^3). The treatment was conducted in an inhalation chamber of 1 m^3 for 5 days/week for 1 hour. 12 rats died during treatment period (no exact time-to-death reported) due to pneumonia and purulent inflammations of nasal cavity. Hence, treatment was stopped after 130 days. Of the 15 rats that survived treatment 5 died with squamous cell carcinoma in nasal cavity (3 animals), glio-sarcoma in cerebellum (1 animal) and lymphosarcoma within the thorax (1 animal). In a second treatment group, 20 rats were exposed to 3 ppm (17 mg/m^3). Despite of the lower concentration, almost all animals developed a purulent inflammation of nasal cavity. A few animals died, therefore treatment was also stopped after 130 days. Beyond non-carcinogenic lethal causes, one animal each died with peripheral glioma of trigeminal nerve, of an esthesioneuroepithelioma, and of squamous cell carcinoma, the latter two

in the nasal cavity. The authors conclude that the severe purulent inflammations seem to inhibit tumor formation due to necrosis of cells.

Schlögel (1972) conducted a cancer study with inhalation exposure on male and female Wistar rats, NMRI mice, and Syrian golden hamsters. The animals were exposed either to 2.6 mg dimethyl sulfate /m^3 (0.5 ppm) (6 hours/day, 2 days/week on Tuesday and Friday), to 10.5 mg/m^3 (2 ppm) (6 hours/day, 1 day/2 week), or to 178 mg/m^3 (34 ppm) (rats), 252 mg/m^3 (48 ppm) (mice), and 105 mg/m^3 (20 ppm) (hamsters) every 4th month for 1 hour. For further study details see Section 3.3. The incidence of malignant nose and lung cancer (nasal and lung carcinoma) was slightly elevated in the 0.5 ppm group (5/97 animals compared to 2/70 animals of control group) and moderately elevated in 2 ppm groups (10/74 animals) (see Table 6). Rats were most sensitive to tumor induction (3/37 animals at 0.5 ppm, 6/27 animals at 2 ppm), while hamsters were the least sensitive (0/28 and 1/22 animals at 0.5 and 2 ppm, respectively). Female animals were more sensitive than male animals of all species (5 females out of 18 exposed animals with tumors). 4 exposures to the sublethal dose led to malignant tumor induction only in rats (34 ppm). Control animals revealed no malignant tumors in the lungs, however 2 tumors occurred at other unspecified sites.

TABLE 6. Tumor Incidences in Rats/Mice/Hamsters (Schlögel 1972)

tumors	control	0.5 ppm	2 ppm	sublethal
malignant lung tumor	36664	36574	36695	36543
malignant nose tumor	36664	36525	37013	36543
benign lung tumor	36560	36650	36622	36924

A very limited reported carcinogenic study was conducted by Molodkina et al. (1986) in 90 male and female CBAxC57BC/GI mice after 6-month inhalation to 3 different dimethyl sulfate concentrations (0.38 ± 0.08 mg/m^3 [0.074 ± 0.015 ppm], 1.62 ± 0.17 mg/m^3 [0.31 ± 0.06 ppm] or 20.26 ± 1.34 mg/m^3 [3.93 ± 0.26 ppm]) for 2 hours per day, 5 days a week. In high and intermediate dose group a significant increase in tumor incidence, mainly lung adenoma, was observed.

According to WHO (1985) concentrations of 3 mg/m^3 (0.6 ppm) induce respiratory tract tumors in nasal cavity and air passages in rats after a 15 month treatment period (literature source not indicated). Low incidence of carcinogenicity detected in animals exposed intravenously to dimethyl sulfate seems to be closely related to the rapid disappearance of dimethyl sulfate from the bloodstream and the low level of DNA alkylation (WHO 1985). The German DFG (Henschler 1972) classified dimethyl sulfate as a substance with a moderate carcinogenic potential presumably due to the rapid hydrolysis on mucous tissues. Almost all observed tumors developed from inhalation exposure occurred locally at the site of exposure.

3.8. Summary

Dimethyl sulfate is classified as very toxic by inhalation in various animals (1-hour LC_{50} between 32 and 98 ppm) causing irritation of respiratory tract and eyes at low concentration as well as respiratory impairment and tissue damage (pulmonary edema, emphysema, peribronchitis) beginning at concentrations of 10 ppm, with aggravation of symptoms at increasing concentration (Weber 1902; Flury and Zernik 1931; Ghiringhelli et al. 1957). At higher concentrations, congestion, hemorrhage, and parenchymatous degeneration of various organs (liver, kidneys, spleen, lung) were observed (Weber 1902; Mohlau 1920;

Ghiringhelli et al. 1957). As cause of death, Hein (1969) stated pulmonary emphysema, pulmonary edema (at animals with short period of survival) or bronchopneumonia (at animals with long period of survival).

Dermal application of lethal dimethyl sulfate doses leads to injuries of respiratory tract and death is caused by respiratory failure (Weber 1902). Dimethyl sulfate is of moderate acute oral toxicity in animals, with LD_{50} values of about 140 - 440 mg/kg (Schmezer and Schmähl 1987; Smyth et al. 1951; Molodkina et al. 1979) and is classified as toxic in terms of acute toxicity. Lesions of gastrointestinal tract and effects on the nervous system were observed by Weber et al. (1902) following single applications of a lethal dose. Alterations in respiration and apnea seem to be the most important physiological alterations observed after systemic administration via intravenous and subcutaneous pathway (Weber 1902; Wachtel 1920).

The cardinal symptoms observed within most of the animals after acute nonlethal inhalation dimethyl sulfate treatment are coughing and sneezing as well as closed eyes and conjunctivitis (Schlögel 1972). These symptoms are observed at 0.5 - 2 ppm. At higher concentrations of 10 ppm and above respiratory embarrassment and lesions, e.g. breathing difficulties, inflammations, dyspnea and hyperemic zones, as well as eye effects (lacrimation, salivation, cornea lesions) were observed (Weber 1902; Flury and Zernik 1931; Hein 1969; Mathison et al. 1995).

No effects on fetal development were seen by Alvarez et al. (1997) in rats after inhalation exposure to 0.1, 0.7 or 1.5 ppm dimethyl sulfate during gestation. A slight alteration in number of small follicles, leading to a marginal reduced litter size in dimethyl sulfate-treated mice after intraperitoneal injection of 75 mg/kg was reported by Bishop et al. (1997).

Sensitization was positively tested by Ashby et al. (1995) in the murine local lymph node assay. Therefore, dimethyl sulfate is classified as a potential sensitizer (risk phrase R43: May cause sensitization by skin contact).

The methylating potency of dimethyl sulfate was observed in several studies (Löfroth et al. 1974; Mathison et al. 1995; ECB 2002). From in vivo and in vitro investigations, methylation of the N7 position (MeAd and MeGua) were mainly produced, besides this the methylating products N3-MeAd and O^6-MeGua were detected. Most of the conducted mutagenicity tests revealed positive test results (Skopek et al. 1978; Braun et al. 1977; Quillardet et al. 1985; Hoffmann et al. 1988; Nakamura et al. 1987; Connell and Medcalf 1982; Couch et al. 1978; Newbold et al. 1980; Wolff et al. 1977; Cleaver et al. 1977; Probst et al. 1981) however no mutagenicity was observed in in vivo tests (Epstein and Shafner 1968; Braun et al. 1984). Although results on in vivo test deserves more relevance than a bacterial or in vitro cell test for human mutagenic effects, the negative results from mammal test do not invalidate the majority of positive results obtained in vitro.

Although the quality of the carcinogenicity studies is restricted, there is sufficient evidence for the tumor inducing potential of dimethyl sulfate after prolonged inhalation exposure in animals. All tumors observed by Schlögel (1972) in rats, mice and hamsters occurred locally (nasal and lung carcinoma after 0.5, 2 and 34 ppm). Additionally to tumors of nasal cavity Druckrey et al. (1970) observed gliosarcoma in cerebellum and lymphosarcoma (3 and 10 ppm, 5 days / week for 1 hour for 130 days). The authors assume that tumors of the deep respiratory tract (e.g. bronchus or lung) cannot occur, because dimethyl sulfate breakdown already happens in the upper mucosal tissues. However, malignant lung tumors were observed by Schlögel (1972).

A carcinogenic risk calculation conducted by ECB (2002) lists a risk for malignant tumors for occupational exposure scenario after repeated inhalation exposure. These calculations to assess the carcinogenic activity of dimethyl sulfate are based on the Schlögel-study after exposure to 2 ppm

(Schlögel 1972). This study is only considered as a rough indication of the carcinogenic potency due to the limited quality (low number of animals, short duration, high dose level). No dose-response relationship can be observed in this study at concentrations of 0.5 ppm, 2 ppm and sublethal dose (rats 48 ppm, mice 34 ppm, golden hamsters 20 ppm). Calculations based on the 2 ppm exposure gained a carcinogenic activity attributable to the exposure to the substance per unit concentration (expressed per mg/m^3), expressed as I_{conc}. The carcinogenic activity for life span exposure is calculated with $I_{conc} = 2.2\ mg/m^3$ (for detailed calculations see Appendix B).

