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INTERIM ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

1,4-DIOXANE

(CAS Reg. No. 123-91-1)

for
NAS/COT Subcommittee for AEGLs

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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. AEGL-2 and AEGL-3 levels, and AEGL-1 levels as appropriate, will be developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and will be distinguished by varying degrees of severity of toxic effects. It is believed that the recommended exposure levels are applicable to the general population including infants and children, and other individuals who may be sensitive or susceptible. The three AEGLs have been defined as follows:

AEGL-1 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 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 odor, taste, and sensory irritation, or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL level, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL level. Although the AEGL values represent threshold levels for the general public, including sensitive subpopulations, it is recognized that certain individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL level.

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EXECUTIVE SUMMARY

1,4-Dioxane is a colorless combustible liquid with a characteristic unpleasant odor. Hellman and Small (1974) reported an odor detection threshold of 1.8 ppm and an odor recognition threshold of 5.7 ppm. Several studies reported that the initial strong odor diminished rapidly during exposure. In a toxicokinetic study on humans, exposure to 50 ppm for 6 h led to eye irritation (Young et al., 1977). In other experimental studies, exposure to 300 ppm for 15 min led to irritation of eyes, nose and throat; after exposure for an unspecified exposure time, irritation was quite distinct at 1400 ppm and at 2800 ppm subjects complained of very strong initial irritation and slight pressure in the chest (Wirth and Klimmer, 1936). Yant et al. (1930) reported eye irritation, resulting in blinking, squinting and lacrimation, a burning sensation in nose and throat and slight vertigo in subjects exposed to 5500 ppm dioxane for 1 minute; 1600 ppm for 10 minutes resulted in an immediate slight burning of the eyes accompanied by lacrimation and nasal irritation. A few lethal cases have been reported after repeated occupational exposure to unknown dioxane concentrations. Initial signs and symptoms comprised nausea and vomiting, described as "stomach trouble" by the workers, followed after 2-3 days by oliguria and anuria. About 3-7 days after the first symptoms, coma developed, followed by death. Microscopic examinations revealed centrilobular liver necrosis, almost symmetrical necrosis of the outer renal cortex and hemorrhages around the glomeruli. Studies on exposed workers did not reveal evidence of genotoxic or carcinogenic effects of dioxane.

Acute toxic effects in animals are mainly central nervous system depression, kidney and liver damage as well as irritation effects. At lethal concentrations, narcosis has been observed in rats and guinea pigs. Pozzani et al. (1959) reported a 4-hour LC_{50} for dioxane of 14,300 ppm in rats. A similar LC_{50} value of 12,800 ppm for 4 hours was reported by Pilipyuk et al. (1977). Rats exposed for 2x1.5 hours per day at 5000 ppm died after 3-5 consecutive exposure days (Fairley et al., 1934). Necropsy findings included evidence of serious kidney and liver damage, such as patchy cell degeneration of the cortical tubules, inter- and intratubular hemorrhages and liver cell degeneration varying from cloudy swelling to large areas of complete necrosis. A 2-hour LC_{50} value of 18,000 ppm in mice has been reported (Pilipyuk et al., 1977). Goldberg et al. (1964) studied the effect of dioxane on avoidance behavior (conditioned response) and on escape behavior (unconditioned response) of rats using a pole climbing test. After the training period, rats were exposed 4 hours/day, 5 days/week for 2 weeks. Behavior measurements were performed after every exposure. At 6000 ppm, 6/8 rats showed a delay of the conditioned response behavior after the 1st exposure, while in the subsequent exposures between 3 and 8 of a total of 8 rats were affected. Effects on the escape response were not observed. Drew et al. (1978) reported significantly increased serum activities of liver enzymes (ornithine carbamyl transferase, aspartate aminotransferase and alanine aminotransferase) in rats after a single 4-hour exposure at 1000 or 2000 ppm dioxane. Frantik et al. (1994) studied the inhibition of propagation and maintenance of the electrically evoked seizure discharge in rats and mice. Of six different time characteristics recorded, the duration of tonic extension of hindlimbs in rats and the velocity of tonic extension in mice were the most sensitive and reproducible response measures. The authors suggested the EC_{10} as the effect threshold, which was 1200 ppm for 4 hours in rats and 580 ppm for 2 hours in mice. No indication of teratogenic or fetotoxic effects was found in rats after dosing at up to 517 mg/kg/d by gavage on gestational days 6-15. Dioxane did not induce gene mutations in Salmonella typhimurium. It did not induce TK gene mutations in mouse lymphoma L5178 tk+/- cells or HGPRT gene mutations or chromosomal aberrations in Chinese hamster ovary cells. However, it did induce a slight increase in sister chromatid exchange in the absence of metabolic activation and caused morphological transformation of BALB/c 3T3 mouse cells. Oral

administration of high doses to rats caused DNA strand breaks and micronuclei formation in liver cells. No induction of unscheduled DNA synthesis was observed in rat hepatocytes at up to 2 % dioxane in drinking water. Of six bone-marrow micronucleus tests, five were negative, while one was positive. When administered orally at 0.5 % or higher in drinking water (corresponding to about 500 mg/kg/day), dioxane produced malignant tumors of the nasal cavity and liver in rats and tumors of the liver and gallbladder in guinea pigs. It was also active as a promotor in a two-stage skin carcinogenesis study in mice. A lifetime bioassay exposing rats at 111 ppm for 7 hours/day, 5 days/week found no evidence for carcinogenic effects.

For the derivation of AEGL-1 values, irritation was considered the most relevant endpoint. As key study, the pharmacokinetic study of Young et al. (1977) was chosen, because this was the only adequately reported and analytically controlled study available for this endpoint. Four healthy men reported eye irritation at 50 ppm throughout the 6-hour exposure period. Since this was a pharmacokinetic study, no emphasis was put on reporting of symptoms and the authors did not define the severity level of the eye irritation. The irritation was nevertheless considered to be below the notable discomfort level as described in the AEGL-1 definition because the authors (Young et al., 1977) considered 50 ppm as an adequate workplace standard and a level of 50 ppm has been used as a workplace standard in the past. A total uncertainty factor of 3 was used because for local effects, the toxicokinetic differences do not vary considerably within and between species. Since the study by Young et al. (1977) reported eye irritation throughout the whole exposure period of 6 hours and did not report an increase of the effect with time, the same exposure concentration was applied to all time points. Using a constant value for the AEGL-1 is also supported by the observation of Yant et al. (1930) who reported no eye irritation, squinting and lacrimation in Guinea pigs exposed to 1000 ppm for up to 6 hours, while at 2000 ppm or higher these symptoms were observed within 8 minutes or less.

A level of distinct odor awareness (LOA) for 1,4-dioxane of 1.7 ppm was derived on the basis of the odor detection threshold from the study of Hellman and Small (1974). The LOA represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, about 10 % of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odor perception.

For the AEGL-2, two independent derivations based on central nervous system effects and liver effects were elaborated. The two approaches led to identical AEGL-2 values and were mutually supportive. With regard to central nervous system effects, Goldberg et al. (1964) reported that exposure at 6000 ppm for 4 hours affected the performance of rats in an conditioned response test (pole climbing in response to buzzer to avoid electrical shock), but did not affect the escape response to an electrical shock. This observation was made after one as well as after repeated exposures. The exposure level of 6000 ppm for 4 hours was considered a NOEL for central nervous system depression. Higher concentrations caused narcosis in mice (8300 pm for 3.5 hours; Wirth and Klimmer, 1936) and guinea pigs (30,000 ppm for 1-2 hours; Yant et al., 1930). A total uncertainty factor of 30 was used. The interspecies factor was reduced to 3 because the toxicodynamic differences between species were considered limited for CNS depression and because application of the default factor would have lowered the AEGL-2 values to a level that humans are known to tolerate without adverse effects (Young et al., 1977). An intraspecies factor of 10 was applied. The other exposure duration-specific values were derived by time scaling according to the dose-response regression equation $C^n * t = k$, using the default of n=3 for shorter exposure periods and

n=1 for longer exposure periods, due to the lack of suitable experimental data for deriving the concentration exponent. Time extrapolation was continued to the 10-minute period because even at considerably higher concentrations of 1600 ppm for 10 minutes (Yant et al., 1930) volunteers did not experience more severe effects than moderate eye, nose and throat irritation.

With regard to liver effects, the study by Drew et al. (1978) reported increased the serum activities of liver enzymes after a single exposure of rats at 2000 ppm for 4 hours. While the reported 2-3-fold increase in liver enzymes was considered a weak, reversible liver damage because chemicals, viruses or tumors can easily increase aminotransferase levels by 10- to 100-fold in rats and humans, lethal liver and kidney damage occurred in rats after exposure at 5000 ppm for 2x1.5 hours/day after at few days from (Fairley et al., 1934). Therefore, the level of 2000 ppm for 4 hours was considered an adequate basis for AEGL-2 derivation. A total uncertainty factor of 10 was used. An interspecies uncertainty factor of 1 was applied because metabolism in humans and rats is very similar, involving the same metabolic steps and intermediate metabolites and because application of a total uncertainty factor of 30 would reduce the AEGL-2 level to an exposure concentration of 17 ppm for 8 hours and 33 ppm for 4 hours, which humans are known to tolerate without adverse effect (pharmacokinetic study exposing subjects to 50 ppm for 6 hours; Young et al., 1977). An intraspecies factor of 10 was applied. The other exposure duration-specific values were derived by time scaling according to the dose-response regression equation $C^n * t = k$, using the default of n=3 for shorter exposure periods and n=1 for longer exposure periods, due to the lack of suitable experimental data for deriving the concentration exponent. Time extrapolation was continued to the 10-minute period because even at considerably higher concentrations of 1600 ppm for 10 minutes (Yant et al., 1930) exposed subjects did not experience more severe effects than moderate eye, nose and throat irritation.

The AEGL-3 was based on a 4-hour LC_{50} for dioxane of 14,300 ppm in rats (Pozzani et al., 1959) because this was the only acute inhalation study described in sufficient detail. This study was supported by the study of Pilipyuk et al. (1977), which was reported in insufficient detail to serve as key study. For extrapolation from the LC_{50} value to the threshold for lethality, a divisor of 3 was used. This divisor was considered adequate because available data indicated a very steep dose-response curve for lethality after inhalation exposure (Pilipyuk et al., 1977; Yant, 1930). A total uncertainty factor of 10 was used. An interspecies uncertainty factor of 1 was applied because metabolism in humans and rats is very similar, involving the same metabolic steps and intermediate metabolites and because a higher uncertainty factor would have resulted in AEGL-3 values of 480 ppm for 10 and 30 minutes, which contrasts with the observation that exposure of human subjects to 1600 ppm for 10 minutes (Yant et al., 1930) resulted in moderate irritation, but not in more severe effects. An intraspecies factor of 10 was applied. The other exposure duration-specific values were derived by time scaling according to the dose-response regression equation $C^n * t = k$, using the default of n=3 for shorter exposure periods and n=1 for longer exposure periods, due to the lack of suitable experimental data for deriving the concentration exponent. For the 10-minute AEGL-3 the 30-minute value was applied because the derivation of AEGL values was based on a long experimental exposure period and no supporting studies using short exposure periods were available for characterizing the concentration-time-response relationship.

The calculated values are listed in the table below.

SUMMARY TABLE OF PROPOSED AEGL VALUES FOR 1,4-DIOXANE [a]

Classification	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour	Endpoint (Reference)
AEGL-1 (Nondisabling)	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)	irritative effects in humans (Young et al., 1977)
AEGL-2 (Disabling)	580ppm (2100 mg/m³)	400 ppm (1400 mg/m³)	320 ppm (1200 mg/m³)	200 ppm (720 mg/m³)	100 ppm (360 mg/m³)	central nervous system effects in rats (no narcosis) (Goldberg et al., 1964); liver enzyme increase in rats (no severe necrosis) (Drew et al., 1978)
AEGL-3 (Lethal)	950 ppm (3400 mg/m³)	950 ppm (3400 mg/m³)	760 ppm (2700 mg/m³)	480 ppm (1700 mg/m³)	240 ppm (860 mg/m³)	extrapolated NOEL for acute lethality in rats (Pozzani et al., 1959; Pilipyuk et al., 1977)

[a] Cutaneous absorption may occur; direct skin contact with the liquid should be avoided.

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1. INTRODUCTION

1,4-Dioxane is a colorless combustible liquid with a characteristic unpleasant odor (NIOSH, 1977).

There are three types of production processes for dioxane: 1) the most important synthesis is by acid-catalyzed conversion of diethylene glycol (or other ethylene glycols) by ring closure in a closed system; 2) catalyzed cyclo-dimerization of ethylene oxide on acid ion exchange resins via oligo-ethylene sulphonates; 3) ring closure of 2-chloro-2'-hydroxyethyl ether through heating with 20 % sodium hydroxide (ECB, 1999). The technical grade product is >99.9 % pure, but may contain bis(2-chloroethyl)-ether as an impurity (DeRosa et al., 1996). ECB (1999) states as impurities water (<=0.1 %), 2-methyl-1,3-dioxane (<=0.1 %), 2-ethyl-1,3-dioxane (<=0.03 %) and hydrogen peroxide (<=0.001 %); 2,6-tert.-butyl-p-cresol is found as a stabilizing additive).

The world-wide production capacity in 1995 was estimated at 8000-10000 metric tons with a production volume in Europe of 2000-2500 metric tons per year (for 1997) (ECB, 1999) and in the US of about 7500 metric tons per year (for 1977) (NIOSH, 1977).

Dioxane has a great variety of applications. Because of its physical-chemical properties it is used mainly as a processing solvent (waxes, fat, lacquers, varnishes, cleaning and detergent preparation, pharmaceuticals, pesticides, adhesives, cosmetics, cellulose derivatives, magnetic tape). It is also used as extraction medium for animals and vegetable oils and as a laboratory chemical (ECB, 1999).

Chemical and physical properties of 1,4-dioxane are listed in Table 1.

TABLE 1: CHEMICAL AND PHYSICAL DATA

Parameter	Value	Reference
Molecular formula	$C_4H_8O_2$	IARC, 1999
Molecular weight	88.11	IARC, 1999
CAS Registry Number	123-91-1	IARC, 1999
Synonyms	diethylene-1,4-dioxide; 1,4-dioxacyclohexane; diethylene ether; tetrahydro-p-dioxane	ECB, 1999
Physical state	liquid	IARC, 1999
Color	colorless	IARC, 1999
Density	1.034 g/cm³	ECB, 1999
Vapor pressure	40 hPa at 20 ˚C	ECB, 1999
Vapor density	3.0 (relative to air = 1)	NICNAS, 1998
Melting point	11.8 ˚C	IARC, 1999

Boiling point	101.1 ˚C	IARC, 1999
Solubility	miscible in water and most organic solvents	IARC, 1999
Explosive limits in air	upper, 22 %(v/v); lower, 2 %(v/v)	IARC, 1999
Conversion factors	1 ppm = 3.6 mg/m³ 1 mg/m³ = 0.278 ppm	ECB, 1999

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

A few case reports on delayed lethal effects in humans after inhalation exposure at the workplace are available. No fatalities have been reported after oral or dermal contact with 1,4-dioxane. The health effects of dioxane on humans are summarized in Table 2.

2.2.1. Case Studies

Barber (1934), reported on the death of 5 men, aged 29-38, exposed to dioxane in an artificial silk plant in England (further described by Henry, 1933). The exposures occurred in an experimental plant where two similar machines were used to treat cellulose acetate yarn with dioxane. After process installation in 1932, the process in one of the two machines was altered in October 1933. The vessel containing dioxane was enclosed without exhaust ventilation. Therefore, workers were exposed to concentrated dioxane vapor when the enclosure was opened for manipulation of the yarn. Dioxane concentrations were not reported. The exposures probably involved inhalation and dermal contact. According to Barber (1934), 16 men were definitely exposed to dioxane, and 8 or 9 of these had worked on the machine where the process was altered. Seven of these became ill between the 5th and 19th of November, and 5 men died between the 11th and 25th of November. Signs and symptoms of poisoning comprised nausea and vomiting, described as "stomach trouble" by the workers, followed after 2-3 days by oliguria and anuria; no signs of jaundice were observed. Leukocytosis was present in all cases. About 3-7 days after the first symptoms, coma developed, followed by death. Pathological findings included enlarged pale livers, swollen hemorrhagic kidneys, and edematous lungs and brains. Microscopic examinations revealed centrilobular liver necrosis, almost symmetrical necrosis of the outer renal cortex and hemorrhages around the glomeruli. Nothing was reported about the two workers who survived.

Johnstone (1959) reported the case of a worker who had placed an open container of dioxane between his feet with no ventilation while using the solvent during working hours to manually remove glue form a table top and also for cleaning his hands (i.e. additional dermal exposure occurred). Later measurements of the atmosphere showed a dioxane concentrations between 208 and 650 ppm. After 6 days of work, the man (aged 21) became hospitalized with severe epigastric pain. The patient developed oliguria, became comatose on the 6th day and died one day later. Upon postmortem examination, the liver showed uniformly severe centrilobular necrosis and the kidneys showed cortex necrosis with extensive interstitial hemorrhage. The exposure from the additional dermal contact with dioxane was not estimated quantitatively.

2.2. Nonlethal Toxicity

Several experimental studies were performed regarding odor perception and irritative effects as well as toxicokinetic properties of dioxane. Two reports investigated possible effects of occupational exposure to dioxane. The health effects of dioxane on humans are summarized in Table 2.

2.2.1. Experimental Studies

Young et al. (1977) performed a pharmacokinetic study on humans. Four healthy male subjects, 40-49 years old (smoking status not reported), were exposed for 6 hours at 50 ppm. In the dynamic chamber (26.7 m^3) an airflow of 3.7-4.2 m^3/min was maintained throughout the exposure. Dioxane vapor was generated by pumping dioxane with a syringe pump into a glass vaporization flask heated to 90-100 ˚C. A nitrogen flow of 5 l/min was conducted through the flask to sweep the dioxane vapor into the chamber airstream. A circulating fan was used inside the chamber to achieve uniform distribution. Analytical monitoring of the dioxane concentration in the chamber was done using a Wilks Miran 1 infrared analyzer. The subjects received an extensive physical examination including chest X-ray, electrocardiogram, respiratory function tests, conventional blood chemistry determinations and urinalysis prior to the study. Following exposure, all tests, except for the radiograph, were repeated at 24 hours and at 2 weeks. Samples of blood and urine collected during and after the exposure were analyzed for dioxane and its metabolite, 2-hydroxy-ethoxyacetic acid, by gas chromatography and mass spectrometry. Eye irritation was a frequent complaint throughout the exposure. The perception of odor diminished with time; two of the subjects could not perceive the odor after 4 and 5 hours in the chamber, while the other two subjects could still detect the odor at the end of the exposure period. Results relating to pharmacokinetics are summarized in Section 4.1. Liver enzyme measurements were not performed after the exposure.