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Following inhalation dimethyl sulfate is well absorbed to over 84 % (Cartlidge et al. 1996). A rapid respiratory absorption is observed up to 50.3 mg/m^3 (about 10 ppm), which decreased at higher dose levels, probably due to a decreased minute volume (ECB 2002). From dermal exposure it can be assumed, that intoxication occurs via inhalation pathway to a large part due to the vaporization of dimethyl sulfate.

Dimethyl sulfate is readily absorbed through skin and mucosa (Vyskocil and Viau 1999), however ECB (2002) remarks that data on dermal absorption are limited and insufficient to draw conclusions. Schettgen et al. (2002; 2004) assume a considerable dermal absorption due to their measurements of the dimethyl sulfate-specific globin adduct N-methylvaline in 62 dimethyl sulfate exposed workers. A maximum of 184.7 µg N-methylvaline /l blood was measured in dermally exposed persons. Unexposed control persons (n = 12) usually showed concentrations of about 10 µg N-methylvaline /l blood. 40 µg/l correspond to 0.2 mg/m^3 (0.385 ppm) dimethyl sulfate.

Mathison et al. (1995) exposed adult male CrlCD:BR rats for either 20 or 40 minutes to dimethyl sulfate concentrations of 0.9, 1.4, 3.1, 9.6, or 24.2 ppm in a nose/head-only chamber for characterization of vapor uptake kinetics. Dimethyl sulfate uptake from the chamber was measured at 1-min intervals. Blank chambers were run intermittently at the same levels to evaluate dimethyl sulfate decomposition rates. Dimethyl sulfate vapor clearance appears to increase with increasing concentration between 0.9 and 10 ppm and was calculated from 69 % for 0.9 ppm dimethyl sulfate to 89 % for 9.6 ppm dimethyl sulfate after 40 minutes. For 24.2 ppm a noticeable decrease in uptake was observed (74.5 % clearance), although the animals revealed no signs of discomfort or stress in response to the dimethyl sulfate exposure. Decomposition of dimethyl sulfate in the blank chamber was of about 10 - 20 % of initial dimethyl sulfate concentration. This study provides information of dimethyl sulfate uptake, however allows no quantification of absorption rate.

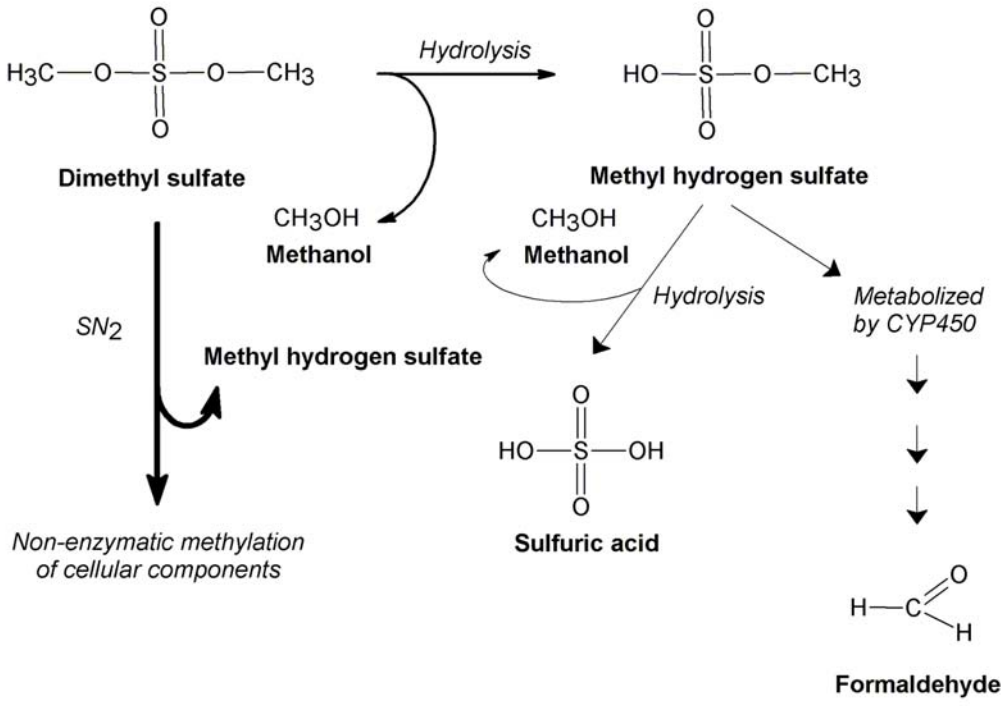
Dimethyl sulfate hydrolyzes readily in contact with water or in humidity to form sulfuric acid, methanol and methyl hydrogen sulfate (monomethyl sulfate) (Wang et al. 1988). In aqueous solution dimethyl sulfate hydrolyzation occurs with a half-time of about 20 minutes at 35 °C to methanol and methyl hydrogen sulfate (Cartlidge et al. 1996). A hydrolyzation half-time of 1.2 hours at 25 °C and pH 7 was reported by NLM (2002). WHO (1985) reported a half-time of 4.5 hours in pH 7 buffered aqueous solution for hydrolysis to methyl hydrogen sulfate and methanol in mammalian tissues, while conversion to sulfuric acid occurs more slowly. Natural decomposition is dependent on humidity. The in vivo breakdown of dimethyl sulfate occures faster, probably due to the high reactivity with cellular constituents. In rats Swann and McGee (1968) reported no detectable dimethyl sulfate 5 minutes after a single i.v. injection of 75 mg/kg body weight to Wistar rats.

Dimethyl sulfate binds to tissue proteins and nucleobases of DNA (Leng and Lewalter 2002). It is metabolized in humans and animals via enzymatic and non-enzymatic pathways. The enzymatic pathways involve cytochrome P450, glutathion S-transferase, and β-lyases (Leng and Lewalter 2002; Dahl and Hadley 1983). Metabolization via cytochrome P450 enzymes was investigated by Dahl and Hadley (1983). Incubation of rat liver and nasal microsomes with dimethyl sulfate (2 mM in water) led to little amounts of formaldehyde in liver microsomes, and to traces in nasal microsomes. For phase II biotransformation the glutathion-dependent pathway is qualitatively considered as the major one.

Löfroth et al. (1974) exposed 4 mice to vapors of radiolabeled dimethyl sulfate in a 6-l-glass (whole-body) at about 3 ppm for 135 minutes and 60 ppb for 60 minutes. About 70 % of the total dimethyl sulfate was collected in the urine up to 24 hours after exposure to both concentrations.

In the blood and urine of guinea pigs exposed to vapors of dimethyl sulfate (75 ppm) for 18 minutes methanol was found (Ghiringhelli et al. 1957). A maximum level of 2 mg / 100 mg was indicated for blood concentration at various intervals (not specified). Concentrations of methanol in urine were reported between 0.064 and 0.156 mg in the first two days after exposure. The amounts of methanol were far less than expected from extensive hydrolysis and were regarded as toxicologically not significant. Methanol was found as the only urinary metabolite (WHO 1985).

Figure 1: Main Pathways for the Methylation of Dimethyl Sulfate (most relevant pathways are illustrated by thick arrows).



4.2. Mechanism of Toxicity

Only the intact dimethyl sulfate-molecule has alkylating properties. This step is non-enzymatic and should already begin within the first minutes after tissue contact, leading to methylation (and deactivation) of proteins, essential amines, nucleobases and other cellular molecules. Also in the extracellular compartments dimethyl sulfate exerts methylating properties, simple hydrolysis being a competitive reaction. Cells may be damaged in multiple ways. After cleavage of the first methylester function (leading to methyl hydrogen sulfate) the second has no alkylating properties. This can be assumed from comparative in vitro investigations with dimethyl sulfate and methyl hydrogen sulfate by Tan et al. (1983), where only dimethyl sulfate was cytotoxic and mutagenic to CHO cells. The methylgroup may be hydroxylated via cytochromes with a subsequent formation of formaldehyde and sulfuric acid which upon intracellular formation may contribute to the total cytotoxic impact.