Silverman et al. (1946) studied the sensory response to industrial solvent vapors including dioxane. An average number of 12 subjects of both sexes were exposed for 15 minutes, the exact number of subjects exposed to dioxane was not given. The subjects were aware of the exposure, no control exposure to air was performed. A motion picture was shown to the subjects to divert their attention from the exposure. Air-vapor concentrations were produced in a dynamic exposure chamber by continuously adding a known quantity of air saturated with dioxane to the measured flow of air being continuously forced into the chamber. The subjects were exposed to 200 or 300 ppm technical grade dioxane. The majority of subjects exposed to dioxane at 300 ppm reported irritation to eyes, nose and throat, although they did not find the odor objectionable. The authors concluded that "... sensory tests show 200 ppm to be the highest concentration acceptable" for an 8-hour exposure; however, they did not state whether or not the exposed subjects experienced irritative effects at 200 ppm. No further details or experimental results were reported.

Yant et al. (1930) exposed 5 volunteers for 1 minute at 5500 ppm dioxane vapor. The subjects reported irritation to the eyes, resulting in blinking, squinting and lacrimation, and a burning sensation in nose and throat. Three of the subjects noticed a slight vertigo which disappeared quickly after ending the exposure. When the same subjects were exposed at 1600 ppm for 10 minutes, they noted an immediate slight burning of the eyes accompanied by lacrimation, slight irritation of the nose and throat and an alcohol-like odor, which decreased in intensity with continued exposure. Lacrimation and nasal irritation persisted throughout the test. No vertigo was noted. One person complained of an "upset stomach" after exposure. The specifications of the exposure chamber, the purity of dioxane and the methods of

generating and measuring the dioxane atmospheres were not reported.

Wirth and Klimmer (1936) exposed 5 subjects (probably the authors themselves and institute coworkers) to dioxane concentrations of 0.7, 1.4, 2.8, 5.6, 8.4, 280, 1400 or 2800 ppm in a glass and stoneware exposure chamber for unspecified durations. The lower concentrations (up to 8.4 ppm) were generated by evaporating the calculated amount of dioxane from a filter paper with the aid of a fan. The higher concentrations were obtained by dispersing dioxane using a compressed-air sprayer. Slight mucous membrane irritation was reported at 280 ppm. At 1400 ppm, the irritation was quite distinct with slight stinging in the nose and scratchiness and dryness in the throat. At 2800 ppm, irritation was initially very strong and complaints of slight pressure in the chest were expressed. The subjects became accustomed to the irritation and odor after a few minutes, but continued to experience an unpleasant, metallic, bitter taste.

Fairley et al. (1934) exposed groups of 4 and 6 subjects in an exposure chamber at 1000 ppm for 5 minutes or 2000 ppm for 3 minutes, respectively. The concentrations were obtained by vaporizing a 1:4 dioxane-water mixture in a 10-m^3 chamber. At 1000 ppm, a rather sickly odor was detected immediately. The subjects observed a sensation of warmth in the throat and chest, which rapidly faded. One subject experienced a sense of constriction in the throat. At 2000 ppm, the initial strong ethereal or spirituous odor appeared to diminish rapidly during exposure. No lacrimation or desire to cough were noted.

The American Industrial Hygiene Association evaluated odor threshold studies and reported a range of 0.8-172 ppm with a geometric mean of 12 ppm for the odor detection threshold and a range of 1.8-278 ppm with a geometric mean of 22 ppm for the odor recognition threshold (AIHA, 1989). In a review article, Amoore and Hautala (1983) reported a geometric mean odor detection threshold of 24 ppm using odor thresholds reported in the literature, but "omitting extreme points and duplicate quotations".

Hellman and Small (1974) reported the absolute (detection) and recognition thresholds of 101 petrochemicals, determined using a trained odor panel in the Union Carbide Technical Center, South Charleston, WV. An odor fountain was placed about 14 inches below the vent pipe which carried the odorous stream out of the exposure chamber. Details of the procedure used are not reported. The odor detection threshold was 1.8 ppm. At this concentration "50 % of the odor panel observed an odor in the working fountain". The odor recognition threshold was the concentration at which 50 % "of the odor panel defined the odor as being representative of the odorant being studied". The odor recognition threshold was 5.7 ppm.

May (1966) reported an odor detection threshold of 170 ppm and a recognition threshold of 270 ppm. In this experiment, a panel of 8 men and 8 women sniffed graded dilutions of dioxane from wide-mouth flasks.

Wirth and Klimmer (1936), using exposure of 5 subjects (probably the authors themselves and institute coworkers) to different dioxane concentrations in an exposure chamber, reported thresholds of 2.8 ppm for recognition and 5.6 ppm for detection.

2.2.2. Occupational Exposure

Thiess et al. (1976) published a study of 74 workers (aged 32-62) with a cumulative potential

exposure of 1840 man-years and an average duration of 25 years with estimated dioxane exposure concentrations of 0.006-13.3 ppm. Hematological and clinical chemistry parameters were analyzed in 24 current workers. Six of these workers had evidence of liver toxicity, as determined by increased serum aminotransferase levels (aspartate aminotransferase and alanine aminotransferase). All six workers who had elevated aminotransferase levels were known to have consumed about 80 g of alcohol daily for several years. When five of these men reduced their alcohol consumption, their aminotransferase levels returned to normal. Company medical records were evaluated for another 23 previously dioxane-exposed workers; this group was medically examined and chest radiography and blood analyses were performed. Six persons showed elevated aminotransferase levels. All of these had an usual daily ethanol consumption of more than 80 g. Medical records of 27 retired workers were evaluated and showed no higher incidences of liver or kidney diseases. Statistical epidemiological analyses did not reveal differences between observed and expected death rates and cancer incidences.

Another occupational study (Buffler et al., 1978) of 165 workers exposed for at least one month during a 21-year interval to dioxane at average concentrations ranging between 0.1 and 17 ppm and typical maximum concentrations ranging between 1.5 and 32 ppm also found no differences between observed and expected incidences of cancer. Part of the workers were also exposed to vinyl chloride or other, chlorinated solvents.

NIOSH (1977) cited written communications of two representatives (cited by NIOSH as C.U. Dernehl in 1976 and R.E. Peele in 1977) from another manufacturer: air samples were taken during 1974 and 1975 in both production and drum filling facilities. Air samples of 50 ml were directly injected into a gas chromatograph. Sampling in the breathing zone showed an average concentration of 11.36 ppm (range 0.05-51 ppm, n=30). During the 42 years of dioxane production in the plant, about 80 workers were thought to have been potentially exposed to dioxane. In 1976, 42 persons, who were identified as having worked in the dioxane unit at some time or other, were given complete physical examinations, chest X-rays, electrocardiograms and a series of liver profile tests. It was reported that abnormalities were not found in any of the 42 employees. Cancer surveillance which had begun about 20 years ago, revealed four deaths from malignancy (one each of colon cancer, lymphosarcoma, lung carcinoma and glioblastoma) in the dioxane-exposed workers.

TABLE 2: SUMMARY OF EFFECTS IN HUMANS AFTER INHALATION OF DIOXANE

Concentration (ppm)	Exposure Time	Study type and effects	Reference
unknown	workshift, several days	case report on 5 men a man who became ill with nausea and epigastric pain, developed oliguria and after a few days became comatose and died	Barber (1934)
5500	1 min	5 subjects; reported irritation to eyes, resulting in blinking, squinting and lacrimation, and a burning sensation in nose and throat; 3/5 subjects reported slight vertigo.	Yant et al. (1930)
2800	not specified	5 subjects; very strong initial irritation, slight pressure in the chest	Wirth and Klimmer (1936)
2000	3 min	4-6 subjects; initial strong ethereal odor, no lacrimation or cough were noted	Fairley et al. (1934)
1600	10 min	5 subjects; immediate burning of the eyes with lacrimation, slight nose and throat irritation, alcohol-like odor	Yant et al. (1930)
1400	not specified	5 subjects; distinct irritation with slight stinging in the nose and scratchiness and dryness in the throat	Wirth and Klimmer (1936)
1000	5 min	4-6 subjects; sickly odor detected immediately, warm sensation in the throat and chest, which faded rapidly; one subject experienced constriction in the throat	Fairley et al. (1934)
208-650	workshift/d, 6 d	case report of a man who was hospitalized with epigastric pain, developed oliguria, became comatose after 6 d and died one day later	Johnstone (1959)
300	15 min	12 subjects; irritation to eyes, nose and throat	Silverman et al. (1946)
280	not specified	5 subjects; slight mucous membrane irritation	Wirth and Klimmer (1936)
200	15 min	12 subjects; report does not state presence or absence of symptoms; authors concluded that 200 ppm was highest acceptable concentration	Silverman et al. (1946)
50	6 h	pharmacokinetic study on 4 men, eye irritation, odor perception, which diminished with time	Young et al. (1977)
22	not stated	odor recognition threshold	AIHA (1989)
12	not stated	odor detection threshold	AIHA (1989)

2.3. Developmental/Reproductive Toxicity

No studies documenting developmental or reproductive effects of 1,4-dioxane in humans were identified (ECB, 1999; Medline and Toxline databases searched in 2/2002; ATSDR, 2004).

2.4. Genotoxicity

In lymphocytes obtained from 6 workers employed in dioxane production and exposed to unspecified concentrations for 6-15 years, no increase in chromosomal aberrations was found relative to that observed in an equal number of controls (Thiess et al., 1996) (see Section 2.2.2). No other studies documenting genotoxic effects of dioxane in humans were identified (IARC, 1999).

2.5. Carcinogenicity

In the cross sectional study by Thiess et al. (1976) (see Section 2.2.2) no evidence of liver or kidney damage or higher incidence of cancer deaths than in the general population were observed in group of 74 workers. In the study by Buffler et al. (1978) (see Section 2.2.2) no significant difference in observed deaths from overall cancer in 165 employees compared to the expected numbers were found.

2.6. Summary

Volunteer studies reported odor detection thresholds between 1.8 and 170 ppm and odor recognition thresholds between 5.6 and 270 ppm (Wirth and Klimmer, 1936; May, 1966; Hellman and Small, 1974). AIHA (1983) reported a geometric mean odor detection threshold of 12 ppm and a geometric mean odor recognition threshold of 22 ppm. Several studies reported that the initial strong ethereal odor diminished rapidly during exposure (Fairley et al., 1934; Yant et al., 1930; Young et al., 1977).

Volunteers reported eye irritation during exposure at 50 ppm for 6 hours in toxicokinetic study (Young et al., 1977). Subjects exposed at 300 ppm for 15 minutes experienced irritation to eyes, nose and throat; they did not find the odor objectionable (Silverman et al., 1946). Wirth and Klimmer (1936) reported that exposure to 280 ppm (time period not specified) led to a slight mucous membrane irritation in exposed subjects, at 1400 ppm the irritation was quite distinct and at 2800 ppm subjects complained of very strong initial irritation and slight pressure in the chest. Eye irritation, resulting in blinking, squinting and lacrimation, and burning sensation in nose and throat developed in subjects exposed at 5500 ppm for 1 minute (Yant et al., 1930). Three of the subjects noticed a slight vertigo which disappeared quickly after leaving the exposure. Immediate slight burning of the eyes accompanied by lacrimation and nasal irritation resulted from exposure at 1600 ppm for 10 minutes. Fairley et al. (1934) reported that subjects exposed at 2000 ppm for 3 minutes experienced an initial strong odor, which diminished rapidly, but no strong irritation effects, such as lacrimation or cough.

Barber (1934) described 5 fatalities after repeated exposure to unknown concentrations of dioxane at the workplace. Exposure probably also comprised dermal contact. The men experienced nausea and vomiting, described as "stomach trouble", followed after 2-3 days by oliguria and anuria.

About 3-7 days after the first symptoms, coma developed, followed by death. Microscopic examinations revealed centrilobular liver necrosis, almost symmetrical necrosis of the outer renal cortex and hemorrhages around the glomeruli. Johnstone (1959) reported a similar case of a man who worked near to an open container of dioxane (additional dermal exposure occurred). After 6 days on work, the man became hospitalized with severe epigastric pain; he developed oliguria, became comatose on the 6th day and died one day later. Later measurements of the atmosphere showed a dioxane concentrations between 208 and 650 ppm; no quantitative estimation of the dermal exposure was performed.

Case control studies did not reveal evidence of genotoxic or carcinogenic effects of dioxane (Thiess et al., 1996; Buffler et al., 1978; IARC, 1999).

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

Acute inhalation toxicity tests were performed in rats, mice, Guinea pigs, rabbits and cats. However, no LC_{50} study complying with today‘s standards is available. The lethality data are summarized in Table 6.

3.1.1. Rats

Pozzani et al. (1959) determined the LC_{50} values for 24 chemical solvents and a total of 51 binary to quaternary mixtures of these solvents in female Carworth Farms-Nelson rats. Exposure time was either 4 or 8 hours. Dioxane or other solvents and mixtures were delivered by a motor-driven syringe into a heated Pyrex evaporator through which an appropriate amount of air was metered. The resultant vapors were conducted into a 9-liter desiccator which served as inhalation chamber for groups of 6 rats. The LC_{50} values were calculated by the method of moving averages. The 4-hour LC_{50} for dioxane was 14,300 ppm (51.3 mg/l). The number of different dioxane concentrations used was not stated. No clinical or necropsy observations were reported.

BASF AG (1980) exposed groups of male and female Sprague-Dawley rats for 1 hour (12 rats), 3 hours (12 rats) or 7 hours (18 rats) at saturated dioxane vapor at 20 ˚C (estimated concentration 40,000 ppm). The postexposure observation period was 14 d. Lethality was observed in 0/12, 6/12 and 4/18 rats, respectively. During exposure, animals showed escape behavior, eye and nose irritation, dyspnea, unsteady gait, apathy and narcosis. At necropsy, acute heart dilatation, hemorrhagic erosions of the stomach mucosa and acute lung dilatation were observed. No alterations were found in animals surviving until day 14. In a similar test (BASF AG, 1973) rats were exposed for 1 hour (12 rats), 3 hours (6 rats) or 4 hours (6 rats) at saturated dioxane vapor at 20 ˚C. Mortality was observed in 0/12, 6/6 and 6/6 animals, respectively. The authors did not discuss the somewhat inconsistent findings from the two studies.

Pilipyuk et al. (1977) reported the following values for an 4-hour inhalation exposure of white rats: LC_{16} = 11,100 ppm, LC_{50} = 12,800 ppm and LC_{84} = 14,500 ppm. No experimental details were described.

Studies with repeated inhalation exposure

Fairley et al. (1934) exposed guinea pigs, rats, mice and rabbits at 1000, 2000, 5000 or 10,000

ppm dioxane. Animals were exposed twice daily for 1.5 hours (total 3 hours/day) on 5 days per week and one time for 1.5 hours at the 6th day; no exposure was performed on the 7th day. The total exposure time was not clearly stated by the authors: at the highest exposure concentration, all animals died during the first 3 days; for 5000 and 2000 ppm, the longest exposure period was about 3 weeks; for 1000 ppm animals were exposed for up to about 6 weeks. Exposure was done in a 1-m^3 static chamber. The dioxane concentration was obtained by vaporizing the calculated quantity of a 1:4 dioxane-water mixture. The authors did not mention whether the chamber air was mixed and did not perform analytical measurements. The 1000-ppm vapor was obtained by heating the mixture; for the other concentrations, the mixture was sprayed into the chamber. The mean temperature of the chamber was maintained at 27 ˚C to prevent condensation. At 10,000 ppm, all animals noticed the presence of something unusual at once, and rapidly displayed evidence of slight lacrimation. In all cases breathing was slightly distressed and this was more marked in the rats compared to other species. On opening the chamber after the first 1.5-hour exposure, all animals seemed drowsy, but recovered rapidly. At the two lowest concentrations, authors noted signs of slight discomfort in the animals; rabbits took up their characteristic defense attitude, but this and other symptoms tended to lessen in the latter part of the several exposures.

In experiments with rats, 1/3 rats died after 2 exposures for 1.5 hours on the same day at 10,000 ppm; the other two rats died after the 2nd exposure day. At 5000 ppm, rats died after several exposure days. At 10,000 ppm, rats died of pulmonary lesions, which varied from an acute vascular congestion to an advanced infiltration of red blood cells. Evidence of serious kidney damage included patchy cell degeneration of the cortical tubules, vascular congestion and inter- and intratubular hemorrhages. The liver showed cell degeneration that varied from cloudy swelling to large areas of complete necrosis. At lower exposure concentrations, no lung damage from dioxane exposure was found and the main necropsy findings consisted of kidney and liver lesions.

Studies with non-inhalation exposure

Pozzani et al. (1959) determined the oral LD_{50} values for 24 chemical solvents and a total of 51 binary to quaternary mixtures of these solvents in female Carworth Farms-Nelson rats. Chemicals were applied undiluted by gavage to groups of 6 rats. The number of different dioxane concentrations used was not stated. The LD_{50} for dioxane was 6370 mg/kg (6.16 ml/kg). No clinical or necropsy observations were reported.

Other authors reported oral LD_{50} values in rats of about 5170 mg/kg (30 % aqueous solution; BASF, 1973), 5345 mg/kg (not stated if administered pure or as solution; Laug et al., 1939), about 6200 mg/kg (not stated if administered pure or as solution; Nelson, 1951), 6500 mg/kg (not stated if administered pure or as solution; BASF, 1958) and 7339 mg/kg (aqueous solution of unstated concentration; Smyth et al., 1939). Argus et al. (1973) reported a LD_{50} of 5.60±0.06 ml/kg (5790±62 mg/kg) in Sprague-Dawley rats after intraperitoneal injection of phenol in saline.

Studies with repeated non-inhalation exposure

David (1964) exposed 50 white rats of an unspecified strain to drinking water containing 5 % dioxane for 1-10 days (corresponding to about 4150 mg/kg/d). Thirty five rats died during exposure. No details were reported on which days animals died; no necropsy was performed. Microscopic examination of kidneys from rats sacrificed after 3 days showed swollen epithelial cells in the proximal section of the nephron. Vesicular degeneration of tubular epithelium was first observed at day 5 and became more severe at day 7 or later. An accumulation of intracellular hyaline droplets was observed by electron

microscopy. Subsequent changes were noted in the tubular epithelium followed by degeneration and ultimately resulting in necrosis.

3.1.2. Mice

Wirth and Klimmer (1936) exposed mice of an unspecified, white strain to two grades of dioxane by inhalation. One grade was a very pure product that contained 99.8 % dioxane with 0.2 % water and was completely free of aldehydes and other impurities. The other, a technical dioxane grade of 96.4 % purity, contained 1.5 % aldehyde and acetal, 2.1 % water and trace amounts of alcohol and acids. Experiments were carried out in static 32-liter anesthesia flasks with both grades at concentrations ranging from 1400 ppm for about 8.3 hours to 39,000 ppm for approx. 1 h. Eye irritation was observed at all concentrations. Concentrations, exposure time and effects are summarized in Table 3. No difference between the two grades of dioxane was found. There was a considerable interindividual variation in the time until death.