Exposure to dimethyl sulfate results in local and systemic effects depending on extent and duration of exposure. In evaluating the toxicity of dimethyl sulfate local effects are in the foreground for nonlethal and lethal intoxication and occur at concentrations much lower than those producing systemic effects. Roux et al. (1977) assume, that toxic effects are caused by the corrosive potential of sulfuric acid and the effects of methanol to the nervous system. Formation of methanol leads to headache, dizziness, wariness, visual disturbances, seizures, coma, paralysis, and kidney injury (Roux et al. 1977). It can be supposed from methanol toxicity data, that the concentrations produced via hydrolysis of dimethyl sulfate are not high enough to cause the mentioned effects. For example, Chuwers et al. (1995) conducted a study on 26 volunteers exposed to 200 ppm methanol and observed only slight alterations in neurological and psychological tests, that were judged as not meaningful. In accordance with Roux et al. (1977) Hein (1969) reported, that the toxicity of dimethyl sulfate is caused on the one hand by the intact molecule, and by sulfuric acid formed by hydrolysis on the other hand. Sulfuric acid should be responsible for the local corrosive injuries, but the systemic effects are caused by the absorbed dimethyl sulfate molecule. A major cause of effects in inhalation dimethyl sulfate intoxication is respiratory failure as consequence of mucosal inflammation and edema of respiratory tract.

TABLE 7. Characterization of Local and Systemic Effects of Dimethyl Sulfate

local effects	systemic effects
caused by methylation of dimethyl sulfate	caused by intact molecule
latency period	onset of effects immediately
irritation and corrosion	hypotension, decrease in respiration rate, apnea
lesions of respiratory tract, inflammation, demucosation, edema	organ failure (kidney, liver, heart)
secondary effects of inflammation	convulsion, paralysis, delirium, coma

From investigations on high concentration inhalation (undiluted mist of dimethyl sulfate) in guinea pigs DuPont (1943) concluded, that irritation is confined to the bronchi and bronchiole, and dimethyl sulfate-mist does not get far enough to cause irritation of the lung tissue itself, indicated by lacking of congestion or edema of alveolar walls and exudate in alveolar cavity.

Usually a latency period of 4 to 12 hours between exposure and onset of effects was reported from human case studies. From experimental studies on animals latency periods of few minutes were reported after administration of high doses via different pathways (Weber 1902). As indicated above, the local effects on skin and mucosa are not due to the intact molecule, but to sulfuric acid. The delayed hydrolysis of dimethyl sulfate to sulfuric acid, as described by WHO (1985), is presumably responsible for this latency period. Delayed effects after several hours are also described for S-Lost and phosgene (NRC 2003, 2002) and may be a characterization of irritative acting gas, where decomposition products (e.g. hydrochloric acid) contribute to the effects.

Von Nida (1947) assumes from a lethal intoxication after accidental oral intake of dimethyl sulfate, that the first slight symptoms are caused by decomposition of dimethyl sulfate at the mucosa, which set in immediately. The more dangerous component is the intact molecule that evaporates in the oral cavity and is spread out in the respiratory and digestive tract.

No neurotoxicity was seen after inhalation exposure, besides local analgesic effects.

Experimental studies revealed a tumorigenic potential of dimethyl sulfate after inhalation exposure to concentrations of 17 mg/m^3 (3.3 ppm), and 2.6 mg/m^3 (0.5 ppm), respectively (Druckrey et al. 1970; Schlögel 1972). Dimethyl sulfate acts as a directly genotoxic agent due to its potential to react with nucleophilic groups of nucleic acids (Löfroth et al. 1974; Mathison et al. 1995; Swann and McGee 1968).

4.3. Other Relevant Information

Due to the only slightly pronounced odor and the anesthetic effect on mucosa, what leads to anosmia, respiratory tract and lungs alarm signs are absent. Littler and McConnell (1955) reported for one case of dimethyl sulfate intoxication, that the patient required no analgesics for one week. From there it is possible to be exposed to high toxic or even lethal concentrations without noticing. Very different onset of first perceptions of poisoning is reported in literature. For example, a slight dyspnea was noticed 1 hour after exposure to “one breath” reported by Thiess and Goldmann (1967), however 4 hours of exposure to a deadly concentration remained unnoticed in a case study described by Weber (1902). For all cases a delay in developing symptoms of toxicity were reported after inhalation, and time of appearance correlates with exposure extent in general. For lethal poisoning health effects started after 4 hours in average (Weber 1902; Roßmann and Grill 1952) and for nonlethal exposure after 10 hours in average for low dose exposure (Roßmann and Grill 1952; Nebelung 1957; Wang 1988).

4.3.1. Species Variability

No relevant data on species specific differences in absorption, metabolism, or elimination are known for dimethyl sulfate. Non-enzymatic hydrolysis is not expected to differ greatly between different species. For irritation and corrosive effects, no major toxicodynamic differences are relevant. Moreover, very similar lesions are observed in various species. Due to the damage resulting from direct contact of dimethyl sulfate with epithelial surfaces an order-of-magnitude variability among species is not likely. However, existing data on quantitative species differences are limited and moderate species differences are documented in case of lethality data.

In comparing LC_{50} or benchmark concentrations for lethality data from Hein (1969; see Table 3) some variability in effect sizes may be observed, although clearly less than an order of magnitude. Guinea pigs seem to be more susceptible against vapors of dimethyl sulfate (1-hour exposure) than mice. Rats and hamsters show sensitivity in between these two species. Guinea pigs show a very steep time-mortality

curve with a maximal period of survival of 50 hours at 71 ppm (first death 3 hours after exposure), compared to mice, where no lethality was observed during this period (first death approximately 80 hours after exposure).

According to investigations on carcinogenic potential of dimethyl sulfate Schlögel (1972) reveals, that rats are more susceptible than mice and hamsters regarding tumor induction. Limited data from dimethyl sulfate exposure on monkeys reported by Roßmann and Grill (1952) and on cats reported by Flury and Zernik (1931) suggest some but no extensive differences in susceptibility between species.

It can be assumed, that dimethyl sulfate causes lesions in other parts of the respiratory tract of humans than in experimental animals due to differences in breathing technique. Kalberlah et al. (1999) report that the extrathoriatic region shows a lesser filtering potency in humans than in experimental animals, therefore higher concentrations of contaminants are able to reach the pulmonary region. However this is partly compensated by a larger surface of the pulmonary region in humans leading to similar area doses in animals and humans.

4.3.2. Susceptible Populations

There are no major toxicokinetic differences between individuals expected to be relevant after dimethyl sulfate exposure. Unspecific irritating and corrosive action is also assumed to be similar between different individuals. However, susceptibility to dimethyl sulfate effects may differ as evidenced by some human case studies and the variability in lethality concentrations within one animal species (Hein 1969).

Ip et al. (1989) reported exposure of two workers: one developing slight discomforts and the other suffering from severe injuries of respiratory tract. Although, no statement can be made according to underlying factors of intraspecies variability it is conspicuous, that intoxication with similar exposure scenario and exposure concentrations can show a varying course of disease, including nonlethal and lethal effects. This can be at least partly explained by different first aid measures and therapy. Due to use medication to handle respiratory tract secondary infections, e.g. laryngitis, bronchitis, pneumonia, caused by mucosa injury dimethyl sulfate intoxications show a more advantageous disease progress nowadays. In summary, some degree of heterogenic response to exposure towards dimethyl sulfate can not be excluded.

4.3.3. Concentration-Exposure Duration Relationship

As demonstrated by Schlögel (1972) and Hein (1969) irritative effects on respiratory tract aggravated with longer exposure duration.

Roßmann and Grill (1952) calculated effect concentrations for human exposure based on animal data using Habers law and gave a C^1 x t product of 5200 (mg/m^3 x hours) for lethal effects and of 1320 for severe disease process in monkeys. This calculation has not been validated and existing data are too poor to derive an exponent to be used for time extrapolation based on these calculations. However, LC_{50} values derived in rats of 64 ppm for an 1-hour duration (Hein 1969) and of 32 ppm for a 4-hour exposure (Kennedy and Graepel 1991) support the equation C^2 x t = k. A similar time relationship was observed within mice, for which LC_{50} values of 98 ppm and 54 ppm were reported for an 1-hour and a 4-hour exposure, respectively (Hein 1969; Molodkina et al. 1986).

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

There are no scientific human data to be used for derivation of an AEGL-1. Some suggestions of irritating concentrations or thresholds are reported in literature (Wang 1988; Smyth 1956), but they are not supported by study reports. Wang et al. (1988) assume from literature data and own investigations (not shown) that concentrations below 1 ppm can cause slight irritation of eyes. No details are provided.

5.2. Summary of Animal Data Relevant to AEGL-1

Frame et al. (1993, abstract publication) reported changes in the nasal cell proliferation (decreased labeling index for respiratory epithelium; increased labeling index for olfactory epithelium) in rats repeatedly nose-only exposed to 0.1, 0.7 and 1.5 ppm for 6 hours/d (2 weeks, 10 exposures). At 0.7 and 1.5 ppm, but not at 0.1 ppm, histopathology revealed dimethyl sulfate-related lesions (erosion, ulceration and atrophy of respiratory and olfactory epithelia), that increased in severity with higher exposure concentration.

At 0.5 and 2 ppm dose-dependent effects on eyes and respiration tract were reported by Schlögel (1972) in rats, mice and golden hamster in a repeated study with 6-hour inhalation duration. The lowest exposure concentration of 0.5 ppm led to changes in behavior and clinical findings in some animals of all species already after 20 minutes (closed or half-closed eyes; ruffled fur). After 6 hours exposure, the animals developed delayed breathing problems. They coughed and sneezed occasionally and revealed asthmatic-like breathing sounds. After inhalation exposure to 2 ppm dimethyl sulfate for 6 hours all effects described at 0.5 ppm aggrevated, additionally conjunctivitis with sensitivity to light and expiration sounds were reported. All described effects occurred already after first exposure as reported in personal communication (Schlögel 2003).

No substance related effects have been reported by Alvarez et al. (1997) at clinical examination during exposure in a repeated nose-only inhalation study with dimethyl sulfate concentrations of 0.1, 0.7, and 1.5 ppm (10 exposures) in rats. However, due to the kind of investigations as a developmental study, detailed examination on local irritating effects might not have been not performed or observations were not described.

5.3. Derivation of AEGL-1

For the derivation of AEGL-1 values irritations of eyes and respiratory tract are the most relevant effects. No human studies with single exposure investigating irritative effects are available. At concentrations relevant for AEGL-1 no qualified observations at workplace are documented. In laboratory animals, at 0.5 and 2 ppm irritative effects of dimethyl sulfate were observed by Schlögel (1972) after 6-hour exposure in rats, mice and golden hamster. The irritating symptoms aggravated clearly with increasing concentration. Effects observed at 0.5 ppm after 6 hours (sneezing, cough, asthmatic-like breathing sounds) are already judged as above AEGL-1 level. In the same study, rough fur and eye lid closure were observed in rats soon after onset of exposure (after 20 minutes). These effects seem of questionable relevance for health risk assessments in humans. Also, these effects were not reported in repeated exposure studies with similar exposure concentrations (Frame et al. 1993; Alvarez et al. 1997) or in human studies with low exposure. Therefore, only the well established effects after 6 hours exposure are regarded further. Slight effects are reported from studies with repeated exposure. Frame et al. (1993) describe altered nasal cell proliferation without histopathological findings at 0.1 ppm for 6-hour exposures in rats in an abstract publication. Due to the lack of adequate data from single exposure the study by Frame

et al. (1993) with repeated exposure is used for AEGL-1 derivation. It is supported by the data from Schlögel (1972) with more pronounced effects in rats after 6-hour exposure to 0.5 ppm. It was tried to get additional information by several ways concerning the study by Frame et al. (1993), reported only as an abstract, however without success to date. Because the conducting laboratory (Haskell Laboratory for Toxicology and Industrial Medicine, DuPont Company) is well known and judged as trustworthy, this study was used for the derivation despite the limited reporting.

As discussed in Section 4.3.1 and 4.3.2 no major differences in toxicokinetics and toxicodynamics (irritating effects) between species are expected after exposure to dimethyl sulfate. However, some species are shown to be moderately more susceptible than others. The interspecies factor is reduced to 3 because the critical study is with repeated exposure. No large differences in susceptibility between individuals are expected for unspecific irritating effects. Hence, the uncertainty factor to account for susceptible subpopulations may also be reduced to 3, leading to an overall uncertainty factor of 10.

The experimental derived exposure values were scaled to AEGL time frames using the equation $C^n \times t = k$ (Ten Berge et al. 1986). As demonstrated in Section 4.3.3 LC_{50} values derived in rats and mice support the equation $C^2 \times t = k$. Thus, the value of n = 2 in the exponential function was used for extrapolation from the 6-hour exposure to 30 minutes, 1 hour, 4 hour, and 8 hour. Because extrapolation from 6 hours to short durations of less than 30 minutes leads to very high uncertainty, the values for 10 minutes are set equal to the values for 30 minutes.

TABLE 8. AEGL-1 Values for Dimethyl Sulfate *)

10-minute	30-minute	1-hour	4-hour	8-hour
0.035 ppm (0.18 mg/m^3)	0.035 ppm (0.18 mg/m^3)	0.024 ppm (0.12 mg/m^3)	0.012 ppm (0.062 mg/m^3)	0.0087 ppm (0.045 mg/m^3)

*) Relevant skin uptake and sensitizing properties of dimethyl sulfate can not be excluded. Dimethyl sulfate is a methylating and mutagenic substance, classified as suspected human carcinogen (A2: ACGIH, 1991; 2A: IARC, 1999; Carc. Cat. 2, R45: BAuA, 2001).

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

There are no scientific human data to be used for derivation of an AEGL-2. Some suggestions of relevant effect concentrations or thresholds are reported in the literature, but they are not supported by study reports. Roßmann and Grill (1952) assumed from animal non-lethal 3-hour exposure to 1.35 ppm dimethyl sulfate vapor to cause severe symptoms on eyes (conjunctivitis, keratitis) and respiratory tract (cough, bronchospasm, dyspnea) by extrapolation. Wang et al. (1988) reported that the observed moderate irritative reactions on eyes and the upper respiratory tract, without abnormal breathing sounds in 19 persons, occur at concentrations in excess of 1 ppm for a 10-minute exposure duration.

6.2. Summary of Animal Data Relevant to AEGL-2

Schlögel (1972) conducted investigations on inhalation exposure to dimethyl sulfate in rats, mice, and golden hamster in a study with repeated exposure. The lowest exposure concentration of 0.5 ppm for 6 hours led to changes in behavior within some animals of all species already after 20 minutes (ruffled fur; closed or half-closed eyes). Following exposure, the animals developed breathing problems (they coughed and sneezed occasionally, and breathed sometimes similar to asthmatics). After exposure to 2 ppm dimethyl sulfate for 6 hours additionally conjunctivitis with sensitivity to light were reported. All described effects occurred already after first exposure as reported in personal communication (Schlögel 2003). A higher incidence of inflammation of the lungs was observed after repeated exposure to 0.5 and 2 ppm.

At 0.7 and 1.5 ppm Frame et al. (1993, abstract publication) observed dimethyl sulfate-related lesions (erosion, ulceration and atrophy of respiratory and olfactory epithelial) in rats repeatedly exposed for 6-hours nose-only (10 exposures). Effects increased in severity with higher exposure concentration.

At 0.7 or 1.5 ppm dimethyl sulfate (nose-only exposure for 6 hours/day, 10 exposures), but not at 0.1 ppm pregnant Crl:CD®BR rats (25 animals) revealed a significant reduced body weight gain between day 7 and day 16 of gestation (72 % of the controls at 0.7 ppm, 30 % at 1.5 ppm) (Alvarez et al. 1997).

At 10 ppm Hein (1969) reported lacrimation and salivation during an 1-hour exposure, and corneal injuries several hours after cessation of exposure in guinea pigs, but not in rats and mice. However closed eyes and cleaning reflexes were observed within all species at this concentration. Occasionally hemorrhage lung zones, pulmonary congestion, emphysema, and edema as well as enlargement and blue-red discoloration of livers were observed. At histopathology extension and demucosation of trachea and bronchi was observed.

6.3. Derivation of AEGL-2

Breathing problems and asthmatic-like breathing sounds observed at 0.5 ppm at 6-hour exposure after first exposure in a repeated study in rats, mice, and golden hamster by Schlögel (1972) are relevant effects for the derivation of the AEGL-2. Above this value irreversible lesions must be expected, what is supported by injuries of respiratory and olfactory epithelial (erosion, ulceration and atrophy) observed by Frame et al. (1993) at 0.7 ppm after repeated 6-hour exposure (10 exposures).

Effects (ruffled fur, eye lid closure) observed already after 20-minute exposure to 0.5 ppm are of questionable relevance for health risk assessments in humans. The author was not able to exclude some experimental influence caused by air circulation (personal communication, Schlögel 2003), however

closed eyes and eye troubles were reported by other authors at higher concentration with experimental animals (Flury 1931; Hein 1969), as well as in human lethal and non-lethal case studies (Weber 1902; Strothmann 1929; Thiess and Goldmann 1967; Savic 1971; Roux et al. 1977; Zhao 1989; Testud et al. 1999). Eye effects were not reported in repeated exposure studies with similar exposure concentrations as by Schlögel (Frame et al. 1993; Alvarez et al. 1997). Moreover, the effect size is judged to below AEGL-2 level, therefore the well established effects seen at 0.5 ppm after 6 hours are used as a starting point for AEGL-2 derivation.

AEGL-2 values are based on the effect concentrations in rats, mice, and golden hamsters following a 6-hour exposure to 0.5 ppm investigated by Schlögel (1972). As verified in personal communication (Schlögel 2003) effects observed after 6-hour exposure to 0.5 ppm are reversible within a few hours.