TABLE 3: EFFECTS IN MICE AFTER ACUTE INHALATION EXPOSURE, adopted from Wirth and Klimmer (1936)

Concentration (ppm)	Exposure duration (min) to a) pure / b) technical dioxane	Number of animals exposed to pure/ technical dioxane	Exposure time (min) until onset of symptom for pure / technical dioxane			Time until death after end of exposure (h)
			loss of equilibrium	prostration	narcosis	
39000	55 56	2 2	21, 25 26, 29	32, 40 39, 41	55, 55 56, n.o.[1]	6.5, 67 20, 51
28000	100 100	2 2	45, 48 52, 53	55, 85 60, 65	n.o., n.o. 100, n.o.	9.25, n.o. 100, n.o.
25000	94 95	2 2	47, 47 45, 45	66, 66 55, 65	n.o., n.o. 85, 95	15, 17 8, 15
17000	115 115	2 2	45, 53 53, 53	68, 70 80, 85	115, 115 n.o., n.o.	3.3, 7.3 192, 192
12500	155 158	2 2	60, 75 83, 84	90, 110 138, 138	150, n.o. 153, n.o.	49, 49 26, 48
8300	212 212	1 1	90 120	110 117	135 153	0.2 43
2800	575 578	2 2	405, 420 420, 420	n.o., n.o. 540, 540	n.o., n.o. n.o., n.o.	n.o., n.o. n.d.
2800	480 n.d.[2]	2 n.d.	295, 295 n.d.	n.o., n.o. n.d.	n.o., n.o. n.d.	n.o., n.o. n.o., n.o.
2100	480 480	2 2	360, 420 420, 455	445, n.o. n.o., n.o.	n.o., n.o. n.o., n.o.	0.3, n.o. 21.5, n.o.

Concentration (ppm)	Exposure duration (min) to a) pure / b) technical dioxane	Number of animals exposed to pure/ technical dioxane	Exposure time (min) until onset of symptom for pure / technical dioxane			Time until death after end of exposure (h)
			loss of equilibrium	prostration	narcosis	
1400	500 500	2 2	n.o., n.o. n.o., n.o.	n.o., n.o. n.o., n.o.	n.o., n.o. n.o., n.o.	n.o., n.o. n.o., n.o.

[1] n.o., not observed
[2] n.d., not done

Pilipyuk et al. (1977) reported the following values for a 2-hour inhalation exposure of white mice: LC_{16} = 17,000 ppm, LC_{50} = 18,000 ppm and LC_{84} = 19,300 ppm. No experimental details were described.

Izmerov et al. (1982) reported an LC_{50} of 10,109 ppm for 2 hours in mice. No experimental details were reported.

Studies with repeated inhalation exposure

In the study by Fairley et al. (1934) (described in Section 3.1.1) 3/3 mice died after 2 exposures for 1.5 hours on the same day at 10,000 ppm. At 5000 ppm, 1/3 mice died after the first exposure day and the other animals died after several exposures. At 10,000 ppm there appeared to be some degree of lung edema. Evidence of serious kidney damage included patchy cell degeneration of the cortical tubules, vascular congestion and inter- and intratubular hemorrhages. The liver showed cell degeneration that varied from cloudy swelling to large areas of complete necrosis. At lower exposure concentrations, no lung damage from dioxane exposure was found and the main necropsy findings consisted of kidney and liver lesions.

Studies with non-inhalation exposure

Laug et al. (1939) reported an oral LD_{50} of 5850 mg/kg in mice.

3.1.3. Guinea Pigs

Yant et al. (1930) exposed an unspecified number of guinea pigs to dioxane concentrations of 1000, 2000, 3000, 10,000 or 30,000 ppm and observed the duration of exposure in minutes to up to a maximum of 8 hours required to produce nasal irritation, eye irritation, retching movements, changes in respiration and narcosis. The composition of the dioxane-air mixture was calculated from the quantity of liquid dioxane vaporized and the air volume in or flowing through the exposure chamber. The chamber concentration was checked by sorption of the vapor from a measured volume by activated charcoal and determination of the weight gain (authors made no statement how measured concentrations compared to target values). Animals exposed at 30,000 ppm for 3 hours developed a state of marked narcosis during exposure and died within 2 days. No narcosis was seen after exposure at 10,000 ppm or lower for up to 8 hours. Congestion of the lungs, hyperemia of the surface of the brain and paleness of the liver were seen

in guinea pigs that were killed immediately after the exposure at 30,000 ppm for 30 minutes. Nonlethal effects are summarized in Section 3.2.3.

Studies with repeated inhalation exposure

Lethal effects reported in the study by Fairley et al. (1934) (described in Section 3.1.1) are summarized in Table 4. Necropsy of the kidneys showed cortical lesions ranging from patchy swelling to complete necrosis as the dioxane concentration increased. Hemorrhages and vascular congestion were also observed. At 10,000 ppm, the lungs showed pulmonary lesions that varied from an acute vascular congestion to an advanced infiltration of red blood cells and these pulmonary lesions were the cause of death in these animals. The livers showed changes ranging from vascular congestion to cellular degeneration as the concentration increased. At lower exposure concentrations, no lung damage from dioxane exposure was found and the main necropsy findings consisted of kidney and liver lesions.

TABLE 4: EFFECTS AFTER REPEATED INHALATION EXPOSURE OF RATS, MICE, GUINEA PIGS AND RABBITS, adopted from Fairley et al. (1934)

Concentration (ppm)	Species; total number of animals	Individual total exposure hours	Effect or procedure
10,000	guinea pig; 6	3, 3, 3, 4.5, 4.5, 7.5	died
10,000	rat; 3	3, 4.5, 7.5	died
10,000	mouse; 3	3, 3, 3	died
5000	guinea pig; 6	7.5, 21, 43.5, 94.5, 94.5, 94.5	first two animals removed due to pregnancy (outcome was stillbirth); only one animal died on exposure day 15
5000	rat; 3	9, 13.5, 15	died
5000	mouse; 3	3, 22.5, 51	died
5000	rabbit; 4	16.5, 49.5, 49.5, 49.5	were killed at termination (no explanation for earlier killing time)
2000	guinea pig; 4	48, 102, 102, 102	were killed at termination (no explanation for earlier killing time)
2000	rat; 6	48, 102, 102, 102, 102, 102	were killed at termination (no explanation for earlier killing times)
2000	mouse; 5	102, 102, 102, 102, 102	were killed at termination
2000	rabbit; 4	45, 69, 99, 99	the 2nd animal died; others were killed (no explanation for earlier killing times)
1000	guinea pig; 3	106.5, 147, 202.5	were killed at termination (no explanation for earlier killing times)

Concentration (ppm)	Species; total number of animals	Individual total exposure hours	Effect or procedure
1000	rat; 3	78, 147, 202.5	were killed at termination (no explanation for earlier killing times)
1000	mouse; 4	12, 106.5, 147, 202.5	were killed at termination (no explanation for earlier killing times)
1000	rabbit; 2	144, 196.5	were killed at termination (no explanation for earlier killing time)

Studies with non-inhalation exposure

Oral LD_{50} values of 4000 mg/kg (not stated if administered pure or as solution; Laug et al., 1939) and 3256 mg/kg (aqueous solution of unstated concentration; Smyth et al., 1941) have been reported.

3.1.4. Rabbits

Studies with repeated inhalation exposure

In the study by Fairley et al. (1934) (described in Section 3.1.1), no deaths occurred after several exposures at 5000 ppm for 2x1.5 hours/day. No rabbits were exposed at 10,000 ppm. After killing, animals exposed at 5000 ppm showed serious kidney damage with patchy cell degeneration of the cortical tubules, vascular congestion and inter- and intratubular hemorrhages. The liver showed cell degeneration that varied from cloudy swelling to large areas of complete necrosis. At 2000 or 1000 ppm, the main necropsy findings consisted of kidney and liver lesions.

Studies with non-inhalation exposure

Oral LD_{50} values of about 2100 mg/kg (not stated if administered pure or as solution; Nelson, 1951) and 6500 mg/kg (not stated if administered pure or as solution; Knoefel, 1935) have been reported. De Navasquez (1935) reported minimal lethal doses of 2100 mg/kg for the oral route (groups of 5 rabbits, 1:10 dilution in water, gavage application) and 1600 mg/kg for the intravenous route (groups of 5 rabbits, 1:4 dilution in water).

3.1.5. Other Species

Wirth and Klimmer (1936) exposed groups of 2 cats at 1200 ppm for 430 minutes, 1800 ppm for 258 minutes, 2400 ppm for 240 minutes or 3100 ppm for 182 minutes using to two grades of dioxane (see Section 3.1.2). Marked irritation with salivation and lacrimation was observed at all concentrations. Concentrations, exposure time and effects are summarized in Table 5. Necropsy findings were fatty livers and inflamed respiratory organs and lung edema; no kidney lesions were reported.

The authors also exposed three male cats at an average of 1400 ppm for about 6.5 hours/day for 14 d. From the 4th day to the end of the experiment, the cats seemed sleepy during exposure. Retching and vomiting were observed occasionally. None of the animals died.

TABLE 5: EFFECTS IN CATS AFTER SINGLE INHALATION EXPOSURE, adopted from Wirth and Klimmer (1936)

Concentration (ppm)	Exposure duration (min) to a) pure / b) technical dioxane	Number of animals (sex) exposed	Exposure time (min) until onset of symptom for pure / technical dioxane		Lethality after end of exposure (h)
			loss of equilibrium	prostration	
3100	a) 182 b) 180	a) 2 (m) b) 2 (m, f)	a) 74 ,94 b) 55, 70	a) 105, 125 b) 180, 180	a) n.o.[1], 0.03 b) 35, 8
2400	a) 240 b) 245	a) 2 (m f) b) 2 (f)	a) 173, 165 b) 125, 150	a) 215, 215 b) 245, 240	a) 50, 39 b) 96, 96
1800	a) 258 b) 258	a) 2 (f) b) 2 (m)	a) 150, 150 b) 180, 200	a) 250, n.o. b) n.o., 240	a) 96, 120 b) 120, 120
1200	a) 430 b) 430	a) 2 (f) b) 2 (m)	a) 270, 270 b) n.o., n.o.	a) n.o., n.o. b) n.o., n.o.	a) 96, 240 b) n.o., n.o.

[1] n.o., not observed

Gross (1943) reported that 21/28 animals (mice, rats, guinea pigs and rabbits) died from an 8-hour exposure at 4000-11,000 ppm and 4/10 animals died after exposure at 37,500 ppm for 3 hours.

TABLE 6: SUMMARY OF ACUTE LETHAL INHALATION DATA IN LABORATORY ANIMALS

Species	Concentration (ppm)	Exposure Time	Effect	Reference
rat	saturated vapor (estimated 40,000)	7 h	death in 4/18 animals	BASF AG (1980)
rat	saturated vapor (estimated 40,000)	4 h	death in 6/6 animals	BASF AG (1973)
rat	saturated vapor (estimated 40,000)	3 h	death in 6/6 animals	BASF AG (1973)
rat	saturated vapor (estimated 40,000)	3 h	death in 6/12 animals	BASF AG (1980)
rat	saturated vapor (estimated 40,000)	1 h	no deaths in 12 animals	BASF AG (1980)

Species	Concentration (ppm)	Exposure Time	Effect	Reference
rat	saturated vapor (estimated 40,000)	1 h	no deaths in 12 animals	BASF AG (1973)
rat	14,300	4 h	LC_{50}	Pozzani et al. (1959)
rat	12,800	4 h	LC_{50}	Pilipyuk et al. (1977)
rat	10,000	2 * 1.5 h /d (same day)	death of 1/3 rats on first day, other animals died on subsequent exposures	Fairley et al. (1934)
rat	5000	2 * 1.5 h /d (same day)	no deaths on first day, but all animals died on subsequent exposures	Fairley et al. (1934)
mouse	39,000	1 h	4/4 animals died	Wirth and Klimmer (1936)
mouse	28,000	1 h	2/4 animals died	Wirth and Klimmer (1936)
mouse	25,000	1 h	4/4 animals died	Wirth and Klimmer (1936)
mouse	18,000	2 h	LC_{50}	Pilipyuk et al. (1977)
mouse	17,000	1 h	4/4 animals died	Wirth and Klimmer (1936)
mouse	12,500	1 h	4/4 animals died	Wirth and Klimmer (1936)
mouse	10,109	2 h	LC_{50}	Izmerov et al. (1982)
mouse	10,000	2 * 1.5 h /d (same day)	death of 3/3 animals on first exposure day	Fairley et al. (1934)
mouse	8300	1 h	2/2 animals died	Wirth and Klimmer (1936)
mouse	5000	2 * 1.5 h/d (same day)	death of 1/3 animals on first day, other animals died on subsequent exposures	Fairley et al. (1934)
mouse	2800	1 h	no deaths in 6 animals	Wirth and Klimmer (1936)
guinea pig	30,000	3 h	death of exposed animals (number not stated)	Yant et al. (1930)

Species	Concentration (ppm)	Exposure Time	Effect	Reference
guinea pig	10,000	2 * 1.5 h /d (same day)	no deaths on first day, but death of 6/6 animals on subsequent exposures	Fairley et al. (1934)
cat	3100	182 min	4/4 animals died	Wirth and Klimmer (1936)
cat	2400	245 min	4/4 animals died	Wirth and Klimmer (1936)
cat	1800	258 min	4/4 animals died	Wirth and Klimmer (1936)
cat	1200	430 min	2/4 animals died	Wirth and Klimmer (1936)

3.2. Nonlethal Toxicity

Experimental data are available for effects of inhalation exposure to dioxane on the central and peripheral nervous system, on liver cytotoxicity and on irritative effects. These data are summarized in Table 8.

3.2.1. Rats

Goldberg et al. (1964) (experimental system described in Goldberg et al., 1962) studied the effect of dioxane exposure on conditioned pole-climbing avoidance response to a buzzer and an unconditioned escape response to a buzzer and an electrical shock. Behavioral experiments were performed in a 1x1x2 foot plastic chamber with a stainless steel grid floor. A wooden pole with a rough surface is attached to the top of the chamber and serves as a safety or escape area. During the training phase which started at 30-40 days of age, female Carworth Farms Elias rats were placed in the chamber for 15 seconds with no stimulus. A series of shocks (100 V pulses of 20 ms, 10 pulses/s) was delivered to the floor for 30 seconds concurrent with the activation of a buzzer. After several exposures to these associated stimuli, the rats learned that the pole is the safety area. If a rat successfully climbed the pole, the stimuli were immediately terminated. When the animal consistently manifests the proper escape, the stimuli are dissociated and the rat climbs the pole in response to the buzzer alone (conditioned stimulus). An avoidance-escape conditioned response is considered to have developed. The response to the shock and the buzzer is considered an unconditioned response. After many more exposures to the situation, the rats learned to climb the pole when it was first accessible, in the absence of the above stimuli. Positive response during the environmental adjustment period is considered to be a secondary conditioned response. Rats were trained to respond consistently to the above procedures and develop a secondary conditioned response of less than 12 seconds, with conditioned response and unconditioned response of less than 2 seconds. With suitable training, about 90 % of all animals manifest these requirements. Trained rats were randomized and divided into groups.

The testing procedure comprised the following: the rat was placed in the testing chamber for 15 seconds. When the animal climbed the pole (secondary conditioned response), it was placed back on the grid and the buzzer was activated. An additional successful climb (conditioned response) was followed by again placing the animal on the floor, this time the unconditioned stimuli (buzzer and shock) were used and response time measured. Effect measurement was done on a quantal basis, i.e., the percentage of rats which showed an inhibition of the conditioned response. The authors considered an effect of dioxane to be evident by abolishment of the secondary conditioned response and an abolishment or prolongation of the conditioned response and/or unconditioned response time of greater than 6 seconds, with 15 seconds as the maximum period during which each stimulus was applied. Testing for the unconditioned response (electrical shock) was only done if an animal manifested a blockage or significant prolongation of the conditioned response.

Eight to 10 rats were used in both control and experimental groups with different chemicals, including dioxane at 1500, 3000 or 6000 ppm. Rats were exposed 4 hours/day, 5 days/week for 2 weeks. Rats were exposed in a dynamic 200-l exposure chamber at an airflow of approximately 95 l/min. Vapors were generated by flowing the dioxane, pumped by a motor-driven syringe assembly, down a vertical, electrically-heated, spiral Pyrex tube connected to the air inlet of the chamber. Air flows were adjusted so that the actual vapor concentrations as determined with a Zeiss interferometer were within ±10 % of the nominal concentrations.

Responses were determined on days 1, 2, 3, 4, 5 and 10 before, during and 2 hours after removal from exposure. At 1500 ppm, only one rat was affected and its responses were not consistent from day to day. At 3000 ppm, the avoidance reaction (conditioned response) was delayed in 2/8 rats after the first and in 2-3/8 rats after the subsequent exposures. At 6000 ppm, about 6/8 rats showed a delay of the avoidance response (conditioned response) after the 1st exposure, and 3-8/8 rats were affected in the subsequent exposures. No effects were found on escape response (unconditioned stimulus) after the first exposure; an effect was found in 3/8 animals after the 2nd exposure to 6000 ppm, but not in the subsequent exposures (for any of the exposure conditions). Results on the secondary conditioned response were not reported. At the end of the two weeks, growth rate was significantly reduced in the 6000-ppm group compared to controls.

Drew et al. (1978) exposed male CD1 rats for 4 hours to 1000 or 2000 ppm dioxane or other organic solvents. The serum enzymes aspartate aminotransferase (glutamate oxalacetate transaminase), alanine aminotransferase (glutamate pyruvate transaminase), glucose-6-phosphatase and ornithine carbamyl transferase were measured prior to exposure, immediately after exposure and 24 and 48 hours after exposure. No effect on glucose-6-phosphatase was found. The activities of ornithine carbamyl transferase and aspartate aminotransferase were dose-dependently increased (about 2-3-fold) at 24 and 48 hours; the activity of alanine aminotransferase was about 2-fold increased at 2000 ppm for 24 or 48 hours while it was only marginally increased at 1000 ppm.

Frantik et al. (1994) studied the inhibition of propagation and maintenance of the electrically evoked seizure discharge in rats and mice. Effect-air concentration regressions were determined for 48 common solvents using 4-hour exposures in Wistar rats. The exact exposure concentrations were not stated. Dynamic 80-liter glass chambers were used for exposure. The authors stated that 16 rats, 4 controls exposed to ambient air and 4 in each concentration group were exposed and measured in one trial and that at least two such trials were performed with each compound. A short electrical impulse was

applied through ear electrodes. Of six different time characteristics recorded, the duration of tonic extension of hindlimbs in rats and the velocity of tonic extension in mice were the most sensitive and reproducible response measures. The median of individual control values was subtracted from the values observed after exposure. Group means of differences were corrected for the difference in the simultaneously tested control group and converted to relative values, i.e., to percentage of the arbitrary maximum values, which for rats were 8 seconds and for mice 0.5 per second. All data were processed using linear regression analysis. The estimate of concentration in air evoking 37 % of the maximum possible effect (shortening of the duration of tonic extension of hindlimbs) was 1860 ppm (one-sided 90 % confidence interval 200 ppm). The slope of the regression was 0.041 %/ppm. The authors suggested the EC_{10} as a threshold because the lowest effect level which could be proven statistically in most solvents was about 10 %. For dioxane, the EC_{10} can be calculated as:

$EC_{10, rat, 4h}$ = 1860 ppm - 27 %/0.041 %/ppm = 1200 ppm

3.2.2. Mice

Frantik et al. (1994) studied the inhibition of propagation and maintenance of the electrically evoked seizure discharge in rats and H-strain mice (see Section 3.2.1 for description). Effect-air concentration regressions were determined for 48 common solvents using 2-hour exposures in mice. The exact exposure concentrations were not stated. The authors stated that 32 mice, 8 controls exposed to ambient air and 8 in each concentration group were exposed and measured in one trial and that at least two such trials were performed with each compound. A short electrical impulse was applied through ear electrodes. The estimate of concentration in air evoking 30 % of the maximum possible effect (reduction of the velocity of tonic extension in the hindlimbs was the most sensitive effect) in mice was 2400ppm (one-sided 90 % confidence intervall 420 ppm). The slope of the regression was 0.011 %/ppm. The authors suggested the EC_{10} as a threshold because the lowest effect level which could be proven statistically in most solvents was about 10 %. For dioxane, the EC_{10} can be calculated as:

$EC_{10, mouse, 2h}$ = 2400 ppm - 20 %/0.011 %/ppm = 580 ppm

3.2.3. Guinea pigs

Yant et al. (1930) (see study description in Section 3.1.3) exposed an unspecified number of guinea pigs at 1000, 2000, 3000, 10,000 or 30,000 ppm and observed the duration of exposure in minutes to up to a maximum of 8 hours required to produce nasal irritation, eye irritation, retching movements, changes in respiration and narcosis. The results are summarized in Table 7.