As discussed in Section 4.3.1 and 4.3.2 no major differences in toxicokinetics and toxicodynamics (irritating effects) between species are expected after exposure to dimethyl sulfate. However, some species are shown to be moderately more susceptible than others. Therefore, the interspecies factor is reduced to 3. No large differences in susceptibility between individuals are expected for unspecific irritating effects. Hence, the uncertainty factor to account for susceptible subpopulations may also be reduced to 3, leading to an overall uncertainty factor of 10.

The experimental derived exposure values were scaled to AEGL time frames using the equation $C^n \times t = k$ (Ten Berge et al. 1986). As demonstrated in Section 4.3.3 LC_{50} values derived in rats and mice support the equation $C^2 \times t = k$. Thus, the value of n = 2 in the exponential function was used for extrapolation from the 6-hour exposure to 30 minutes, 1 hour, 4 hour, and 8 hour. Because extrapolation from 6 hours to short durations of less than 30 minutes leads to very high uncertainty, the values for 10 minutes are set equal to the values for 30 minutes.

The derived AEGL-2 values are assumed to be appropriate to avoid relevant cell damage in respiratory tract, which may contribute to cell replication and may be viewed as a risk factor for development of malignant effects.

TABLE 9. AEGL-2 Values for Dimethyl Sulfate *)

10-minute	30-minute	1-hour	4-hour	8-hour
0.17 ppm (0.88 mg/m^3)	0.17 ppm (0.88 mg/m^3)	0.12 ppm (0.62 mg/m^3)	0.061 ppm (0.32 mg/m^3)	0.043 ppm (0.22 mg/m^3)

*) Relevant skin uptake and sensitizing properties of dimethyl sulfate can not be excluded. Dimethyl sulfate is a methylating and mutagenic substance, classified as suspected human carcinogen (A2: ACGIH, 1991; 2A: IARC, 1999; Carc. Cat. 2, R45: BAuA, 2001).

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

No adequate human experiences are available to derive AEGL-3 values.

Two calculations for assumed human lethal concentrations of dimethyl sulfate were reported by Roßmann and Grill (1952) (5.4 ppm, 3 hours) and Wang et al. (1988) (97 ppm, 10 minutes) based on data from animal studies and exposure modeling. However for none of the reported case studies measurements of concentrations were conducted.

7.2. Summary of Animal Data Relevant to AEGL-3

As presented in Section Species Variability (Section 4.3.1) guinea pigs and hamsters are the most susceptible species against vapors of dimethyl sulfate. A LC_{50} value for guinea pigs of 32 ppm was derived for an 1-hour exposure (Hein 1969). Hamsters reveal a LC_{50} of 56 ppm (Hein 1969). Compared to this, LC_{50} values for rats range from 64 ppm (Hein 1969) to 100 ppm (DuPont 1971) for an 1-hour exposure period. For a 4-hour exposure of rats a LC_{50} value of 32 ppm was reported by Kennedy and Graepel (1991). At 10 ppm disease process that lasted several days but no lethality was reported by Hein (1969) in rats and guinea pigs for an 1- hour exposure, however 1/20 mice died at this concentration as well as at 42 ppm. The highest LC_0 for rats have been 49 ppm (Hein 1969) and 58 ppm (DuPont 1971) for an 1-h exposure, and 15 ppm (Smyth 1956) for a 4-h exposure. In mice no lethality was seen at 49 ppm at the highest for an 1-h exposure, and in guinea pigs at 10 ppm at the highest, also for an 1-h exposure (Hein 1969).

Studies with "sublethal" concentrations in rats (34 ppm), mice (48 ppm) and golden hamsters (20 ppm) for an 1-hour exposure (4x per year) revealed severe dyspnea and breathing problems within all species 4 hours after cessation of exposure, that aggravated the following 2 days (Schlögel 1972). A recovery was observed not until one week after exposure. Isolated lethality was reported after 1st exposure, especially in rats and hamsters, as can be derived from the supplied figures. However, no figures were given for the unexposed control group.

7.3. Derivation of AEGL-3

The most qualified study to derive a lethal concentrations below LC_{100} is the study from Hein (1969). In this experiment, after 1-hour exposure most species showed no lethality after 10 ppm. Only with mice there was an incidence of lethal effect in 1/20 animals after exposure to 10 ppm. However, there was no clear dose response with no lethality after 49 ppm in mice. The highest non-lethal concentration of 49 ppm (rats, 1-h exposure) was used for the derivation of the AEGL-3 values. Accuracy of the derived LC_0 in hamster by Hein (1969) was largely confirmed by BMCL calculations:

Rat $BMCL_{05}$	32 ppm (log Probit)
Mouse $BMCL_{05}$	44 ppm (log Probit)
Guinea Pig $BMCL_{05}$	5.8 ppm (Quantal Quadratic)
Hamster BMC_{01}	12.6 ppm (Multistage)

The study bei Hein (1969) was chosen due to the comprehensively reported effects and the long follow-up observation period of 3 weeks. This LC_0 is further supported by the LC_0 of 58 ppm derived from a study by DuPont (1971), which is however less extensive reported.

As discussed in Section 4.3.1 and 4.3.2 no major differences in toxicokinetics and toxicodynamics (irritating effects) between species are expected after exposure to dimethyl sulfate. However, some species are shown to be moderately more susceptible than others. The rat as the species used for the derivation of the AEGL-3 values is less susceptible as for example the guinea pig. Because not the most susceptible species was used, an interspecies factor of 10 was applied. No large differences in susceptibility between individuals are expected for lethality. Hence, the uncertainty factor to account for susceptible subpopulations may be reduced to 3, leading to an overall uncertainty factor of 30, which is applied on the highest valid LC_0 of 49 ppm (rats, 1-h exposure) received in the study from Hein (1969).

The experimental derived exposure values were scaled to AEGL time frames using the equation $C^n \times t = k$ (Ten Berge et al. 1986). As demonstrated in Section 4.3.3 LC_{50} values derived in rats and mice support the equation $C^2 \times t = k$. Thus, the value of n = 2 in the exponential function was used for extrapolation from the 1-hour exposure to all durations.

TABLE 10. AEGL-3 Values for Dimethyl Sulfate *)

10-minute	30-minute	1-hour	4-hour	8-hour
4.0 ppm (21 mg/m^3)	2.3 ppm (12 mg/m^3)	1.6 ppm (8.3 mg/m^3)	0.82 ppm (4.3 mg/m^3)	0.58 ppm (3.0 mg/m^3)

*) Relevant skin uptake and sensitizing properties of dimethyl sulfate can not be excluded. Dimethyl sulfate is a methylating and mutagenic substance, classified as suspected human carcinogen (A2: ACGIH, 1991; 2A: IARC, 1999; Carc. Cat. 2, R45: BAuA, 2001).

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity Endpoints

The derived AEGL values for various levels of effects and duration of exposure are summarized in Table 11. The AEGL-1 value is based on nasal cell proliferation in rats (Frame et al. 1993). The AEGL-2 value is based on breathing problems observed in different species (rat, mouse, hamster) (Schlögel 1972). The AEGL-3 values is based on emphysema and edema of the lung, observed in rats, mice, hamsters, and guinea pigs, that can result in lethality (Hein 1969; DuPont 1971) .

TABLE 11. Summary of AEGL Values

Classification	Exposure Duration				
	10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
AEGL-1 (Nondisabling)	0.035 ppm (0.18 mg/m^3)	0.035 ppm (0.18 mg/m^3)	0.024 ppm (0.12 mg/m^3)	0.012 ppm (0.062 mg/m^3)	0.0087 ppm (0.045 mg/m^3)
AEGL-2 (Disabling)	0.17 ppm (0.88 mg/m^3)	0.17 ppm (0.88 mg/m^3)	0.12 ppm (0.62 mg/m^3)	0.061 ppm (0.32 mg/m^3)	0.043 ppm (0.22 mg/m^3)
AEGL-3 (Lethal)	4.0 ppm (21 mg/m^3)	2.3 ppm (12 mg/m^3)	1.6 ppm (8.3 mg/m^3)	0.82 ppm (4.3 mg/m^3)	0.58 ppm (3.0 mg/m^3)

*) Relevant skin uptake and sensitizing properties of dimethyl sulfate can not be excluded. Dimethyl sulfate is a methylating and mutagenic substance, classified as suspected human carcinogen (A2: ACGIH, 1991; 2A: IARC, 1999; Carc. Cat. 2, R45: BAuA, 2001).

An useful presentation to evaluate the derived AEGL values in context of the existing empirical effect concentration is presented in Figure 2. For this plot, the toxic responses are placed into severity categories according to the AEGL levels: no effect, discomfort, disabling, some lethality, lethality (100 %). For humans, the only available indication of an adverse effect, i.e. irritative symptoms of the eyes at 1 ppm for a 10 minute exposure, which is however based on an assumption by Wang et al. (1988), was used to better categorize the animal data.

These comparisons with available experimental data indicate, that the derived AEGL values are protective for humans at any of the 3 levels of severity.

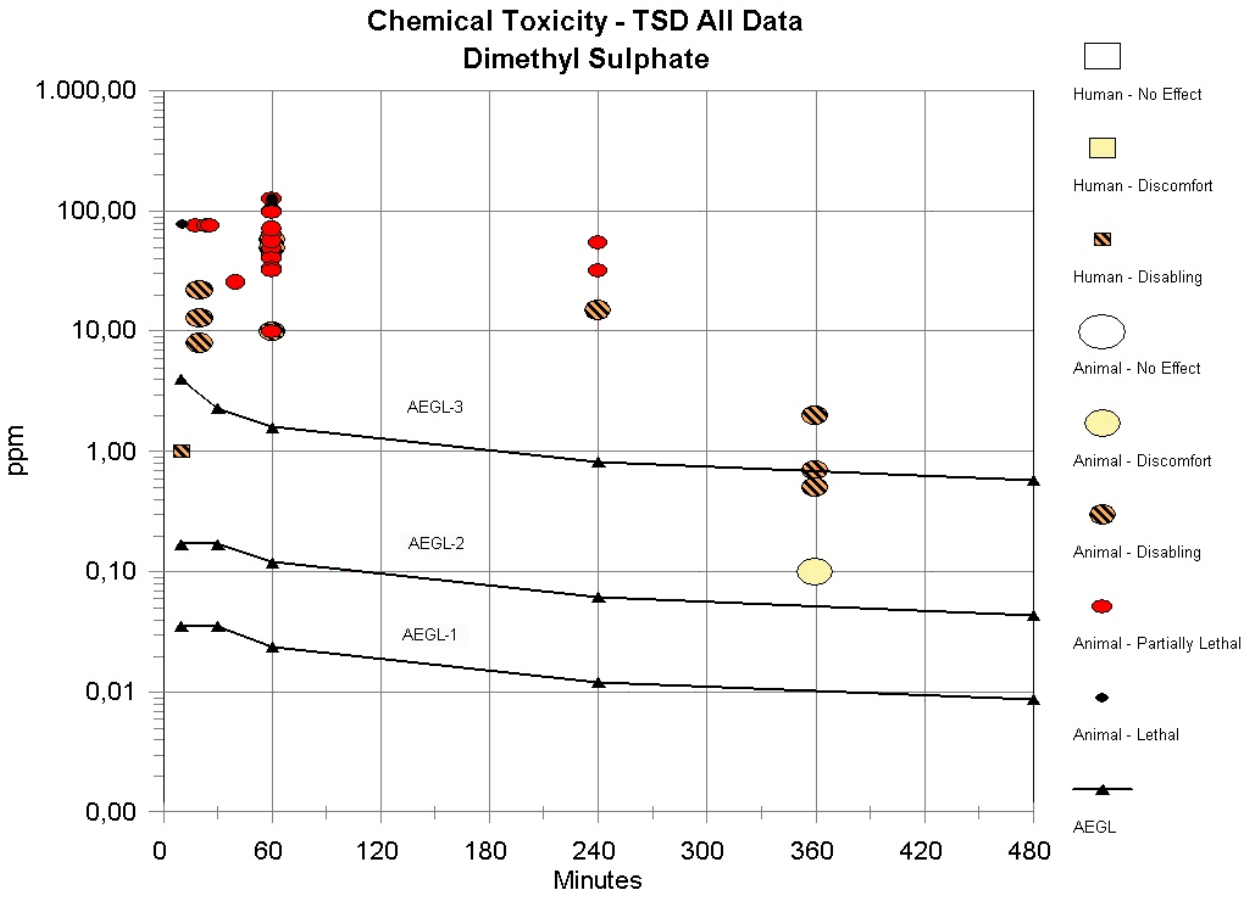


Figure 2: Category Plot of Toxicity Data compared to AEGL Values

8.2. Comparison with Other Standards and Guidelines

Cartlidge et al. (1996) proposed a MEL (maximum exposure limit) of 0.05 ppm (0.26 mg/m^3) for occupational exposure (8-hour TWA) for the UK Health and Safety Executive (HSE). Additionally, a precautionary "Sk" skin notation was considered to be necessary due to the absorption of dimethyl sulfate through skin.

German guidance concentrations (TRK) for dimethyl sulfate production were regulated with 0.02 ppm (0.1 mg/m^3) and 0.04 ppm (0.2 mg/m^3) for dimethyl sulfate use, not based on toxicological consideration.

In Denmark the occupational limit value for dimethyl sulfate is 0.01 ppm (0.05 mg/m^3) (Cartlidge et al. 1996).

The U.S. ACHPPM (1999) derived 1-hour MAG-values (Military Air Guidelines) of 0.3 ppm (minimal effects level), 1 ppm (significant effects level) and 7 ppm (severe effects level). An 1 - 14 day MAG with skin notation of 0.01 ppm is proposed for no significant health effects in the deployed

population for continuous exposure (24 hours to 14 days in duration). Data base for derivation of MAG-values are existing values, however no guideline for dimethyl sulfate is specified.

ECB (2002) calculated a theoretical Health-based Occupational Reference Value (HBORV) of 0.1 mg/m^3 (0.02 ppm) based on a fictive NOAEL of 9 mg/m^3 from a semichronic inhalation study or a NOAEL of 0.9 mg/m^3 from a chronic inhalation study. However it is remarked, that it cannot be excluded, that systemic effects occur even at 0.02 ppm by comparison of these fictive NOAEL´s with available toxicological data from Frame et al. (1993), Alvarez et al. (1997), and Schlögel (1972).

TABLE 12. Existent Standards and Guidelines for Dimethyl Sulfate

Guideline	Exposure Duration				
	10 minute	**30 minute**	**1 hour**	**4 hour**	**8 hour**
AEGL-1	0.035 ppm	0.035 ppm	0.024 ppm	0.012 ppm	0.0087 ppm
AEGL-2	0.17 ppm	0.17 ppm	0.12 ppm	0.061 ppm	0.043 ppm
AEGL-3	4.0 ppm	2.3 ppm	1.6 ppm	0.82 ppm	0.58 ppm
ERPG-1 (AIHA)[a]			-		
ERPG-2 (AIHA)			0.2 ppm		
ERPG-3 (AIHA)			1 ppm		
EEGL (NRC)[b]					
PEL-TWA (OSHA)[c]					0.1 ppm
PEL-STEL (OSHA)[d]					
IDLH (NIOSH)		7 ppm			
REL-TWA (NIOSH)[f]					0.1 ppm
REL-STEL (NIOSH)[g]					
TLV-TWA (ACGIH)[h]					0.1 ppm “skin” notation
TLV-STEL (ACGIH)[i]					
MAK (Germany)[j]					
MAK Peak Limit (Germany)[k]					
MAC (The Netherlands)[l]					0.1
OEL (Denmark)					0.01
HBORV					0.02

[a]**ERPG (Emergency Response Planning Guidelines, American Industrial Hygiene Association** (AIHA 1994)
The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor. An ERPG-1 for dimethyl sulfate was not derived.
The ERPG-2 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 irreversible or other serious health effects or symptoms that could impair an individual's ability to take protection action. The ERPG-2 for dimethyl sulfate is based on experiences in humans.
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 dimethyl sulfate is based on experiences in humans (lethal intoxication after intake of 28 mg (calculated)).

[b]**EEGL (Emergency Exposure Guidance Levels, National Research Council**
The EEGL is the concentration of contaminants that can cause discomfort or other evidence of irritation or intoxication in or around the workplace, but avoids death, other severe acute effects and long-term or chronic injury. An EEGL for dimethyl sulfate was not derived.

[c]**OSHA PEL-TWA (Occupational Health and Safety Administration, Permissible Exposure Limits - Time Weighted Average)** (OSHA 1998)
is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[d]**OSHA PEL-STEL (Permissible Exposure Limits - Short Term Exposure Limit)** (OSHA 1989)
is defined analogous to the ACGIH-TLV-STEL.

[e]**IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)** (NIOSH 1996)
represents the maximum concentration from which one could escape within 30 minutes without any escape-impairing symptoms, or any irreversible health effects. The IDLH for dimethyl sulfate is based on acute inhalation toxicity data in humans.

[f]**NIOSH REL-TWA (National Institute of Occupational Safety and Health, Recommended Exposure Limits - Time Weighted Average)** (NIOSH 1996)
is defined analogous to the ACGIH-TLV-TWA.

[g]**NIOSH REL-STEL (Recommended Exposure Limits - Short Term Exposure Limit)** (NIOSH 1996)
is defined analogous to the ACGIH TLV-STEL.

[h]**ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average)** (ACGIH 1991)
is 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.