TABLE 7: NONLETHAL EFFECTS IN GUINEA PIGS FROM THE STUDY OF YANT et al. (1930)

	Exposure time (min) until onset of symptoms at different concentrations				
Type of symptom	**30,000 ppm**	**10,000 ppm**	**3000 ppm**	**2000 ppm**	**1000 ppm**
Nasal irritation, scratching at nose	immediate onset, intensity increased with increasing concentration				

	Exposure time (min) until onset of symptoms at different concentrations				
Type of symptom	**30,000 ppm**	**10,000 ppm**	**3000 ppm**	**2000 ppm**	**1000 ppm**
Eye irritation, squinting, lacrimation	immediate onset, intensity increased with increasing concentration		8 min	5 min	no symptoms (480 min)
Retching movements or marked expiratory effort, spasmodic contraction of abdominal wall, head lifted, mouth open	2-10	19-27	not observed until 480		
Dyspnea	45-116 min	no symptoms (480 min)			
Shallow, rapid respiration	75-123 min	no symptoms (480 min)			
Gasping respiration	116 min	no symptoms (480 min)			
Shallow, slow respiration	508-540 min	no symptoms (480 min)			
Narcosis - fall to sides, remain quiet	87-141 min	no symptoms (480 min)			

TABLE 8: SUMMARY OF NON-LETHAL EFFECTS IN LABORATORY ANIMALS

Species	Concentration (ppm)	Exposure Time	Effect	Reference
rat	6000	4 h/d, 5 d/w, 2 w	6/8 rats showed an inhibition of a conditioned response after the first exposure; an effect on the unconditioned escape response was only found after the second exposure; growth rate was significantly reduced after 2 w	Goldberg et al., 1964
rat	3000	4 h/d, 5 d/w, 2 w	2/8 rats showed an inhibition of a conditioned response after the first exposure; no effect on unconditioned escape response and growth rate	Goldberg et al., 1964
rat	2000	4 h	increased serum activity of ornithine carbamyl transferase, aspartate aminotransferase and alanine aminotransferase at 24 and 48 h	Drew et al., 1978
rat	1500	4 h/d, 5 d/w, 2 w	no inhibition of a conditioned response after the first exposure	Goldberg et al., 1964
rat	1200	4 h	threshold for shortening of the duration of tonic extension of hindlimbs	Frantik et al., 1994
rat	1000	4 h	increased serum activity of ornithine carbamyl transferase and aspartate aminotransferase at 24 and 48 h	Drew et al., 1978

Species	Concentration (ppm)	Exposure Time	Effect	Reference
mouse	580	2 h	threshold for reduction of the velocity of tonic extension in the hindlimbs	Frantik et al., 1994
Guinea pig	30,000	variable	immediate nasal irritation, nose scratching, eye irritation, squinting, lacrimation; respiratory distress after 2-10 min; dyspnea after 45-116 min; narcosis after 87-141 min; gasping respiration after 116 min; shallow, slow respiration after 508-540 min	Yant et al., 1930
Guinea pig	10,000	variable	immediate nasal irritation, nose scratching, eye irritation, squinting, lacrimation; respiratory distress after 19-27 min; no additional effects	Yant et al., 1930
Guinea pig	3000	variable	immediate nasal irritation, nose scratching; eye irritation, squinting, lacrimation after 8 min; no other effects	Yant et al., 1930
Guinea pig	2000	variable	immediate nasal irritation, nose scratching; eye irritation, squinting, lacrimation after 5 min; no other effects	Yant et al., 1930
Guinea pig	1000	variable	immediate nasal irritation, nose scratching; no eye irritation; no other effects	Yant et al., 1930

3.3. Developmental/Reproductive Toxicity

No studies documenting developmental or reproductive effects of 1,4-dioxane after inhalation exposure were identified (ECB, 1999; Medline and Toxline databases searched in 2/2002; ATSDR, 2004).

Studies with non-inhalation exposure

Giavini et al. (1985) exposed groups of 17-20 pregnant Sprague-Dawley rats by gavage to 0, 0.25, 0.5 or 1.0 ml dioxane/kg b.w. in water during gestational days 6-15 (corresponding to 0.26, 0.52 and 1.03 mg/kg/day). The animals were killed on gestational day 21. At the highest dose, females showed a slightly smaller weight gain during treatment, which continued during the rest of gestation. Food consumption in these females was decreased during treatment. The average weight of live fetuses at the highest dose was significantly less than controls. Number of implantations and number of fetuses alive was slightly decreased and preimplantation loss was slightly increased at 1.03 mg/kg/d. At this dose also a delay of sternum ossification was found. There was no indication for teratogenicity. The NOEL for maternal and embryotoxicity was established at 0.52 mg/kg/day.

3.4. Genotoxicity

A large number of genotixicity tests have been done and these are reviewed in ATSDR, 2004; ECB (1999), IARC (1999), DeRosa et al. (1996), ECETOC (1983) and NIOSH (1977). All mutation tests

887 carried out in Salmonella typhimurium were negative both with and without metabolic activation (Morita
888 and Hayashi, 1998; Nestmann et al., 1984; Haworth et al., 1983; Stott et al., 1981; BASF, 1979a; 1979b;
889 1979c). A HGPRT gene mutation assay in Chinese hamster ovary (CHO) cells (BASF, 1991) as well as a
890 TK gene mutation assay in mouse lymphoma L5178 tk+/- cells (Morita and Hayashi, 1998) gave negative
891 results with and without metabolic activation. Also negative results were observed in a test for
892 chromosomal aberrations in CHO cells both with and without metabolic activation (Morita and Hayashi,
893 1998; Galloway et al., 1987) and an in vitro micronucleus assay in CHO cells (Morita and Hayashi,
894 1998). Tests for sister chromatid exchanges in CHO cells were positive without metabolic activation but
895 negative with metabolic activation in one study (Galloway et al., 1987) and negative with and without
896 activation in another study (Morita and Hayashi, 1998). Dioxane was negative in an UDS test using
897 primary isolated rat heptocytes (Goldsworthy et al., 1991). A cell transformation test with Balb 3T3 cells
898 without metabolic activation was positive (Sheu et al., 1988).

899 Several in vivo micronucleus tests were performed. In C57BL/6 mice, oral administration of
900 dioxane resulted in both positive (Mirkova, 1994) and negative (Tinwell and Ashby, 1994) results in bone
901 marrow cells. Negative results in bone marrow cells were obtained after oral administration in BALB/c
902 (Mirkova, 1994) and CBA (Tinwell and Ashby, 1994) mice as well as after intraperitoneal injection in
903 $B6C3F_1$ mice (McFee et al., 1994). Negative results were also reported for peripheral blood reticulocytes
904 after oral administration or intraperitoneal injection in CD-1 mice (Morita and Hayashi, 1998; Morita,
905 1994). However, statistically significant dose-dependent increases in micronucleated hepatocyte
906 frequency was observed in male CD-1 mouse liver after single oral treatment at 2000 mg/kg or more
907 (Morita and Hayashi, 1998).

908 In a study by Goldsworthy et al. (1991) neither a single 1000 mg/kg administration nor treatment
909 with 1 % dioxane in drinking water for 2 weeks or with 2 % for 1 week resulted in unscheduled DNA
910 synthesis in primary rat hepatocytes. Negative results for unscheduled DNA synthesis were also found in
911 rat nasal respiratory epithelial cells after treatment with 1 % in drinking water for 8 days or after the same
912 treatment plus an additional gavage dose of up to 1000 mg/kg. Kitchin and Brown (1990; 1994) reported
913 that dioxane induced significant single strand breaks in rat liver DNA in the alkaline elution test after a
914 gavage dose of 2550 mg/kg, but not at 840 mg/kg. Sina et al. (1983) reported DNA single strand breaks in
915 an alkaline elution test in vitro when rat hepatocytes were exposed at cytotoxic dioxane concentrations
916 (Sina et al., 1983).

917 **3.5. Carcinogenicity**

918 ***Studies with repeated inhalation exposure***
919 Torkelson et al. (1974) exposed 288 male and 288 female Wistar rats at 111 ppm dioxane for 7
920 hours/day, 5 days/week for a total of 2 years. Control groups of 192 rats/sex were used. Dioxane
921 concentration in the exposure chamber was measured by infrared spectrometric analysis. The authors
922 stated that no adverse effects were noted with respect to appearance, eye and nasal irritation, respiratory
923 distress, demeanor, growth, mortality, hematological and clinical chemistry studies, organ weights or
924 gross and microscopic pathological examination. Upon gross and microscopic examination, no dioxane
925 characteristic nasal and liver tumors, as observed after oral administration, were seen. It is however not
926 clear from the text whether or not the nasal cavity was adequately examined. The incidence of tumors
927 observed in other organs and tissues appeared to be unrelated to exposure. The only difference from the
928 controls was an increase in lymphoreticular cell sarcomas in males (18 % (37/206) vs. 12 % (18/150)) and

in mammary gland adenomas in females (13 % (29/271) vs. 8 % (11/139)), which were not statistically significant.

Studies with non-inhalation exposure

Kociba et al. (1974) exposed groups of 60 male and 60 female Sherman rats to drinking water containing 0, 0.01, 0.1 or 1 % dioxane for 716 days. The corresponding body doses for males/females were 0, 9.6/19, 94/148 and 1015/1599 mg/kg/day. The high dose group showed reduced body weights throughout the study and increased mortality during the first 4 months. Tumor incidences, combined for both sexes, were 1/106, 0/110, 1/106 and 10/66, respectively, for hepatocellular carcinomas and 0/106, 0/110, 0/106 and 3/66 for nasal carcinomas. The increased incidences in the high dose group were statistically significant compared to the control group.

NCI (1978) administered 0, 0.5 or 1.0 % dioxane in drinking water to groups of 35 male and 35 female Osborne-Mendel rats (corresponding body doses for males/females were 0, 240/350 and 530/640 mg/kg/day) and to groups of 50 male and 50 female $B6C3F_1$ mice (corresponding body doses for males/females were 0, 720/380 and 830/860 mg/kg/day) for 110 weeks (rats) or 90 weeks (mice). In rats, squamous cell carcinomas in the nasal turbinates occurred in a dose-related fashion at incidences of 0/33 controls, 12/33 low-dose and 16/34 high-dose males and 0/34, 10/35 and 8/35 females, respectively. The incidences of hepatocellular adenomas were significantly increased in female rats, with incidences of 0/31, 10/33 and 11/32, respectively. In mice, hepatocellular carcinomas were observed at incidences of 2/49 control males, 18/50 low-dose males and 24/47 high-dose males and in 0/50, 12/48 and 29/37 females, respectively. The incidences of hepatocellular carcinomas or adenomas for rats were 8/49, 19/50 and 28/47, respectively, in males and 0/50, 21/48 and 35/37, respectively, in females. The incidences were statistically significant for dose-related trend and for direct comparison with controls.

In the JBRC (1998) study, groups of Fischer 344/DuCrj rats (50/sex/dose level) received 1,4-dioxane in the drinking water at levels of 200, 1,000, or 5,000 ppm for 2 years (0, 16, 81, and 398 mg/kg/day for males; 0, 21, 103, and 514 mg/kg/day for females). Survival was significantly decreased in the high-dose groups due to liver and nasal tumors. Twenty-two of 50 high-dose male rats survived compared to 40/50 in controls; 24/50 of high-dose females survived compared to 38/50 in controls. In high-dose males (398 mg/kg/day), the incidence of nasal cavity tumors was 7/50 ($p<0.01$) compared to none in the other groups; in high-dose females (514 mg/kg/day), the incidence was 8/50 ($p<0.01$) compared to none in the other groups. The nasal tumors included squamous cell carcinomas, sarcomas, rhabdomyosarcoma, and esthesioneuroepithelioma. The incidence of combined hepatocellular adenoma or carcinoma in males was 0/50, 2/50, 4/49, and 33/50 ($p<0.01$) in the control, low-, mid-, and high-dose male rats; the corresponding incidences in females were 1/50, 0/50, 5/50, and 40/50 ($p<0.01$). High-dose males also had an increased incidence of mesothelioma of the peritoneum (28/50 compared to 2/50 in controls). High-dose females had an increased incidence of mammary gland adenomas (16/50 compared to 6/50 in controls). In the same study groups of Crj:BDF1 mice (50/sex/dose level) received 1,4-dioxane in the drinking water at levels of 500, 2,000, or 8,000 ppm for 2 years (0, 66, 251, and 768 mg/kg/day for males; 0, 77, 323, and 1,066 mg/kg/day for females). Early mortality occurred in female mice, and this was attributed to liver tumors. Survival rates at 104 weeks in females were 29/50, 29/50/ 17/50, and 5/50 in control, low-, mid-, and high-dose groups, respectively. A significant and dose-related increase in the incidence of liver adenomas and carcinomas of the liver was found in female mice. The incidences of combined adenomas and carcinomas in control, low-, mid-, and high-dose females were 4/50, 34/50, 41/40, and 46/50 ($p<0.01$ for all treated groups). High-dose males (768 mg/kg/day) also showed a

significant increased incidence of hepatocellular carcinomas; the combined incidences of adenomas and carcinomas, as the dose increased, were 21/50 (controls), 31/50, 37/50, and 39/50 ($p<0.01$). There were no nasal cavity tumors in male or female mice.

Several other studies reporting liver tumors in rats and guinea pigs, nasal cavity tumors in rats and gall bladder tumors in guinea pigs after oral administration have been reviewed in Stickney et al. (2003), ECB (1999), IARC (1999), DeRosa et al. (1996), ECETOC (1983) and NIOSH (1977).

Perone et al. (1976) treated C3H/HeJ Agouti mice by topical applications of 0.05 ml of various grades of dioxane 3 times/week for 78 weeks. Compared with ethanol-treated controls, no evidence of increased hepatic or skin tumors was found.

In two studies, dioxane showed tumor promoting activity. Increased number of skin, lung and kidney tumors were found in Swiss-Webster mice after topical treatment with 50 µg dimethylbenzanthracene as an initiator followed by 0.2 ml dioxane in acetone for 3 times/week for 60 weeks (King et al., 1973). In another tumor promotion study (Lundberg et al., 1987), increased number of liver foci was observed in Sprague-Dawley rats that had received 30 mg/kg diethylnitrosoamine by intraperitoneal injection one day after partial hepatectomy, followed by administration of 100 or 1000 mg dioxane/kg/day, 5 days/week for 7 weeks.

3.6. Summary

Acute toxic effects in animals are mainly central nervous system depression, kidney and liver damage, peripheral nervous system effects as well as irritative effects. At lethal concentrations, narcosis has been observed in rats (BASF AG, 1980) and guinea pigs (Yant et al., 1930). Pozzani et al. (1959) reported a 4-hour LC_{50} for dioxane of 14,300 ppm in rats. A similar LC_{50} value of 12,800 ppm for 4 hours was reported by Pilipyuk et al. (1977). For exposure to saturated dioxane atmosphere (estimated concentration 40,000 ppm), BASF AG (1973; 1980) reported no deaths of rats for a 1-hour exposure, while in the two experiments 100 % and 50 %, respectively, of the animals died after 3 hours of exposure. At necropsy, acute heart dilatation, hemorrhagic erosions of the stomach mucosa and acute lung dilatation were observed. Fairley et al. (1934) reported death of 1/3 rats after a single exposure day comprising two 1.5-hour exposures to 10,000 ppm. At 5000 ppm rats died after 3-5 consecutive exposure days. For mice, 2-hour LC_{50} values of 18,000 ppm (Pilipyuk et al., 1977) and 10,109 ppm (Izmerov et al., 1982) have been reported.

Goldberg et al. (1964) studied the effect of dioxane on avoidance-escape behavior of rats. Rats were exposed 4 hours/day, 5 days/week for 2 weeks. At 6000 ppm, about 6/8 rats showed a delay of the avoidance response already after the 1st exposure, and 3-8 of 8 rats were affected in the subsequent exposures. No effects were found on escape response; an effect on escape response was only found in 3/8 animals after the 3rd exposure to 6000 ppm. Drew et al. (1978) reported 2-3-fold increased serum activities of liver enzymes (ornithine carbamyl transferase, aspartate aminotransferase and alanine aminotransferase) in rats after a single 4-hour exposure to 1000 or 2000 ppm dioxane. Frantik et al. (1994) studied the inhibition of propagation and maintenance of the electrically evoked seizure discharge in rats and mice. Of six different time characteristics recorded, the duration of tonic extension of hindlimbs in rats and the velocity of tonic extension in mice were the most sensitive and reproducible

response measures. The authors suggested the EC_{10} as the effect threshold, which was 1200 ppm for 4 hours in rats and 580 ppm for 2 hours in mice.

Giavini et al. (1985) found no indication of teratogenic or fetotoxic effects in rats dosed with up to 517 mg/kg/day by gavage on gestational days 6-15.

Dioxane did not induce gene mutations in Salmonella typhimurium (Nestmann et al., 1984; Haworth et al., 1983; Stott et al., 1981; BASF, 1979a; 1979b; 1979c). In Chinese hamster ovary cells, it did not induce HGPRT gene mutations or chromosomal aberrations, although it did induce a slight increase in sister chromatid exchange in the absence of metabolic activation (BASF, 1991; Galloway et al., 1987). It has been reported to cause morphological transformation of BALB/c 3T3 mouse cells (Sheu et al., 1988). Oral administration of high doses to rats caused DNA strand breaks in liver cells (Kitchin and Brown, 1990; 1994). No induction of unscheduled DNA synthesis was observed in rat hepatocytes at up to 2 % dioxane in drinking water (Goldsworthy et al., 1991). Of six studies on the induction of bone-marrow micronuclei, five were negative (Tinwell and Ashby, 1994; Morita, 1994; Mirkova, 1994; McFee et al., 1994), while one was positive (Mirkova, 1994).

When administered orally, dioxane produced malignant tumors of the nasal cavity and liver in rats, liver tumors in mice, and tumors of the liver and gallbladder in guinea pigs (Kociba et al., 1974; NCI, 1978; DeRosa et al., 1996; JBRC, 1998; ECB, 1999; IARC, 1999). It was also active as a promotor in a two-stage skin carcinogenesis study in mice (King et al., 1973). A lifetime bioassay exposing rats at 111 ppm for 7 hours/day, 5 days/week found no evidence for carcinogenic effects (Torkelson et al., 1974).