[i]**ACGIH TLV-STEL (Threshold Limit Value - Short Term Exposure Limit)** (ACGIH 1991)
is defined as a 15-minute TWA exposure which should not be exceeded at any time during the workday even if the 8-hour TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 minutes and should not occur more than 4 times per day. There should be at least 60 minutes between successive exposures in this range.

[j]**MAK (Maximale Arbeitsplatzkonzentration [Maximum Workplace Concentration])** (Deutsche Forschungsgemeinschaft [German Research Association] 2000)
is defined analogous to the ACGIH-TLV-TWA. The MAK Commission classified dimethyl sulfate as category A2 carcinogen, therefore no MAK was derived.

[k]**MAK Spitzenbegrenzung (Peak Limit [give category])** (German Research Association 2000)
constitutes the maximum average concentration to which workers can be exposed for a period up to 30 minutes with no more than 2 exposure periods per work shift; total exposure may not exceed 8-hour MAK The MAK Commission classified dimethyl sulfate as category A2 carcinogen, therefore no MAK peak limit was derived.

[l]**MAC (Maximaal Aanvaaarde Concentratie [Maximal Accepted Concentration])** (SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment], The Hague, The Netherlands 2000)
is defined analogous to the ACGIH-TLV-TWA.

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APPENDIX A: Derivation of AEGL Values

Derivation of AEGL-1

Key Study:	Frame et al. (1993; abstract publication)
Toxicity endpoint:	Altered nasal cell proliferation from repeated exposure to 0.1 ppm for 6 hours (10 exposures).
Time scaling:	$C^2 \times t$ for extrapolation to 30 minutes, 1 hour, 4 hours, 8 hours $k = 0.1^2$ ppm^2 x 360 min = 3.6 ppm^2 x min The 10-min AEGL-1 was set at the same concentration as the 30-min AEGL-1
Uncertainty factors:	3 for interspecies variability 3 for intraspecies variability Combined uncertainty factor of 10
Modifying factor:	None
Calculations:	
10-minute AEGL-1	10-min AEGL-1 = 30-min AEGL-1 = 0.035 ppm (0.18 mg/m^3)
30-minute AEGL-1	C^2 x 30 min = 3.6 ppm^2 x min C = 0.35 ppm 30-min AEGL-1 = 0.35 ppm/10 = 0.035 ppm (0.18 mg/m^3)
1-hour AEGL-1	C^2 x 60 min = 3.6 ppm^2 x min C = 0.24 ppm 1-hour AEGL-1 = 0.24 ppm/10 = 0.024 ppm (0.12 mg/m^3)
4-hour AEGL-1	C^2 x 240 min = 3.6 ppm^2 x min C = 0.12 ppm 4-hour AEGL-1 = 0.12 ppm /10 = 0.012 ppm (0.062 mg/m^3)
8-hour AEGL-1	C^2 x 480 min = 3.6 ppm^2 x min C = 0.087 ppm 8-hour AEGL-1 = 0.087 ppm/10 = 0.0087 ppm (0.045 mg/m^3)

Derivation of AEGL-2

Key Studies:	Schlögel (1972)
Toxicity endpoints:	Breathing difficulties and asthmatic-like breathing sounds at 0.5 ppm for 6 hours.
Time scaling	$C^2 \times t$ for extrapolation to 30 minutes, 1 hour, 4 hours, 8 hours $k = 0.5^2$ ppm^2 x 360 min = 90 ppm^2 x min The 10-min AEGL-2 was set at the same concentration as the 30-min AEGL-2.
Uncertainty factors:	3 for interspecies variability 3 for intraspecies variability Combined uncertainty factor of 10
Modifying factor:	None
Calculations:	
10-minute AEGL-2	10-min AEGL-2 = 30-min AEGL-2 = 0.17 ppm (0.88 mg/m^3)
30-minute AEGL-2	C^2 x 30 min = 90 ppm^2 x min C = 1.7 ppm 30-min AEGL-2 = 1.7 ppm/10 = 0.17 ppm (0.88 mg/m^3)
1-hour AEGL-2	C^2 x 60 min = 90 ppm^2 x min C = 1.2 ppm AEGL-2 = 1.2 ppm/10 = 0.12 ppm (0.62 mg/m^3)
4-hour AEGL-2	C^2 x 240 min = 90 ppm^2 x min C = 0.61 ppm AEGL-2 = 0.61 ppm/10 = 0.061 ppm (0.32 mg/m^3)
8-hour AEGL-2	C^2 x 480 min = 90 ppm^2 x min C = 0.43 ppm AEGL-2 = 0.43 ppm/10 = 0.043 ppm (0.22 mg/m^3)

Derivation of AEGL-3

Key Studies:	Hein (1969)
Toxicity endpoint:	LC_0 of 49 ppm for 1-hour exposure in rats. Calculation of $BMCL_{05}$ gained 32 ppm.
Time scaling	$C^2 \times t$ for extrapolation to 10 minutes, 30 minutes, 4 hours, 8 hours $k = 49^2$ ppm^2 x 60 min = 144060 ppm^2 x min
Uncertainty factors:	10 for interspecies variability 3 for intraspecies variability Combined uncertainty factor of 30
Modifying factor:	None
10-minute AEGL-3	$C^2 \times 10$ min = 144060 ppm^2 x min C = 120 ppm 10-min AEGL-3 = 120 ppm/30 = 4.0 ppm (21 mg/m^3)
30-minute AEGL-3	$C^2 \times 30$ min = 144060 ppm^2 x min C = 69 ppm 30-min AEGL-3 = 69 ppm/30 = 2.3 ppm (12 mg/m^3)
1-hour AEGL-3	C = 49 ppm 1-hour AEGL-3 = 49 ppm/30 = 1.6 ppm (8.3 mg/m^3)
4-hour AEGL-3	$C^2 \times 240$ min = 144060 ppm^2 x min C = 24.6 ppm 4-hour AEGL-3 = 24.6 ppm/30 = 0.82 ppm (4.3 mg/m^3)
8-hour AEGL-3	$C^2 \times 480$ min = 144060 ppm^2 x min C = 17.4 ppm 8-hour AEGL-3 = 17.4 ppm/30 = 0.58 ppm (3.0 mg/m^3)

APPENDIX B: Carcinogenicity Assessment

Cancer Assessment for Dimethyl Sulfate

Based on a carcinogenic study conducted by Schlögel (1972) calculations to elucidate dose-response relationship were conducted using the ppm-hour factor. Schögel exposed rats, mice and golden hamsters to 0.5 ppm, 2 ppm and to a sublethal dose (rats 34 ppm, mice 48 ppm, golden hamsters 20 ppm). The incidences of benign and malignant tumors of respiratory tract, eyes and related organs were determined. An increased incidence of nose and lungs was observed following dimethyl sulfate exposure. This study shows a limited quality concerning number of animals, dose levels, and exposure duration, however there is no other suitable study for derivation of a quantitative risk figure.

Calculation of dose-response relationship

0.5 ppm group

The animals were exposed for about 15 month, twice a week, however higher concentration and frequence of exposure were conducted for the first weeks (about 1 month). No statement can be made on the exact concentration and duration (personal communication). Therefore a ppm-hour calculation results in at least 420 ppm-hour, if 0.5 ppm were used for the whole duration:
1. month (5 6-hour exposures / week) = 20 exposures = 120 hours
2. - 15- month (2 6-hour exposures / week) = 120 exposures = 720 hours
ppm-hour factor = 720 hours x 0.5 ppm = 420
Cancer incidence for all animals = 5.2 %

2 ppm group

All animals were exposed for about 15 month with constant dimethyl sulfate-concentration.
1. - 15- month (1 6-hour exposure every two weeks) = 32 exposures = 192 hours
ppm-hour factor = 192 hours x 2 ppm = 384
Cancer incidence for all animals = 13.5 %

sublethal group

All animals were exposed 4 times within a year.
4 exposures (1 exposure every 3 month for 1 hour) = 4 hours
ppm-hour factor for rats = 4 hours x 34 ppm = 136
ppm-hour factor for mice = 4 hours x 48 ppm = 192
ppm-hour factor for golden hamsters = 4 hours x 20 ppm = 80
Cancer incidence for rats = 3.1 %
No tumors were observed in mice and golden hamster.

The highest ppm-hour factor results from the 0.5 ppm group, but the highest cancer incidence for treatment related tumors was found in the 2 ppm group. Therefore no dose-response relationship can be drawn. At this concentration, as well as at 0.5 ppm cytotoxicity of respiration tract was observed.

*Unit risk calculation*s

As reported in Section 3.8., ECB (2002) conducted a carcinogenic risk estimation for dimethyl sulfate based on the results from Schlögel (1972). The 2 ppm dosage scheme resulted in the highest incidence of malignant tumors and was therefore used for risk assessment.