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

In a pharmacokinetic study (Young et al., 1977), four male volunteers were exposed to 50 ppm dioxane vapor for 6 hours (see study description in Section 2.2.1). The concentration of dioxane in the plasma reached 1 mg/l at 1 hour, 4.5 mg/l at 1.5 hours, 9 mg/l at 2 hours and 10 mg/l at 3 hours, after which a plateau was reached during the rest of the exposure period. The plasma concentration of the metabolite 2-hydroxyethoxyacetic acid was about 2.5 mg/l at 5 hours, 4 mg/l at 6 hours and peaked at 8 mg/l at about 7 hours, i.e. one hour after termination of exposure. Of the total dioxane dose, >99% was excreted in the urine as 2-hydroxyethoxyacetic acid. The half-life for elimination of dioxane from the plasma was 59 ± 7 minutes. The calculated total absorbed dose was 5.4 mg/kg. The data indicated a first-order, one-compartment model that did not become saturated at 50 ppm.

Assuming a body weight of 70 kg for man and an inhalation rate of 20 m^3/d (WHO, 1999), the total inhaled amount of dioxane during the 6-hour exposure can be calculated as:

50 ppm * 3.6 mg/m^3 / ppm * 20 m^3 * 6 h/24 h * 1/70 kg = 12.9 mg/kg

Thus, the lung retention was about: 5.4 mg/kg / 12.9 mg/kg = 43 %

Although exhalation of dioxane was not determined in this experiment, an estimation for the lung retention can be obtained from this data because experiments in rats indicated that a significant elimination of dioxane by exhalation occurred only at much higher doses (Young et al., 1978a; 1978b).

After head-only exposure of 4 male Sprague-Dawley rats at 50 ppm for 6 hours, an absorbed dose of 71.9 mg/kg was estimated, based on the amounts of dioxane and 2-hydroxyethoxyacetic acid excreted in the urine over 48 hours (Young et al., 1978a; 1978b). Over 99.9 % of the total excreted amount was 2-hydroxyethoxyacetic. The concentration of dioxane in the plasma decreased in a first-order kinetic fashion from 7.3 mg/l at the end of exposure to nondetectable levels at 11 hours (5 hours after exposure); the half-life was one hour.

Rhesus monkeys receiving radiolabelled dioxane in either methanol or a skin lotion onto the unoccluded, clipped ventral skin of the forearm for 24 hours, showed a dermal penetration of 2.3 % of the applied dose in methanol and 3.4 % of the applied dose in lotion, as determined from the urinary excretion of radioactivity over five days (Marzulli et al., 1981).

Dermal penetration was determined in diffusion cell studies on human skin (Bronaugh, 1982): up to 3.2 % of applied dioxane (dissolved in a cosmetic lotion) was absorbed under occlusion for 3.5 hours, whereas only 0.3 % absorption occurred under non-occluded conditions; the authors concluded the difference to be most likely accounted for by the high volatility of dioxane.

Young et al. (1978a; 1978b) administered radioactive labelled dioxane in water by gavage to rats at single doses of 10, 100 or 1000 mg/kg or administered multiple doses of 10 or 1000 mg/kg/day for 17 days. Data on the excretion of radioactivity in the urine and of ^{14}C-dioxane and $^{14}CO_2$ in the expired air indicated that after a single oral dose, gastrointestinal absorption was virtually complete within 24 hours of dosing with 10 mg/kg and within 72 hours of dosing with 100 or 1000 mg/kg. After a single oral dose, 99 % of the 10-mg/kg dose was excreted over 24 hours, and 86 % of the 100-mg/kg dose and 76 % of the 1000-mg/kg dose were excreted over 72 hours. The percentage of expired dioxane was 0.43 % of the 10-mg/kg dose, 5 % of the 100 mg/kg dose and 25 % of the 1000-mg/kg dose. Excretion of carbon dioxide in the air (2-3 %) or of radioactivity in the feces (0.95-2 %) collected over 24 hours was not dose-dependent. Virtually complete gastrointestinal absorption of dioxane also occurred after repeated dosing. In urine collected over 480 hours, 99 % and 82 % of the 10- and 1000-mg/kg doses, respectively, were excreted. In the expired air, the percentage of the dose excreted as dioxane was 1 % at 10 mg/kg/d and 8.9 % at 1000 mg/kg/d; the percentage of the dose expired as carbon dioxide was 4 % and 7 %, respectively. After intravenous injection with 3, 10, 30, 100 or 1000 mg/kg, elimination from plasma was linear with a half-life of 1.1 hours at the low doses of 3 and 10 mg/kg. At higher doses, elimination from plasma became progressively slower and biphasic with increasing dose. Metabolic clearances decreased from 2.82 ml/min at 10 mg/kg to 0.17 mg/min at 1000 mg/kg, indicating saturation of metabolic oxidation of dioxane.

The major metabolite of 1,4-dioxane is 2-hydroxyethoxyacetic acid both in humans (Young et al., 1977) and rats (Young et al., 1978a; 1978b). However, a controversy exists whether dioxane is metabolized directly to 2-hydroxyethoxyacetic acid, which can cyclize to the 1,4-dioxane-2-one (Braun and Young, 1977), or whether dioxane is metabolized to 1,4-dioxane-2-one, which is readily converted to 2-hydroxyethoxyacetic acid (Woo et al., 1977, 1978). The uncertainty is the result of the fact that the two candidate chemical structures can readily interconvert under the chemical conditions used in the analysis: at low pH, 2-hydroxyethoxyacetic acid is detected as the major metabolite, while at high pH, 2-hydroxyethoxyacetic acid will be converted to 1,4-dioxane-2-one, which is then identified as the major metabolite (ECB, 1999).

In male Sprague-Dawley rats that received 3000 mg/kg ^{14}C-dioxane by intraperitoneal injection, the urinary secretion of 1,4-dioxane-2-one was about 300 mg metabolite/kg over 24 hours. Pretreatment of rats with phenobarbital or the polychlorinated biphenyl Aroclor 1254, but not methylcholanthrene, prior to dioxane injection significantly increased amounts of the urinary metabolite excreted. In contrast, cytochrome P-450 inhibitor 2,4-dichloro-6-phenylphenoxyethylamine decreased the metabolite excretion, suggesting that the metabolism of dioxane is mediated by cytochrome P-450 enzymes (Woo et al., 1977; 1978). In unpublished studies, Young and Nolan (Young et al., 1978b) have shown that dioxane can induce its own metabolism after repeated oral doses of 1000 mg/kg, but not of 10 mg/kg. In these experiments the high dose led to an increased liver/body weight ratio and to an increased activity in vitro of liver aniline hydroxylase and aminopyrine N-demethylase, suggesting that cytochrome P450 2E1 catalyzes an oxidation step in the dioxane metabolic pathway. In line with an induction of metabolism is the observation that repeated daily administration of 1000 mg/kg resulted in a marked decrease of excretion of dioxane in the expired air (from 25.25 to 8.86 %) and an increase of excretion as $^{14}CO_2$ (from 2.39 to 6.95 %) (Young et al., 1978a; 1978b).

4.2. Mechanism of Toxicity

Death of laboratory animals after acute inhalation was probably due to the narcotic effect of dioxane (BASF AG, 1980) as well as to acute vascular congestion and lung hemorrhage (Fairley et al., 1934). When death occurred after repeated inhalation exposure, the cause of death was kidney and liver damage in rats, mice, Guinea pigs and rabbits (Fairley et al., 1934; David, 1964). In reported human fatalities, which occurred after repeated inhalation exposure at the workplace, death was also caused primarily by liver and kidney necrosis (Barber, 1934; Johnstone 1959).

With regard to its carcinogenic effects, the mode of action of dioxane is not yet clear. Several experiments investigated hepatocyte cell proliferation:

Goldsworthy et al. (1991) investigated the hepatic and nasal epithelial labelling index 24 or 48 hours after a single gavage dose of 1000 mg/kg or a 2-week administration of 1 % dioxane in the drinking water (corresponding to about 1000 mg/kg/day) in male Fisher-344 rats. The percentage of cells in S-phase was determined by administration of ^{3}H-thymidine (single injection or osmotic pump) and subsequent quantitative histoaudiography. In the liver, there was a twofold increase in the labelling index after 2 weeks of exposure. No such effect was seen after the single dose.

Stott et al. (1981) administered dioxane in drinking water at approximately 1000 mg/kg/day for 11 weeks to male Sprague-Dawley rats, a dose at which some increase in liver weight was found. Hepatocytes were isolated by collagenase perfusion and labeled in vitro with ^{3}H-thymidine. Labelling was increased at 1000 mg/kg/day, but not at 10 mg/kg/day. With the same in vitro labelling technique, it was shown that a 1-3 day exposure to 2 % dioxane in drinking water (corresponding to about 2000 mg/kg/day) caused no increases in S-phases, whereas after 8 days and longer exposure a pronounced increase in S-phase was visible.

Miyagawa et al. (1999) found an increased replicative DNA synthesis in male Fisher-344 rats after oral gavage doses of 1000, 1500 or 2000 mg/kg 24 hours, but not 48 hours, after administration using in vitro labelling with.^{3}H-thymidine after collagenase liver perfusion. In liver specimens prepared after the 1000, 1500 or 2000 mg/kg treatments no histopathological changes were found.

On the one hand side, several authors discuss liver cytotoxicity of dioxane at high concentrations as the most likely mechanism of dioxane carcinogenicity (Stickney et al. 2003; ECB, 1999; BUA, 1992; 1993). The cytotoxic effects and organ damage via increased cell turnover may pave the way for liver carcinogenesis. Since dioxane (and 1,4-dioxane-2-ol) has a protein-denaturating effect, one would expect cytostatic as well as proliferating effects, the latter being due to replacement of necrotic cells (AGS, 2001). The non-linear toxicokinetics of dioxane in rats could be in line with this explanation. Saturation of oxidation of dioxane to 2-hydroxyethoxyacetic acid and 1,4-dioxane-2-one at doses between 10 and 1000 mg/kg (Young et al. 1978a; 1978b) could result in the accumulation of dioxane and possibly of its metabolites, such as 1,4-dioxane-2-one and 2-hydroxyethoxy-acetaldehyde, and the induction of cytotoxic effects. Increased hepatocyte cell proliferation has been reported in rats after a single oral dose of 1000 mg/kg or higher (Miyagawa et al., 1999), while in other studies (Stott et al., 1981; Goldsworthy et al., 1991) repeated oral doses of 2000 mg/kg were necessary to induce increases in hepatocyte proliferation. Consistent with this effect level, inhalation exposure of rats at 1000 ppm for 4 hours, corresponding to a body dose of about 630 mg/kg, resulted in increased serum activities of liver enzymes (Drew et al., 1978).

On the other hand side, a genotoxic mechanism cannot be excluded at high doses, at which accumulation of dioxane and its metabolites can occur: increased micronuclei formation in rat hepatocytes was found after a single oral dose of 2000 mg/kg (Morita and Hayashi, 1998); an increased rate of DNA strand breaks was found in rats after a single oral dose of 2550 mg/kg, but not at 840 mg/kg (Kitchin and Brown, 1990; 1994); moreover, dioxane induced sister chromatid exchanges in CHO cells (Galloway et al., 1987) and transformation of Balb 3T3 cells (Sheu et al., 1988) in vitro.

The occurrence of nasal tumors in the drinking water studies cannot be explained easily, because no nasal tumors were found in rats exposed to dioxane vapor for 2 years (Torkelson et al., 1974). Goldsworthy et al. (1991) considered it possible that the manner in which the water was given in the cancer study resulted in the animals having inhaled or sniffed the dioxane-containing water into their nasal passages and that sniffing would result in deposition of the inspired material along the dorsal meatus where the tumors were observed. Reitz et al. (1990) mentioned experiments in which rats were given a dye in the drinking water. Upon examination, significant amounts of dye were present in the turbinates, demonstrating that large amounts of inspired water may be deposited in the nose. It was hypothesized that the nasal lesions are probably irrelevant to man because the nasal tumors in rats were probably a result of repeated direct contact of the nasal mucosa with dioxane-containing drinking water (Reitz et al. 1990; Stickney et al., 2003).

4.3. Other Relevant Information

4.3.1. Pharmacokinetic Modelling

Reitz et al. (1990) developed a physiologically-based pharmacokinetic model to describe tissue levels of dioxane and its metabolites in rats, mice and humans, in order to relate human exposure levels to the positive oral carcinogenicity studies and the negative inhalation carcinogenicity study. The model was formulated to contain six distinct tissue compartments: lung, fat, liver, venous blood, slowly perfused tissues and rapidly perfused tissues. Metabolism was described as a saturable process using Michaelis-Menten kinetics. The model was formulated for four different routes of administration: inhalation, intravenous injection, bolus gavage and consumption via drinking water. The model predictons were compared to the data of Young et al. (1977; 1978a; 1978b).

Once the model had been developed, two dose surrogates were calculated:

1) average area under the liver dioxane concentration time curve per day (AUC-liver): drinking water exposures associated with development of liver tumors in rats (0.5-1.0 % dioxane; NCI, 1978; Kociba et al., 1974) were predicted to give high AUC-liver values of 17,900-64,200 mg*h/l. Similarly, predictions of AUC-liver values for mice at dose levels associated with liver tumor formation (0.5-1.0 % dioxane; NCI, 1978) gave results of 15,200-43,400 mg*h/l. No observed effect levels for liver tumors of 0.1 % dioxane in drinking water (Kociba et al., 1974) and 111 ppm dioxane in air (Torkelson et al., 1974) corresponded to AUC-liver values of 257 and 109 mg*h/l, respectively. The predicted AUC-liver value for humans at a continuous exposure concentration of 10 ppm dioxane in air was 7.36 mg*h/l.

2) average area under the metabolite (2-hydroxyethoxyacetic acid) concentration time curve for the whole body per day (AUC-metabolite): drinking water exposures associated with development of liver tumors in rats and mice (0.5-1.0 % dioxane; NCI, 1978; Kociba et al., 1974) were predicted to AUC-metabolite values of approximately 1500 mg*h/l. No observed effect levels for liver tumors of 0.1 % dioxane in drinking water (Kociba et al., 1974) and 111 ppm dioxane in air (Torkelson et al., 1974) corresponded to AUC-metabolite values of 470 and 197 mg*h/l, respectively. The predicted AUC-metabolite value for humans at a continuous exposure concentration of 10 ppm dioxane in air was 13.5 mg*h/l. The authors pointed at the much smaller ratio of AUC-metabolite values for effect and no-effect levels compared with the ratio for AUC-liver. The AUC-metabolite values were almost identical for the 0.5 and 1.0 % dioxane exposure groups in rats and mice. While the liver tumor frequency in female rats was similar at the two dose levels , the liver tumor frequencies were higher after 1 % dioxane exposures in both, male and female mice (NCI, 1974).

4.3.2. Interspecies Variability

Lethal concentrations were comparable in rats, mice and Guinea pigs. Only one study in cats was available, which suggested a somewhat higher susceptibility. The concentrations at which half of the animals died after a single exposure were:

- for rats about 10,000 ppm for 2x1.5 hours (Fairley et al. 1934), 14,300 ppm for 4 hours (Pozzani et al., 1959), 12,800 ppm for 4 hours (Pilipyuk et al., 1977) and 40,000 ppm for 1-3 hours (BASF AG, 1973; 1980);
- for mice 5000-10,000 ppm for 2x1.5 hours (Fairley et al. 1934), between 2800 ppm for 8-10 hours and 8300 for 3.5 hours (Wirth and Klimmer, 1936), 18000 ppm for 2 hours (Pilipyuk et al., 1977) and 10,109 ppm for 2 hours (Izmerov et al., 1982);
- for Guinea pigs between 10,000 ppm for 8 hours and 30,000 ppm for 3 hours (Yant et al., 1930) and about 10,000 ppm for 2x1.5 hours (Fairley et al. 1934);
- for rabbits >5000 ppm for 2x1.5 hours (Fairley et al. 1934);
- for cats about 1200 ppm for about 7 hours (Wirth and Klimmer, 1936).

The data are displayed in Figure 1. For comparison, the data point for the human case reported by Johnstone (1959) is also displayed. Taking into account that in this case dermal exposure occurred in addition to inhalation exposure and that the worker was exposed repeatedly before falling ill, this case of human exposure is in fairly good agreement with the animal data.

Similar pathological findings, comprising especially liver and kidney necrosis, were reported for fatalities after repeated inhalation exposure at the workplace (Barber, 1934; Johnstone, 1959) and after repeated inhalation and oral exposure of laboratory animals (Fairley et al. 1934; David, 1964).

The metabolism in humans and rats is very similar, involving the same metabolic steps and intermediate metabolites (Young et al., 1977; 1978a; 1978b).

Taken together, the interspecies variability for acute lethal effects is limited and an interspecies uncertainty factor of 3 is considered adequate.

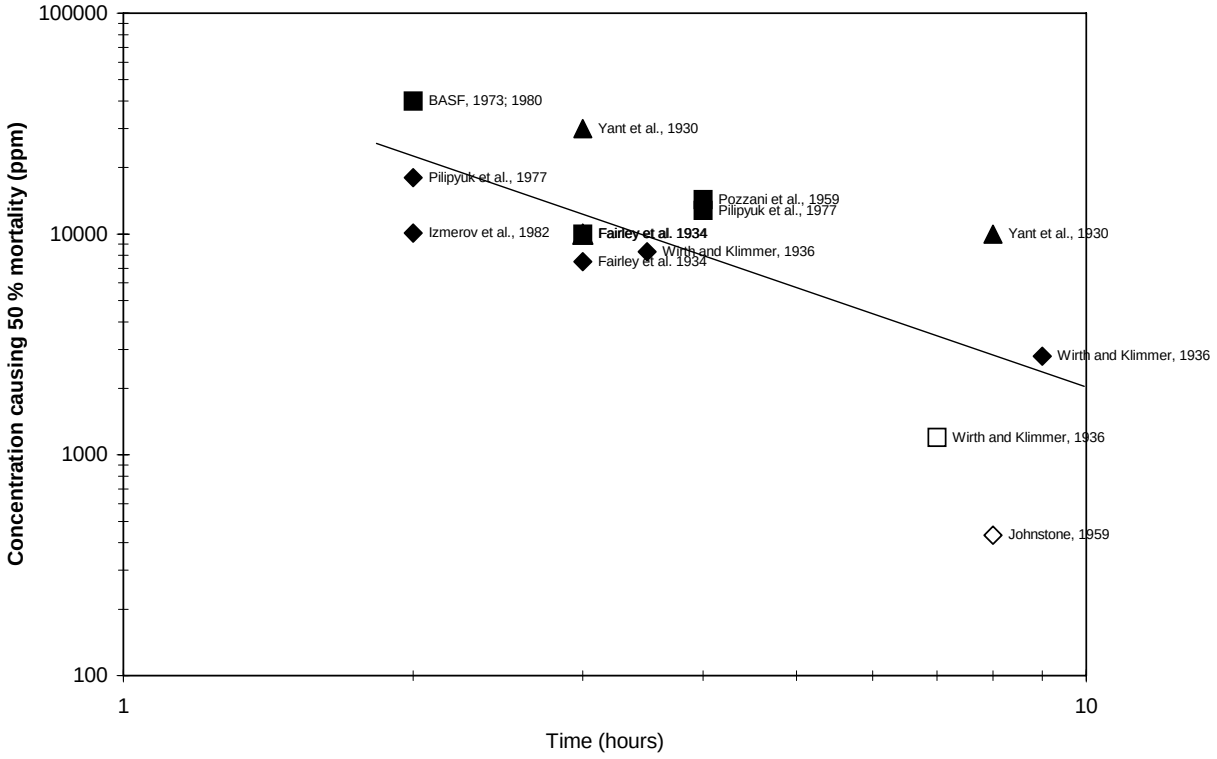


FIGURE 1: SPECIES COMPARISON OF LETHAL INHALATION EXPOSURE
For data points for which a range was given for the exposure concentration or the exposure time, the arithmetic mean of this range was used. Symbols indicate the following species: rat, filled square; mice, filled diamond; guinea pig, filled triangle; cat, open square, and human, open diamond. The line indicates the regression line calculated from all animal data.

4.3.3. Intraspecies Variability

Several studies that evaluated irritative effects of dioxane in humans, did not report marked interindividual differences (Fairley et al. 1934; Yant et al., 1930; Wirth and Klimmer, 1936, Young et al., 1977). However, since occurrence and severity of irritative symptoms were described for the groups of exposed volunteers, but not for each individual, no definitive conclusions can be drawn from these reports.

No studies were located regarding cardiovascular, gastrointestinal, musculoskeletal, hepatic, or renal effects in humans after nonlethal exposure to 1,4-dioxane. Case reports on fatalities reported severe liver and kidney damage. No data on interindividual differences with regard to systemic effects are available. Some interindividual variability in CNS effects was reported by Yant et al. (1930) when 3 or 5 subjects reported vertigo at 5500 ppm for 1 minute.

Due to the lack of data there was no basis for reducing the default intraspecies uncertainty factor.

5. RATIONALE AND PROPOSED AEGL-1

5.1. Human Data Relevant to AEGL-1

Young et al. (1977) exposed 4 healthy male subjects at 50 ppm for 6 hours in the dynamic chamber. Eye irritation was a frequent complaint throughout the exposure. The perception of odor diminished with time; two of the subjects could not perceive the odor after 4 and 5 hours in the chamber, while the other two subjects could still detect the odor at the end of the exposure period. In the study by Silverman et al. (1946), subjects exposed at 300 ppm for 15 minutes reported irritation to eyes, nose and throat; they did not find the odor objectionable. Wirth and Klimmer (1936) reported that exposure to 280 ppm (time period not specified) led to a slight mucous membrane irritation in exposed subjects. At 1400 ppm the irritation was quite distinct.

Hellman and Small (1974) reported an odor detection threshold of 1.8 ppm and an odor recognition threshold of 5.7 ppm. AIHA (1983) published a geometric mean odor detection threshold of 12 ppm and a geometric mean odor recognition threshold of 22 ppm.

5.2. Animal Data Relevant to AEGL-1

Yant et al. (1930) reported no eye irritation, squinting and lacrimation in Guinea pigs exposed to 1000 ppm for up to 6 hours, while at 2000 ppm or higher these symptoms were observed within 8 minutes or less.

Frantik et al. (1994) studied the inhibition of propagation and maintenance of the electrically evoked seizure discharge in rats and mice. Of six different time characteristics recorded, the duration of tonic extension of hindlimbs in rats and the velocity of tonic extension in mice were the most sensitive and reproducible response measures. The authors suggested the EC_{10} as the effect threshold, which was 1200 ppm for 4 hours in rats and 580 ppm for 2 hours in mice.

Drew et al. (1978) reported 2-3-fold increased serum activities of liver enzymes (ornithine carbamyl transferase, aspartate aminotransferase and alanine aminotransferase) in rats after a single 4-hour exposure to 1000 or 2000 ppm dioxane.

5.3. Derivation of AEGL-1

For the derivation of AEGL-1 values, irritation was considered the most relevant endpoint. As key study, the study of Young et al. (1977) was chosen, because this was the only adequately reported and analytically controlled study available for this endpoint. Young et al. (1977) reported eye irritation at 50 ppm throughout the 6-hour exposure period. Since this was a pharmacokinetic study, no emphasis was put on reporting of symptoms and the authors did not define the severity level of the eye irritation. The irritation was nevertheless considered to be below the notable discomfort level as described in the AEGL-1 definition because the authors (Young et al., 1977) considered 50 ppm as an adequate workplace standard and a level of 50 ppm has been used as a workplace standard in the past.

Although no definitive study on the mechanism of eye irritation exists, it is likely that it involves water extraction from the eyes caused by dioxane, which is also compatible the lack of skin irritation by dioxane (ECB, 1999).

Volunteers exposed at 300 ppm complained of irritation to eyes, nose and throat (Silverman et al., 1946). At a similar concentration of 280 ppm, Wirth and Klimmer (1936) found slight mucous membrane irritation in humans. More distinct irritation was observed at 1400-1600 ppm and severe irritation occurred at 2800-5500 ppm (Wirth and Klimmer, 1936; Yant et al., 1930). The shallow increase of irritative effects with concentration also supports the interpretation that the effects found at 50 ppm in the study of Young et al. (1977) can be considered as mild.

Since the study by Young et al. (1977) reported eye irritation throughout the whole exposure period of 6 hours and did not report an increase of the effect with time, it was considered adequate to use the same exposure concentration for all relevant time points. Using a constant value for AEGL-1 is also supported by the observation of Yant et al. (1930) who reported no eye irritation, squinting and lacrimation in Guinea pigs exposed at 1000 ppm for up to 6 hours, while at 2000 ppm or higher these symptoms were observed within 8 minutes or less. The calculations of exposure concentrations scaled to AEGL-1 time points are shown in Appendix A.

A total uncertainty factor of 3 was used because for local effects, the toxicokinetic differences do not vary considerably within and between species.

The values are listed in the table below.

TABLE 9: AEGL-1 VALUES FOR 1,4-DIOXANE

AEGL Level	10 minutes	30 minutes	1 hour	4 hours	8 hours
AEGL-1	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)

A level of distinct odor awareness (LOA) for 1,4-dioxane of 1.7 ppm was derived on the basis of the odor detection threshold from the study of Hellman and Small (1974) (see Appendix B for LOA derivation). The LOA represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, about 10 % of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odor perception.

6. RATIONALE AND PROPOSED AEGL-2

6.1. Human Data Relevant to AEGL-2

Yant et al. (1930) reported eye irritation, resulting in blinking, squinting and lacrimation, a burning sensation in nose and throat in 5 subjects exposed at 5500 ppm for 1 minute. Three of the subjects noticed a slight vertigo which disappeared quickly after leaving the vapor-air mixture. Exposure at 1600 ppm for 10 minutes resulted in an immediate slight burning of the eyes accompanied by

lacrimation, a slight irritation of the nose and throat and an alcohol-like odor, which decreased in intensity with continued exposure. Lacrimation and nasal irritation persisted throughout the test. No vertigo was noted at 1600 ppm.

Wirth and Klimmer (1936) reported that 5 subjects exposed for an unspecified period of time at 2800 ppm complained of very strong initial irritation and slight pressure in the chest; at 1400 ppm, irritation was quite distinct with slight stinging in the nose and scratchiness and dryness in the throat; at 280 ppm, slight mucous membrane irritation was reported. Fairley et al. (1934) reported that subjects exposed at 2000 ppm for 3 minutes experienced an initial strong odor, which diminished rapidly, but no strong irritation effects, such as lacrimation or cough.

6.2. Animal Data Relevant to AEGL-2

Drew et al. (1978) reported 2-3fold increased serum activities of liver enzymes (ornithine carbamyl transferase, aspartate aminotransferase and alanine aminotransferase) in rats after a single 4-hour exposure at 1000 or 2000 ppm dioxane.

Goldberg et al. (1964) studied the effect of dioxane on avoidance-escape behavior (pole climbing in response to buzzer to avoid electrical shock) of rats. Rats were exposed 4 hours/day, 5 days/week for 2 weeks. At 6000 ppm, about 6/8 rats showed a delay of the avoidance response already after the 1st exposure, and 3-8 of 8 rats were affected in the subsequent exposures. No effects were found on escape response; an effect on escape response was only found in 3/8 animals after the 3rd exposure at 6000 ppm.

6.3. Derivation of AEGL-2

For the derivation of AEGL-2 values effects on the central nervous system and effects on liver were considered relevant.

Like other solvents, dioxane can induce narcosis at very high concentrations. Yant et al.(1930) reported that 30,000 ppm induced narcosis in guinea pigs after 1-2 hours exposure, while at 10,000 ppm eye and nose irritation and labored breathing, but no narcosis, were observed. In mice, 8300 ppm for 3.5 hours caused narcosis (Wirth and Klimmer, 1936). Goldberg et al. (1964) reported that 6000 ppm for 4 hours affected the performance of rats in an conditioned response test (pole climbing in response to buzzer to avoid electrical shock), but did not affect the escape response to an electrical shock. The exposure level of 6000 ppm for 4 hours was considered a NOEL for central nervous system depression, while higher concentrations could impair the ability to escape.

A total uncertainty factor of 30 was used. The interspecies factor was reduced to 3 because the toxicodynamic differences between species were considered limited for CNS depression and because application of the default factor would have lowered the AEGL-2 values to a level that humans are known to tolerate without adverse effects (Young et al., 1977). An intraspecies factor of 10 was applied.

Time scaling using the equation $C^n \times t = k$ was carried out to derive exposure duration-specific values. Due to lack of a definitive data set, a default value for n of 3 was used in the exponential function for extrapolation from the experimental period (4 hours) to shorter exposure periods and a default value for n of 1 was used for extrapolation to longer exposure periods. Time extrapolation was continued to the

10-minute period because even at higher concentrations of 1600 ppm for 10 minutes (Yant et al., 1930) or 1400 ppm for 5 minutes (Wirth and Klimmer, 1936) exposed subjects did not experience more severe effects than moderate eye, nose and throat irritation. The calculations of exposure concentrations scaled to AEGL-2 time points are shown in Appendix A.

The endpoint of hepatotoxicity was also considered relevant because liver necrosis occurred in cases of fatal dioxane exposure at the workplace and repeated cytotoxic effects on the liver has been suggested as the mechanism of the carcinogenic effect of dioxane. As shown in the following, derivation of AEGL-2 values on the basis of hepatotoxicity results in identical AEGL-2 values as those derived for central nervous system effects. Drew et al. (1978) reported a 2-3-fold increase in serum activities of liver enzymes in rats after exposure to 1000 or 2000 ppm for 4 hours. The release of liver enzymes into the blood is a sign of cytotoxic liver damage. This effect is, however, normally transient in nature. A 2-3-fold increase in liver enzymes was considered a weak response because liver damage by chemicals, viruses or tumors can easily increase aminotransferase levels by 10- to 100-fold in rats and humans (Hayes et al., 1994). At a higher concentration of 5000 ppm for 2x1.5 hours/day, all rats died after several days from severe liver and kidney damage (Fairley et al., 1934; see Section 3.1.1). Therefore, exposure at 2000 ppm for 4 hours is considered a NOEL for AEGL-2 effects in rats and is used as the basis for AEGL-2 derivation.

A total uncertainty factor of 10 was used. An interspecies uncertainty factor of 1 was applied because metabolism in humans and rats is very similar, involving the same metabolic steps and intermediate metabolites (see Section 4.3.2) and because application of a total uncertainty factor of 30 would reduce the AEGL-2 level to an exposure concentration of 17 ppm for 8 hours and 33 ppm for 4 hours, which humans are known to tolerate without adverse effect (pharmacokinetic study exposing subjects to 50 ppm for 6 hours; Young et al., 1977). An intraspecies factor of 10 was applied.

Time scaling using the equation $C^n \times t = k$ was carried out to derive exposure duration-specific values as explained above. The calculations of exposure concentrations scaled to AEGL-2 time points are shown in Appendix A.

The derived values are considered adequate with respect to the carcinogenicity assessment (see Appendix C). Assuming a body weight of 70 kg, a ventilation rate of 20 m^3/d (WHO, 1999), and an absorption rate of 43 % (Young et al., 1977), the AEGL-2 values correspond to total body doses between 1.8 mg/kg for the 10-minute period and 14 mg/kg for the 8-hour period:

body dose = exposure conc. (mg/m^3) * absorption rate * ventilation rate * 1/body weight

body dose (8 h) = 360 mg/m^3 * 0.43 * 20 m^3 * 8 h/24 h * 1/70 kg = 14 mg/kg

body dose (10 min) = 2100 mg/m^3 * 0.43 * 20 m^3 * 0.167 h/24 h * 1/70 kg = 1.8 mg/kg

This dose level is below that associated with metabolic saturation or proliferative effects on the liver, which has been implicated in dioxane carcinogenicity (see Section 4.2).

The AEGL-2 values are listed in the table below.

TABLE 10: AEGL-2 VALUES FOR 1,4-DIOXANE

AEGL Level	10 minutes	30 minutes	1 hour	4 hours	8 hours

AEGL-2	580 ppm (2100 mg/m³)	400 ppm (1400 mg/m³)	320 ppm (1200 mg/m³)	200 ppm (720 mg/m³)	100 ppm (360 mg/m³)

7. RATIONALE AND PROPOSED AEGL-3

7.1. Human Data Relevant to AEGL-3

Barber (1934) described 5 fatalities after repeated exposure to unknown concentrations of dioxane at the workplace. The workers developed nausea and vomiting, described as "stomach trouble", followed after 2-3 days by oliguria and anuria. About 3-7 days after the first symptoms, coma developed, followed by death. Pathological findings included enlarged pale livers, swollen hemorrhagic kidneys, and edematous lungs and brains. Microscopic examinations revealed centrilobular liver necrosis, almost symmetrical necrosis of the outer renal cortex and hemorrhages around the glomeruli.

Johnstone (1959) reported a similar case of a man who worked near to an open container of dioxane. Later measurements of the atmosphere showed a dioxane concentrations between 208 and 650 ppm (plus additional dermal exposure). After 6 days on work, the man became hospitalized with severe epigastric pain. The patient developed oliguria, became comatose on the 6^{th} day and died one day later. Upon postmortem examination, the liver showed uniformly severe centrilobular necrosis and the kidneys showed cortex necrosis with extensive interstitial hemorrhage.

7.2. Animal Data Relevant to AEGL-3

Pozzani et al. (1959) reported a 4-hour LC_{50} for dioxane of 14300 ppm in rats. A similar LC_{50} value of 12,800 ppm for 4 hours was reported by Pilipyuk et al. (1977). For exposure to saturated dioxane atmosphere (estimated concentration 40,000 ppm), BASF AG (1973; 1980) reported no deaths of rats for a 1-hour exposure, while in the two experiments 100 % and 50 %, respectively, of the animals died after 3 hours of exposure. At necropsy, acute heart dilatation, hemorrhagic erosions of the stomach mucosa and acute lung dilatation were observed. Fairley et al. (1934) reported death of 1/3 rats after a single exposure day comprising two 1.5-hour exposures to 10,000 ppm. At 5000 ppm rats died after 3-5 consecutive exposure days.

For mice, LC_{50} values of 18,000 ppm (Pilipyuk et al., 1977) and 10,109 ppm (Izmerov et al., 1982) have been reported.

7.3. Derivation of AEGL-3

LC_{50} values in rats were considered most relevant for the derivation of the AEGL-3 values. No acute inhalation toxicity study that followed today's standards and guidelines was available for dioxane. The derivation was based on the 4-hour LC_{50} of 14,300 ppm in rats reported by Pozzani et al. (1959). Although this study did not use the most sensitive species (cats), it was used as key study because it was the only study that was adequately described and because study details were far better provided in this study than in the study by Pilipyuk et al. (1977). The LC_{50} reported in the key study is supported by other studies in rats (Pilipyuk et al., 1977; BASF AG; 1980; 1973).

For extrapolation from the LC_{50} value to the threshold for lethality, a factor of 3 was used. This factor was considered adequate because available data indicate a very steep dose-response curve for lethality after inhalation exposure: a) Pilipyuk et al. (1977) reported a factor of 1.3 between the LC_{84} and the LC_{16} (LC_{16} = 11,100 ppm and LC_{84} = 14,500 ppm); b) at 40,000 ppm, BASF AG (1973; 1980) reported no deaths after exposure for 1 hour, while in two experiments 50 and 100 %, respectively, of the rats died after a 3-hour exposure; and c) Yant (1930) reported death of all guinea pigs after 3-hour exposure at 30,000 ppm, while no lethality occurred after 10,000 ppm for 8 hours.

Time scaling using the equation $C^n \times t = k$ was carried out to derive exposure duration-specific values. Due to lack of a definitive data set, a default for n of 3 was used in the exponential function for extrapolation from the experimental period (4 hours) to shorter exposure periods and a default for n of 1 was used for extrapolation to longer exposure periods. For the 10-minute AEGL-3 the 30-minute value was applied because the derivation of AEGL values was based on a long experimental exposure period and no supporting studies using short exposure periods were available for characterizing the concentration-time-response relationship. Moreover, considerable uncertainty exists as to the concentration of dioxane in air at which cytotoxic effects occur in the nasal mucosa, which probably contributes to the mechanism leading to carcinogenic effects of dioxane. The calculations of exposure concentrations scaled to AEGL-3 time points are shown in Appendix A.

A total uncertainty factor of 10 was used. An interspecies uncertainty factor of 1 was applied because metabolism in humans and rats is very similar, involving the same metabolic steps and intermediate metabolites (see Section 4.3.2) and because a higher uncertainty factor would have resulted in AEGL-3 values of 480 ppm for 10 and 30 minutes, which contrasts with the observation that exposure of human subjects to 1600 ppm for 10 minutes (Yant et al., 1930) resulted in moderate irritation, but not in more severe effects. An intraspecies factor of 10 was applied.

The values are listed in the table below.

TABLE 11: AEGL-3 VALUES FOR 1,4-DIOXANE

AEGL Level	10 minutes	30 minutes	1 hour	4 hours	8 hours
AEGL-3	950 ppm (3400 mg/m³)	950 ppm (3400 mg/m³)	760 ppm (2700 mg/m³)	480 ppm (1700 mg/m³)	240 ppm (860 mg/m³)

Discussion of reported lethal human exposures: while in the study of Barber (1934) no (estimation of) exposure concentrations was reported, Johnstone (1959) found dioxane concentrations between 208 and 650 ppm in measurements performed after the death of a worker.

The equivalent body dose for an inhalation exposure of a man (assuming a body weight of 70 kg and a 8-hour workshift inhaled air volume of 10 m³) to 208-650 ppm dioxane for an 8-hour workshift can be calculated as:

resorbed dose (inh.) = (208 to 650) ppm * 3.6 mg/m³/ppm * 20 m³/d * 8 h/ 24 h * 0.43 * 1/70 kg

resorbed dose (inh.) = 31 to 96 mg/kg

using an resorption rate of 43 % (Young et al., 1977) and assuming a body weight of 70 kg and a ventilation rate of 20 m³/d (WHO, 1999).

The dermal exposure is more difficult to estimate. It is assumed that a maximum of 6 g dioxane remained on the hands from each use of dioxane to remove glue from hands and working table and that this procedure was done between 4-16 times per workshift. The skin absorption is assumed to be between the value of about 3 % measured for monkeys and humans (Marzulli et al., 1981; Bronaugh, 1982) and a 10-fold higher value due to skin defattening and skin damage from repeated solvent contact. Thus, a absorbed dermal dose of

absorbed dose (dermal) = 6000 mg * (0.03 to 0.30) * (4 to 16) / 70 kg

absorbed dose (dermal) = 10 to 410 mg/kg

In conclusion, it is likely that the dermal exposure contributed significantly to the total dioxane exposure, which was estimated between 41 and 506 mg/kg.