Calculations gained a carcinogenic activity attributable to the exposure to the substance per unit concentration (expressed per mg/m^3), expressed as I_{conc}.

I_{conc} = (6/27 - 0/36) / (10.5 x 456/728 x 613/728 x 6/24 x 1/14) = 2.2 (mg/m^3)$^{-1}$

I_{conc}	carcinogenic activity attributable to the exposure to the substance per unit concentration (expressed per mg/m$^{3)}$
I_e	6/27 (incidences of malignant tumors in exposed male and female animals)
I_c	0/36 (incidences of malignant tumors in control male and female animals)
C	10.5 (concentration in experiment in mg/m^3)
X_{po}	15 month, 456 days (exposure period)
X_{pe}	613 days (mean survival time found in the exposure group)
L	728 days (mean survival time found in the control group)

Calculation:

To calculate a concentration of dimethyl sulfate that would cause a theoretical excess cancer risk of 10^{-4} the risk is divided by the 1-day carcinogenic activity:

dose = 1x10^{-4} / 2.2 (mg/m^3)$^{-1}$ = 0.045 µg/m^3

To convert a 75-year exposure to a 24-hour exposure, the concentration is multiplied by the number of days in 75 years:

24-hour exposure concentration = 0.045 µg/m^3 x 27375 = 1232 µg/m^3

To convert to an 8-hour exposure an inhalation volume of 10 m^3 for occupational exposure and 20 m^3 for 24-hour is used:

8-hour exposure = 1232 µg/m^3 x 20/10 = 2464 µg/m^3

To adjust for uncertainties in assessing potential cancer risks under short-term exposures the 8-hour exposure is divided by an adjustment factor of 6 (see NAC 2000):

2464 µg/m^3 / 6 = 411 µg/m^3 (0.079 ppm)

Corresponding, calculations for 10^{-5} and 10^{-6} risk levels are conducted:

10^{-5} risk level = 41 µg/m^3 (8-hour exposure)
10^{-6} risk level = 4.1 µg/m^3 (8-hour exposure)

Due to the missing dose-effect relationship calculations on carcinogenic risk levels are uncertain. Dimethyl sulfate reveals the potential to react with nucleophilic groups of nucleic acids and therefore acts as a directly genotoxic agent (Löfroth et al. 1974; Mathison et al. 1995; Swann and McGee 1968). However, the observed cancer incidence in the study conducted by Schlögel (1972) may have been influenced by cytotoxic effects (irritant effects in target tissues) as seen at the exposure concentration.

Concluding remark: The 10^{-4} risk level is above AEGL-2 for 8-hour exposure.

APPENDIX C:
Derivation Summary for Acute Exposure Guideline Levels for Dimethyl Sulfate

AEGL-1 Values				
10 min	30 min	1 h	4 h	8 h
0.035 ppm	0.035 ppm	0.024 ppm	0.012 ppm	0.0087 ppm

Reference:
Frame, S.R., Panepinto, A.S., Bogdanffy, M.S., 1993. Effects of inhalation exposure to dimethyl sulfate on nasal epithelial cell proliferation. The Toxicologist, 13, 389.

Test Species/Strain/Sex/Number:
rats; number not indicated; sex not specified

Exposure Route/Concentration/Durations:
inhalation (nose-only); 0, 0.1, 0.7, or 1.5 ppm for 6 h/d, 2 wk (10 exposures)

Effects:
All concentrations:
Nasal epithelial cell proliferation. Severity of this lesions decreased from anterior to posterior regions. Hypertrophy, hyperplasia, and squamous metaplasia were restricted to respiratory epithelium. For 0.1 ppm these effects were described as slight.
0.7, 1.5 ppm:
Dose-dependent lesions of respiratory and olfactory epithelium (erosion, ulceration, atrophy)

Endpoint/Concentration/Rationale:
6-h exposure to 0.1 ppm resulted in changes in nasal epithelial cell proliferation (decreased labeling index for respiratory epithelium; increased labeling index for olfactory epithelium)

Uncertainty Factors/Rationale: Total uncertainty factor: 10
Interspecies: 3 - little species variability is observed at lethal and non-lethal endpoints
Intraspecies: 3 - no large differences in susceptibility between individuals are expected for unspecific irritating effects.

Modifying Factors: None

Animal to Human Dosimetric Adjustment: Not applied (insufficient data)

Time Scaling: $C^2 \times t$ for extrapolation to 30 minutes, 1 hour, 4 hours, 8 hours. The 10-min AEGL-1 was set at the same concentration as the 30-min AEGL-1

Data Adequacy:
Although the adequacy of data is limited due to the publication as an abstract and due to repeated exposure, the AEGL-1 values are supported by additional observations of slight effects in rats exposed to 0.5 ppm for 6 hours (Schlögel 1972).

AEGL-2 values				
10 min	30 min	1 h	4 h	8 h
0.17 ppm	0.17 ppm	0.12 ppm	0.061 ppm	0.043 ppm
Reference: Schlögel, F. A., 1972. Cancerogenität und chronische Toxizität inhalierten Dimethylsulfats. Med. Inaug.-Dissertation, Universität Würzburg.				
Test Species/Strain/Sex/Number: Wistar rats, NMRI mice, Syrian golden hamsters; males and females; 10 or 15 animals				
Exposure Route/Concentration/Durations: Inhalation (whole-body); 0, 0.5, 2 ppm; 6 h; repeated exposure				
Effects: 0.5 ppm: Changes in behavior within some animals of all species already after 20 minutes (ruffled fur; closed or half-closed eyes). Following exposure, the animals developed breathing problems (cough and sneezing). 2 ppm: Conjunctivitis with sensitivity to light; expiration sounds similar to asthmatics. All described effects occurred already after first exposure.				
Endpoint/Concentration/Rationale: breathing problems following 6-h exposure to 0.5 ppm				
Uncertainty Factors/Rationale: Total uncertainty factor: 10 Interspecies: 3 - little species variability is observed at lethal and non-lethal endpoints Intraspecies: 3 - no large differences in susceptibility between individuals are expected for unspecific irritating effects.				
Modifying Factors: None				
Animal to Human Dosimetric Adjustment: Not applied (insufficient data)				
Time Scaling: $C^2 \times t$ for extrapolation to 30 minutes, 1 hour, 4 hours, 8 hours. The 10-min AEGL-2 was set at the same concentration as the 30-min AEGL-2.				
Data Adequacy: The study was well conducted and was extensively reported (as doctoral thesis). However, it was conducted for investigating the carcinogenicity and chronic effects of dimethyl sulfate, therefore short-term effects not reported in detail, but have been inquired by the author. Concentration was regularly controlled by gas chromatography.				

AEGL-3 Values				
10 min	30 min	1 h	4 h	8 h
4.0 ppm	2.3 ppm	1.6 ppm	0.82 ppm	0.58 ppm

Reference:
Hein, N., 1969. Zur Toxicität von Dimethylsulfat. Med. Inaug.-Dissertation, Universität Würzburg.

Test Species/Strain/Sex/Number:
5 female Wistar rats, 10 or 20 female NMRI mice, 5 golden hamsters, 5 guinea pigs (sex not indicated);

Exposure Route/Concentration/Durations:
Inhalation (whole-body); 10, 49, 64, 71, 127 ppm (rats), 10, 42, 49, 64, 71, 127 ppm (mice), 33, 40, 49, 64, 71, 127 ppm (hamster), 10, 33, 40, 71 ppm (guinea pigs); 1 h

Effects:
The highest LC_0 for rats have been 49 ppm (Hein 1969) and 58 ppm (DuPont 1971) for an 1-h exposure, and 15 ppm (Smyth 1956) for a 4-h exposure. Guinea pigs have been most susceptible to DMA vapor (LC_{50} of 32 ppm).
49 ppm:
During exposure dyspnea with inspiratory stridor and lacrimation were noticed. Necropsy showed severe inflation of stomach and small intestine and occasionally emphysema and edema of lungs.

Endpoint/Concentration/Rationale:
Pulmonary congestion and severe inflations of GIT have been observed at 1-h exposure to 49 ppm.

Uncertainty Factors/Rationale: Total uncertainty factor: 30
Interspecies: 10 - little species variability is observed at lethal and non-lethal endpoints, but rats as the species used for the AEGL-3 derivation was not the most susceptible one.
Intraspecies: 3 - no large differences in susceptibility between individuals are expected for unspecific irritating effects.

Modifying Factors: None

Animal to Human Dosimetric Adjustment: Not applied (insufficient data)

Time Scaling: $C^2 \times t$ for extrapolation to 10 minutes, 30 minutes, 4 hours, 8 hours

Data Adequacy:
The study was well conducted and was extensively reported (as doctoral thesis). Concentration was regularly controlled by gas chromatography. A 3-week follow-up observation was used to cover effects developed after a latency period.