8. SUMMARY OF PROPOSED AEGLs

8.1. AEGL Values and Toxicity Endpoints

The derived AEGL values for various levels of effects and durations of exposure are summarized in Table 12. AEGL-1 were based on a pharmacokinetic study in humans in which eye irritation occurred at 50 ppm throughout the 6-hour exposure period (Young et al., 1977). AEGL-2 values were based on a study in rats in which exposure to 6000 ppm for 4 hours did not affect the ability to escape (Goldberg et al., 1964) and on a study in which exposure to 2000 ppm for 4 hours caused an increased serum activities of liver enzymes (Drew et al., 1978). A 4-hour LC_{50} value of 14,300 ppm (Pozzani et al., 1959), which is supported by another acute lethality study (Pilipyuk et al., 1977), was used for AEGL-3 derivation.

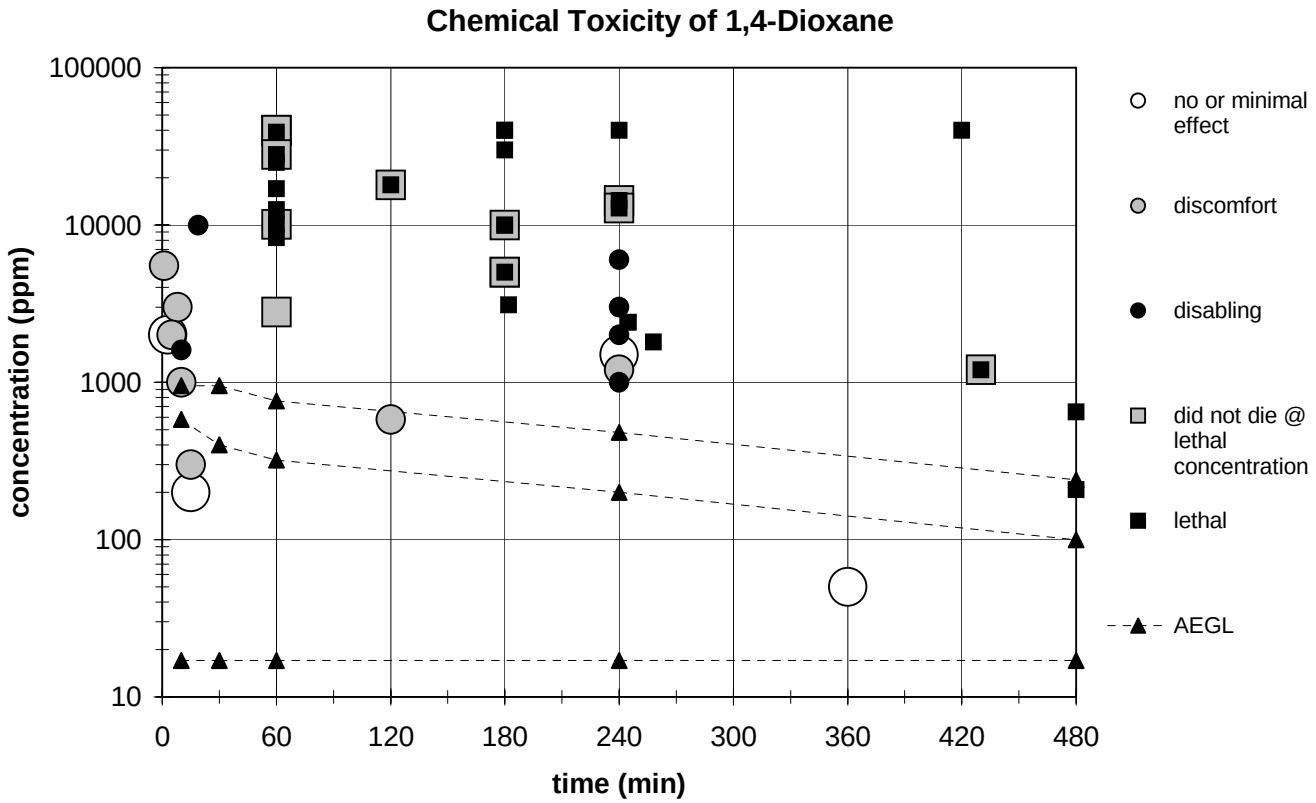
TABLE 12: SUMMARY/RELATIONSHIP OF PROPOSED AEGL VALUES [a]

Classification	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour
AEGL-1 (Nondisabling)	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)	17 ppm (60 mg/m³)
AEGL-2 (Disabling)	580 ppm (2100 mg/m³)	400 ppm (1400 mg/m³)	320 ppm (1200 mg/m³)	200 ppm (720 mg/m³)	100 ppm (360 mg/m³)
AEGL-3 (Lethal)	950 ppm (3400 mg/m³)	950 ppm (3400 mg/m³)	760 ppm (2700 mg/m³)	480 ppm (1700 mg/m³)	240 ppm (860 mg/m³)

[a] Cutaneous absorption may occur; direct skin contact with the liquid should be avoided.

All inhalation data are summarized in Figure 2 below. The data were classified into severity categories chosen to fit into definitions of the AEGL level health effects. The category severity definitions are "No effect"; "Discomfort"; "Disabling"; "Lethal"; "Did not die at a lethal concentration" (at an experimental concentration in which some of the animals died and some did not, this label refers to the animals which did not die) and "AEGL". Note that the AEGL values are designated as a triangle without an indication to their level. The AEGL-3 is higher than the AEGL-2, which is higher than the AEGL-1.

Note: Please note that the two ‘lethality points’ at 208 and 650 ppm for 480 minutes, which seem to be in conflict with the derived AEGL-2 and -3 values, represent the estimated exposure range for the case of lethal outcome of a repeated exposure at the workplace with additional dermal exposure (Johnstone, 1959; cf. discussion in Section 7.3).



1487 **FIGURE 2: CATEGORICAL REPRESENTATION OF ALL DIOXANE INHALATION DATA**

8.2. Comparison with Other Standards and Criteria

Other standards and guidance levels for workplace and community exposures are listed in Table 13.

TABLE 13. EXTANT STANDARDS AND GUIDELINES FOR 1,4-DIOXANE

Guideline	Exposure Duration				
	10 minutes	30 minutes	1 hour	4 hours	8 hours
AEGL-1	17 ppm	17 ppm	17 ppm	17 ppm	17 ppm
AEGL-2	580 ppm	400 ppm	320 ppm	300 ppm	100 ppm
AEGL-3	950 ppm	950 ppm	760 ppm	480 ppm	240 ppm
PEL-TWA (OSHA)[a]					100 ppm
IDLH (NIOSH)[b]		2000 ppm			
REL-TWA (NIOSH)[c]		1ppm [30-min ceiling]			
TLV-TWA (ACGIH)[d]					25 ppm
MAK (Germany)[e]					20 ppm
MAK Spitzen-begrenzung (Germany)[f]	40 ppm [for 15 min]				
MAC (The Netherlands)[g]	24 ppm [for 15 min]				12 ppm

[a] **OSHA PEL-TWA (Occupational Health and Safety Administration, Permissible Exposure Limits - Time Weighted Average)** (OSHA, 1993), is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[b] **IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)** (NIOSH, 1996), is based on acute inhalation toxicity data in animals (Wirth and Klimmer, 1936; Pilipyuk et al., 1977; Yant et al., 1930).

[c] **NIOSH REL-TWA (National Institute of Occupational Safety and Health, Recommended Exposure Limits - Time Weighted Average)** (NIOSH, 1977), is defined analogous to the ACGIH-TLV-TWA. The value was based on the belief that dioxane can cause tumors in exposed workers and on the belief that information allowing the derivation of a safe exposure limit was not available. Thus, the limit was set at the lowest concentration reliably measurable over a short sampling period, which, according to NIOSH, was 1 ppm, based on 30-minute sampling at a sampling rate of 1 l/min. In the past, NIOSH has subscribed to a carcinogen policy which called for "no detectable exposure levels for proven carcinogenic substances". Because of advances in science and in approaches to risk assessment and risk management, NIOSH has

adopted a more inclusive policy (see http://www.cdc.gov/niosh/npg/nengapdx.html). NIOSH recommended exposure limits (RELs) will be based on risk evaluations using human or animal health effects data, and on an assessment of what levels can be feasibly achieved by engineering controls and measured by analytical techniques. To the extent feasible, NIOSH will project not only a no-effect exposure, but also exposure levels at which there may be residual risks.

[d] **ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average)** (ACGIH, 1997). 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.

[e] **MAK (Maximale Arbeitsplatzkonzentration [Maximum Workplace Concentration], Deutsche Forschungs-gemeinschaft [German Research Association], Germany)** (Henschler, 1976/77; Greim, 1996; 1998; 2000), is defined analogous to the ACGIH-TLV-TWA. The MAK values is based on eye irritation at 50 ppm (Young et al., 1977)

[f] **MAK Spitzenbegrenzung (Kategorie I) [Peak Limit Category I, 2]** (Henschler, 1976/77; Greim, 1996; 1998; 2000), constitutes the maximum average concentration to which workers can be exposed for periods up to 15 minutes, with at least 1 hour between exposures and no more than 4 exposures per work shift; total exposure may not exceed 8-hour MAK. The Category I is applied to irritating substances, the excess factor of 2 (over the 8-hour MAK) was chosen by convention and was not derived on substance-specific data.

[g] **MAC ([Maximum Workplace Concentration], Dutch Expert Committee for Occupational Standards, The Netherlands)** (ECB, 1999), is defined analogous to the ACGIH-TLV-TWA.

8.3. Data Adequacy and Research Needs

Older studies have assessed irritative effects of dioxane in humans after a single inhalation exposure. Additionally, experimental studies on the toxicokinetics and the odor perception are available. AEGL-1 values were based on eye irritation in humans reported in a toxicokinetic study. Only few studies are available for the derivation of AEGL-2 values. The AEGL-2 values were based on a study reporting a no effects on the escape response in rats, which was considered a NOEL for depressive effects on the central nervous system that led to narcosis, i.e. the inability to escape, in other studies at higher concentrations. In addition, a study reporting increased liver enzyme activities in serum indicating liver toxicity was used as additional key study. This study was supported by single oral exposure studies demonstrating proliferative and genotoxic effects on rat hepatocytes. For derivation of AEGL-3 values, no LC_{50} study performed and documented according to today‘s standards was available, however, several older studies investigated lethal effects in experimental animals after acute inhalation exposure and reported LC_{50} values. The AEGL-3 values were based on a reported LC_{50} value in rats, which was supported by other acute lethality studies.

Single inhalation exposure studies in animals focusing on lethal effects and irreversible liver and kidney damage would allow for more precisely defining the thresholds for the AEGL-2 and -3 levels.

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APPENDIX A

Time Scaling Calculations for AEGLs

AEGL-1

Key study:	Young et al. (1977)
Toxicity endpoint:	eye irritation occurred at 50 ppm throughout the 6-hour exposure period in this pharmacokinetic study. Since this was a pharmacokinetic study, no emphasis was put on reporting of symptoms and the authors did not define the severity level of the eye irritation. The irritation was nevertheless considered to be below the notable discomfort level as described in the AEGL-1 definition because the authors (Young et al., 1977) considered 50 ppm as an adequate workplace standard and a level of 50 ppm has been used as a workplace standard in the past.
Scaling:	Since the study by Young et al. (1977) reported eye irritation throughout the whole exposure period of 6 hours and did not report an increase of the effect with time, it is considered adequate to use the same exposure concentration for all relevant time points (flat line). C = 50 ppm
Uncertainty/ modifying factors:	3 for intraspecies variability
Calculations:	
10-minute AEGL-1	C = 50 ppm 10-min AEGL-1 = 50 ppm/3 = 17 ppm (60 mg/m³)
30-minute AEGL-1	C = 50 ppm 30-min AEGL-1 = 50 ppm/3 = 17 ppm (60 mg/m³)
1-hour AEGL-1	C = 50 ppm 1-hour AEGL-1 = 50 ppm/3 = 17 ppm (60 mg/m³)
4-hour AEGL-1	C = 50 ppm 4-hour AEGL-1 = 50 ppm/3 = 17 ppm (60 mg/m³)
8-hour AEGL-1	C = 50 ppm 8-hour AEGL-1 = 50 ppm/3 = 17 ppm (60 mg/m³)

AEGL-2

Key study #1:	Goldberg et al. (1964)
Toxicity endpoint:	In rats, exposure to 6000 ppm for 4 hours resulted in a reduced performance in a conditioned response test, but did not affect the escape response.
Scaling:	$C^3 * t = k$ for extrapolation to 1 hours, 30 minutes and 10 minutes $k = 6000^3$ ppm^3 * 4 h = 8.64 * 10^{11} ppm^3 h $C^1 * t = k$ for extrapolation to 8 hours $k = 6000^1$ ppm * 4 h = 24,000 ppm h
Uncertainty/ modifying factors:	Combined uncertainty factor of 30 3 for interspecies variability 10 for intraspecies variability
Calculations:	
10-minute AEGL-2	$C^3 * 0.167$ h = 8.64 * 10^{11} ppm^3 h C = 17,295 ppm 10-min AEGL-2 = 17,295 ppm/30 = 580 ppm (2100 mg/m^3)
30-minute AEGL-2	$C^3 * 0.5$ h = 8.64 * 10^{11} ppm^3 h C = 12,000 ppm 30-min AEGL-2 = 12,000 ppm/30 = 400 ppm (1400 mg/m^3)
1-hour AEGL-2	$C^3 * 1$ h = 8.64 * 10^{11} ppm^3 h C = 9524.0 ppm 1-hour AEGL-2 = 9524 ppm/30 = 320 ppm (1200 mg/m^3)
4-hour AEGL-2	4-hour AEGL-2 = 6000 ppm/30 = 200 ppm (720 mg/m^3)
8-hour AEGL-2	C^1 * 8 h = 24,000 ppm h C = 3000.0 ppm 8-hour AEGL-2 = 3000 ppm/30 = 100 ppm (360 mg/m^3)

AEGL-2

Key study #2:	Drew et al. (1978)
Toxicity endpoint:	In rats, a 2-3fold increased serum activities of liver enzymes (ornithine carbamyl transferase, aspartate aminotransferase and alanine aminotransferase) occurred after a single 4-hour exposure to 1000 or 2000 ppm dioxane. An exposure to 2000 ppm for 4 hours was used as a basis for AEGL derivation.
Scaling:	$C^3 * t = k$ for extrapolation to 1 hours, 30 minutes and 10 minutes $k = 2000^3$ ppm^3 * 4 h = 3.2 * 10^{10} ppm^3 h $C^1 * t = k$ for extrapolation to 8 hours $k = 2000^1$ ppm * 4 h = 8000 ppm h
Uncertainty/ modifying factors:	Combined uncertainty factor of 10 1 for interspecies variability 10 for intraspecies variability
Calculations:	
10-minute AEGL-2	$C^3 * 0.167$ h = 3.2 * 10^{10} ppm^3 h C = 5765.2 ppm 10-min AEGL-2 = 5765 ppm/10 = 580 ppm (2100 mg/m^3)
30-minute AEGL-2	$C^3 * 0.5$ h = 3.2 * 10^{10} ppm^3 h C = 4000.0 ppm 30-min AEGL-2 = 4000 ppm/10 = 400 ppm (1400 mg/m^3)
1-hour AEGL-2	$C^3 * 1$ h = 3.2 * 10^{10} ppm^3 h C = 3174.8 ppm 1-hour AEGL-2 = 3175 ppm/10 = 320 ppm (1200 mg/m^3)
4-hour AEGL-2	4-hour AEGL-2 = 2000 ppm/10 = 200 ppm (720 mg/m^3)
8-hour AEGL-2	C^1 * 8 h = 8000 ppm h C = 1000.0 ppm 8-hour AEGL-2 = 1000 ppm/10 = 100 ppm (360 mg/m^3)

AEGL-3

Key study:	Pozzani et al. (1959)
Toxicity endpoint:	LC_{50} of 14,300 ppm in rats for 4 hours of exposure.
Extrapolation factor:	3 for extrapolation of LC_{50} to lethality threshold 14,300 ppm / 3 = 4767 ppm
Scaling:	$C^3 * t = k$ for extrapolation to 4 hours, 1 hours, 30 minutes and 10 minutes $k = 4767^3$ ppm^3 * 4 h = $4.333 * 10^{11}$ ppm^3 h $C^1 * t = k$ for extrapolation to 8 hours $k = 4767^1$ ppm * 4 h = 19,068 ppm h
Uncertainty/ modifying factors:	Combined uncertainty factor of 10 1 for interspecies variability 10 for intraspecies variability
Calculations:	
10-minute AEGL-3	10-min AEGL-3 = 30-min AEGL-3 = 950 ppm (3400 mg/m^3)
30-minute AEGL-3	$C^3 * 0.5$ h = $4.333 * 10^{11}$ ppm^3 h C = 9533.9 ppm 30-min AEGL-3 = 9534 ppm/10 = 950 ppm (3400 mg/m^3)
1-hour AEGL-3	$C^3 * 1$ h = $4.333 * 10^{11}$ ppm^3 h C = 7567.1 ppm 1-hour AEGL-3 = 7567 ppm/10 = 760 ppm (2700 mg/m^3)
4-hour AEGL-3	4-hour AEGL-3 = 4767 ppm/10 = 480 ppm (1700 mg/m^3)
8-hour AEGL-3	$C^1 * 8$ h = 19,068 ppm h C = 2383.5 ppm 8-hour AEGL-3 = 2384 ppm/10 = 240 ppm (860 mg/m^3)

1901 **APPENDIX B**

1902 **Level of Distinct Odor Awareness**

Derivation of the Level of Distinct Odor Awareness (LOA)

The level of distinct odor awareness (LOA) represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, about 10 % of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odor perception. The LOA derivation follows the guidance given by van Doorn et al. (2002).

For derivation of the odor detection threshold (OT_{50}), two studies are available in which the odor threshold for the reference chemical n-butanol (odor detection threshold 0.04 ppm) have also been determined:

May (1966):
odor detection threshold for dioxane: 170 ppm
odor detection threshold for n-butanol: 11 ppm
corrected odor detection threshold (OT_{50}) for dioxane: 170 ppm * 0.04 ppm / 11 ppm = 0.62 ppm

Hellman and Small (1974):
odor detection threshold for dioxane: 0.8 ppm
odor detection threshold for n-butanol: 0.3 ppm
corrected odor detection threshold (OT_{50}) for dioxane: 0.8 ppm * 0.04 ppm / 0.3 ppm = 0.11 ppm

Since the n-butanol value from the Hellman and Small (1974) study was much closer to the reference value, this study was used to derive the LOA.

The concentration (C) leading to an odor intensity (I) of distinct odor detection (I=3) is derived using the Fechner function:

$I = k_w * \log (C / OT_{50}) + 0.5$

For the Fechner coefficient, the default of $k_w = 2.33$ will be used due to the lack of chemical-specific data:

$3 = 2.33 * \log (C / 0.11) + 0.5$ which can be rearranged to

$\log (C / 0.11) = (3 - 0.5) / 2.33 = 1.07$ and results in

$C = (10^{\wedge}1.07) * 0.11 = 11.8 * 0.11 = 1.30$ ppm

The resulting concentration is multiplied by an empirical field correction factor. It takes into account that in every day life factors, such as sex, age, sleep, smoking, upper airway infections and allergy as well as distraction, increase the odor detection threshold by a factor of 4. In addition, it takes into account that odor perception is very fast (about 5 seconds) which leads to the perception of concentration peaks. Based on the current knowledge, a factor of 1/3 is applied to adjust for peak exposure. Adjustment for distraction and peak exposure lead to a correction factor of 4 / 3 = 1.33

$LOA = C * 1.33 = 1.30 \text{ ppm} * 1.33 = 1.7$ ppm

The LOA for 1,4-dioxane is 1.7 ppm.

APPENDIX C

Preliminary Cancer Assessment of 1,4-Dioxane

Preliminary Cancer Assessment of 1,4-Dioxane

No inhalation slope factor is available for dioxane. As discussed in Section 4.2, the relevance to humans of the nasal tumors in rats observed in the drinking water studies is doubtful. Therefore, dose-response data for liver tumors in rats and mice will be used for calculation.

Stickney et al. analyzed the available tumor dose-response data and calculated a geometric mean oral slope factor of 2.4×10^{-3} $(mg/kg/day)^{-1}$.

As described in Section 3.4, some studies indicate that dioxane or one of its metabolites may exert clastogenic effects in vivo at high oral doses and in vitro at high concentrations: increased micronuclei formation in rat hepatocytes was found after a single oral dose of 2000 mg/kg (Morita and Hayashi, 1998); an increased rate of DNA strand breaks was found in rats after a single oral dose of 2550 mg/kg, but not at 840 mg/kg (Kitchin and Brown, 1990; 1994); moreover, dioxane induced sister chromatid exchanges in CHO cells (Galloway et al., 1987) and transformation of Balb 3T3 cells (Sheu et al., 1988) in vitro. However, there is also considerable evidence that dioxane causes tumors via a non-genotoxic, cytotoxic mechanism (see Section 4.2): increased hepatocyte cell proliferation has been reported in rats after a single oral dose of 1000 mg/kg or higher (Miyagawa et al., 1999), while in other studies (Stott et al., 1981; Goldsworthy et al., 1991) repeated oral doses of 2000 mg/kg were necessary to induce increases in hepatocyte proliferation. Consistent with this effect level, an inhalation exposure of rats to 1000 ppm for 4 hours, corresponding to a body dose of about 630 mg/kg, resulted in increased serum activities of liver enzymes (Drew et al., 1978). The non-linear toxicokinetics of dioxane in rats leads to saturation of the oxidation of dioxane to 2-hydroxyethoxyacetic acid and 1,4-dioxane-2-one at doses between 10 and 1000 mg/kg (Young et al. 1978a; 1978b); this could result in the accumulation of dioxane and possibly of its metabolites, such as 1,4-dioxane-2-one and 2-hydroxyethoxy-acetaldehyde.

Overall, it is concluded that there is little evidence of carcinogenicity from a short-term exposure to dioxane.

Calculation:

The inhalation slope factor can be estimated by dividing the oral slope factor by a body weight of 70 kg and multiplying by the inhalation rate of 20 m^3/day:

Inhalation slope factor $= 2.4 \times 10^{-3}$ $(mg/kg/day)^{-1} * 20$ $m^3/d * 1/70$ kg $= 6.9 \times 10^{-4}$ $(mg/m^3)^{-1}$

To calculate a concentration of dioxane that would cause a theoretical excess cancer risk of 10^{-4} (a virtually safe dose), the risk is divided by the slope factor:

dose = risk/slope factor = $1 \times 10^{-4} / 6.9 \times 10^{-4}$ $(mg/m^3)^{-1} = 0.14$ mg/m^3

To convert a 70-year exposure to a 24-hour exposure, the virtually safe dose is multiplied by the number of days in 70 years:

24-hour exposure concentration = 0.14 mg/m^3 * 25600 days = 3584 mg/m^3

To adjust for uncertainties in assessing potential cancer risks under short-term exposures under the multistage model, the 24-hour exposure is divided by an adjustment factor of 6 (see SOP):

3584 mg/m^3 / 6 = 597 mg/m^3

If the exposure is limited to a fraction (f) of a 24-hour period, the fractional exposure becomes 1/f * 24 h:

24-hour exposure = 597 mg/m³ (166 ppm)
8-hour exposure = 1791 mg/m³ (498 ppm)
4-hour exposure = 3582 mg/m³ (996 ppm)
1-hour exposure = 14328 mg/m³ (3983 ppm)
30-minute exposure = 28656 mg/m³ (7966 ppm)
10-minute exposure = 85968 mg/m³ (23899 ppm)

For 10^{-5} and 10^{-6} risk levels, the 10^{-4} values are reduced by 10-fold and 100-fold, respectively.

These values based on carcinogenicity exceed the AEGL-3 and AEGL-2 values based on non-carcinogenic effects and are, therefore, not proposed for AEGL-3 or AEGL-2. The current scientific knowledge suggests that dioxane will only induce cancer after multiple exposures.

1989 **APPENDIX D**

1990 **Derivation Summary for 1,4-Dioxane AEGLs**

ACUTE EXPOSURE GUIDELINES FOR 1,4-DIOXANE (CAS NO. 123-91-1)

AEGL-1 VALUES				
10 minutes	30 minutes	1 hour	4 hours	8 hours
17 ppm	17 ppm	17 ppm	17 ppm	17 ppm

Reference: Young, J.D., W.H. Braun, L.W. Rampy, M.B. Chenoweth and G.E. Blau, 1977. Pharmacokinetics of 1,4-dioxane in humans. *Journal of Toxicology and Environmental Health*, 3, 507-520.

Test Species/Strain/Number: Humans/ n.a. / 4 males

Exposure Route/Concentrations/Durations: Inhalation / 50 ppm / 6 hours

Effects:
Eye irritation was a frequent complaint throughout the exposure. The perception of odor diminished with time; two of the subjects could not perceive the odor after 4 and 5 hours in the chamber, while the other two subjects could still detect the odor at the end of the exposure period. No other clinical effects were observed in this pharmacokinetic study.

Endpoint/Concentration/Rationale:
For the derivation of AEGL-1 values, irritation was considered the most relevant endpoint. As key study, the pharmacokinetic study of Young et al. (1977) was chosen, because this was the only adequately reported and analytically controlled study available for this endpoint. Young et al. (1977) reported eye irritation at 50 ppm throughout the 6-hour exposure period. Since this was a pharmacokinetic study, no emphasis was put on reporting of symptoms and the authors did not define the severity level of the eye irritation. The irritation was nevertheless considered to be below the notable discomfort level as described in the AEGL-1 definition because the authors (Young et al., 1977) considered 50 ppm as an adequate workplace standard and a level of 50 ppm has been used as a workplace standard in the past. In the study by Silverman et al. (1946) 300 ppm caused irritation to eyes, nose and throat. At a similar concentration, 280 ppm, Wirth and Klimmer (1936) found slight mucous membrane irritation. More distinct irritation was observed at higher concentrations of 1400-1600 ppm and severe irritation occurred at 2800-5500 ppm (Wirth and Klimmer, 1936; Yant et al., 1930). The shallow increase of irritative effects with concentration also supports the interpretation that the effects found at 50 ppm in the study of Young et al. (1977) can be considered as mild and as a basis for AEGL-1 derivation.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: not applicable
Intraspecies: 3 - because for local effects, the toxicokinetic differences do not vary considerably within and between species.

Modifying Factor: Not applicable

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling:
Since the study by Young et al. (1977) reported eye irritation throughout the whole exposure period of 6 hours and did not report an increase of the effect with time, it is considered adequate to use the same exposure concentration for all relevant time points. Using a constant value for AEGL-1 is also supported by the observation of Yant et al. (1930) who reported no eye irritation, squinting and lacrimation in Guinea pigs exposed to 1000 ppm for up to 6 hours, while at 2000 ppm or higher these symptoms were observed within 8 minutes or less.

Level of distinct odor awareness (LOA)
The level of distinct odor awareness (LOA) for 1,4-dioxane is 1.7 ppm. This value is based on the odor detection threshold reported by Hellman and Small (1974). The LOA represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, about 10 % of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odor perception

Data Adequacy:
Although only a small number of subjects were investigated and the irritative effects were not the focus of this pharmacokinetic study, the study was considered adequate as AEGL-1 key study. The AEGL-1 value is between the odor detection and odor recognition thresholds for dioxane of 12 and 22 ppm, respectively (AIHA, 1983). At the derived AEGL-1 concentration, sensitive individuals may experience slight eye irritation which is considered unlikely to exceed the AEGL-1 effect level. The derived AEGL-1 values is, thus, considered to have warning properties, although it should be noted that human exposure studies indicated that individuals get accustomed to the odor after the first minutes (Young et al., 1977; Failey et al., 1934).

ACUTE EXPOSURE GUIDELINES FOR 1,4-DIOXANE (CAS NO. 123-91-1)

AEGL-2 VALUES				
10 minutes	30 minutes	1 hour	4 hours	8 hours
580 ppm	400 ppm	320 ppm	200 ppm	100 ppm

Reference:
#1: Goldberg, M.E., H.E. Johnson, U.C. Pozzani and H.F. Smyth, 1964. Effect of repeated inhalation of vapors of industrial solvents on animal behavior. I. Evaluation of nine solvent vapors on pole-climb performance in rats. *American Industrial Hygienists Association Journal,* 25, 369-375.
#2: Drew, R.T., J.M. Patel and F.-N. Lin, 1978. Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination. *Toxicology and Applied Pharmacology,* 45, 809-819.

Test Species/Strain/Sex/Number: #1: Rats / Carworth Farms Elias female / 8 per group
#2: Rats / CD1 male / number of rats per group not stated

Exposure Route/Concentrations/Durations: #1: Inhalation / 1500, 3000 and 6000 ppm / 4 hours/day, 5 days/week for 2 weeks
#2: Inhalation / 0, 1000 and 2000 ppm / 4 hours

Effects:
#1: A conditioned response (pole climbing in response to buzzer to avoid electrical shock) and escape response (pole climbing to electrical shock without buzzer signal) were determined on days 1, 2, 3, 4, 5 and 10 before, during and 2 hours after removal from exposure. At 1500 ppm, no effects occurred. At 3000 ppm, the conditioned response was delayed in 2/8 rats after the first and in 2-3/8 rats after the subsequent exposures. At 6000 ppm, about 6/8 rats showed a delay of the conditioned response after the 1st exposure, and 3-8/8 rats were affected in the subsequent exposures. No effects were found on escape response (unconditioned stimulus) after the first exposure (for any of the exposure conditions); an effect was found in 3/8 animals after the 2nd exposure to 6000 ppm, but not in the subsequent exposures.
#2: No effect on glucose-6-phosphatase was found. The activities of ornithine carbamyl transferase and aspartate aminotransferase were dose-dependently increased (about 2-3-fold) at 24 and 48 h; the activity of alanine aminotransferase was about 2-fold increased at 2000 ppm at 24 and 48 hours while it was only marginally increased at 1000 ppm.

Endpoint/Concentration/Rationale:
#1: Like other solvents, dioxane can induce narcosis at very high concentrations. Yant et al.(1930) reported that 30,000 ppm induced narcosis in guinea pigs after 1-2 hours exposure, while at 10,000 ppm eye and nose irritation and labored breathing, but no narcosis, were observed. In mice, 8300 ppm for 3.5 hours caused narcosis (Wirth and Klimmer, 1936). Goldberg et al. (1964) reported that 6000 ppm for 4 hours affected the performance of rats in an conditioned response test (pole climbing in response to buzzer to avoid electrical shock), but did not affect the escape response to an electrical shock. The exposure level of 6000 ppm for 4 hours was considered a NOEL for central nervous system depression, while higher concentrations could impair the ability to escape.
#2: Drew et al. (1978) reported a 2-3-fold increase in serum activities of liver enzymes in rats after exposure to 1000 or 2000 ppm for 4 hours. The release of liver enzymes into the blood are a sign of cytotoxic liver damage; this effect is, however, normally transient in nature. A 2-3-fold increase in liver enzymes was considered a weak response because liver damage by chemicals, viruses or tumor can easily increase aminotransferase levels by 10- to 100-fold in rats and humans (Hayes et al., 1994). At a concentration of 5000 ppm for 2x1.5 hours/day, all rats died after several days from severe liver and kidney damage (Fairley et al., 1934). Therefore, an exposure to 2000 ppm for 4 hours is considered a NOEL for AEGL-2 effects in rats and is used as the basis for AEGL-2 derivation.

Uncertainty Factors/Rationale:
#1: The interspecies factor was reduced to 3 because the toxicodynamic differences between species were considered limited for CNS depression and because application of the default factor would have lowered the AEGL-2 values to a level that humans are known to tolerate without adverse effects (Young et al., 1977). An intraspecies factor of 10 was applied.
#2: An interspecies uncertainty factor of 1 was applied because metabolism in humans and rats is very similar, involving the same metabolic steps and intermediate metabolites (see Section 4.3.2) and because application of a total uncertainty factor of 30 would reduce the AEGL-2 level to an exposure concentration of 17 ppm for 8 hours and 33 ppm for 4 hours, which humans are known to tolerate without adverse effect (pharmacokinetic study exposing subjects to 50 ppm for 6 hours; Young et al., 1977). An intraspecies factor of 10 was applied.

Total uncertainty factor:	#1: 30	#2: 10
Interspecies:	#1: 3	#2: 1
Intraspecies:	#1: 10	#2: 10

Modifying Factor: Not applicable

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling:
Time scaling using the equation $C^n * t = k$ was done to derive the other exposure duration-specific values. Due to lack of a definitive data set, an n of 3 was used in the exponential function for extrapolation from the experimental period (4 hours) to shorter exposure periods and an n of 1 was used for extrapolation to longer exposure periods. Time extrapolation was continued to the 10-minute period because even at considerably higher concentrations of 1600 ppm for 10 minutes (Yant et al., 1930) or 1400 ppm for 5 minutes (Wirth and Klimmer, 1936) exposed subjects did not experience more severe effects than moderate eye, nose and throat irritation.

Data Adequacy:
Due to the lack of appropriate human studies, the AEGL-2 values were based on central nervous system effects in rats and liver toxicity in rats. The derived values are considered adequate with respect to the carcinogenicity assessment. Assuming a body weight of 70 kg, a ventilation rate of 10 m^3 during an 8-hour shift, and an absorption rate of 43 % (Young et al., 1977), the AEGL-2 values correspond to total body doses between 1.8 mg/kg for the 10-minute period and 14 mg/kg for the 8-hour period. This dose level was far below that associated with metabolic saturation or proliferative effects on the liver, which has been implicated in dioxane carcinogenicity.

ACUTE EXPOSURE GUIDELINES FOR 1,4-DIOXANE (CAS NO. 123-91-1)

AEGL-3 VALUES				
10 minutes	30 minutes	1 hour	4 hours	8 hours
950 ppm	950 ppm	760 ppm	480 ppm	240 ppm

Reference: a) Pozzani, U.C., C.S. Weil and C.P. Carpenter, 1959. The toxicological basis of threshold limit values. 5. The experimental inhalation of vapor mixtures by rats with notes upon the relationship between single dose inhalation and single dose oral data. *American Industrial Hygiene Assocation Journal*, 20, 364-369; b) Pilipyuk, Z.I., G.M. Gorban, G.I. Solomin and A.I. Gorshunova, 1977. Toxicology of 1,4-dioxane [in Russian]. *Kosmicleskaja Biologiya i Aviakosmicheskaya Medicina*, 11, 53-57.

Test Species/Strain/Sex/Number: a) Rat / Carworth Farms-Nelson / females, number not stated
b) Rat / not stated / not stated

Exposure Route/Concentrations/Durations: a) Inhalation / not stated / 4 hours
b) Inhalation / not stated / 4 hours

Effects: a) LC_{50} for dioxane was 14300 ppm (51.3 mg/l)
b) LC_{16} = 11,100 ppm, LC_{50} = 12800 ppm and LC_{84} = 14,500 ppm

Endpoint/Concentration/Rationale:
LC_{50} values in rats were considered most relevant for the derivation of the AEGL-3 values. No acute inhalation toxicity study that followed today's standards and guidelines was available for dioxane. The derivation was based on the 4-hour LC_{50} of 14,300 ppm in rats reported by Pozzani et al. (1959). Although this study did not use the most sensitive species (cats), it was used as key study because it was the only study that was adequately described and because study details were far better described in this study than in the study by Pilipyuk et al. (1977). The equivalent body dose for an inhalation exposure of female rats (assuming a body weight of 0.250 kg) to 14,300 ppm dioxane for 4 hours can be calculated as 8786 mg/kg. The estimated total inhaled dose is comparable to oral LD_{50} values in rats which were between 5170 and 7339 mg/kg (BASF, 1958; 1973; Laug et al., 1939; Nelson, 1951; Pozzani et al., 1959; Smyth et al., 1939) and thus supports the LC_{50} value of Pozzani et al. (1959) used as basis for AEGL-3 derivation.
For extrapolation from the LC_{50} value to the threshold for lethality, a factor of 3 was used. This factor was considered adequate because available data indicate a very steep dose-response curve for lethality after inhalation exposure: a) Pilipyuk et al. (1977) reported a factor of 1.3 between the LC_{84} and the LC_{16} (LC_{16} = 11,100 ppm and LC_{84} = 14,500 ppm); b) at 40,000 ppm, BASF AG (1973; 1980) reported no deaths after exposure for 1 hour, while in two experiments 50 and 100 %, respectively, of the rats died after a 3-hour exposure; and c) Yant (1930) reported death of all guinea pigs after 3-hour exposure at 30,000 ppm, while no lethality occurred after 10,000 ppm for 8 hours.

Uncertainty Factors/Rationale: Total uncertainty factor: 10 Interspecies: 1 because metabolism in humans and rats is very similar, involving the same metabolic steps and intermediate metabolites (see Section 4.3.2) and because a higher uncertainty factor would have resulted in AEGL-3 values of 480 ppm for 10 and 30 minutes, which contrasts with the observation that exposure of human subjects to 1600 ppm for 10 minutes (Yant et al., 1930) resulted in moderate irritation, but not in more severe effects. Intraspecies: 10
Modifying Factor: Not applicable
Animal to Human Dosimetric Adjustment: Insufficient data
Time Scaling: Time scaling using the equation $C^n * t = k$ was done to derive the other exposure duration-specific values. Due to lack of a definitive data set, an n of 3 was used in the exponential function for extrapolation from the experimental period (4 hours) to shorter exposure periods and an n of 1 was used for extrapolation to longer exposure periods. For the 10-minute AEGL-3 the 30-minute value was applied because the derivation of AEGL values was based on a long experimental exposure period and no supporting studies using short exposure periods were available for characterizing the concentration-time-response relationship. Moreover, considerable uncertainty exists as to the concentration of dioxane in air at which cytotoxic effects occur in the nasal mucosa, which probably contributes to the mechanism leading to carcinogenic effects of dioxane.
Data Adequacy: No well-documented inhalation LC_{50} study in laboratory animals performed to today's standards was available for the derivation of AEGL-3 values. Therefore, a study in rats was used, which was supported by other inhalation as well as acute oral toxicity studies.