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1,3-BUTADIENE
(CAS Reg. No. 106-99-0)

INTERIM ACUTE EXPOSURE GUIDELINE LEVELS
(AEGLs)

For
NAS/COT Subcommittee for AEGLS

2009

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 toxicological and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

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

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m;) 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;) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

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

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EXECUTIVE SUMMARY

1,3-Butadiene (butadiene) is a highly volatile, colorless gas with a mildly aromatic odor. A detection and recognition threshold of 0.45 and 1.1 ppm have been reported, respectively. The odor threshold is reported to be 0.16 ppm (0.3520 mg/m^3). It is soluble in ethanol, diethyl ether, and organic solvents, and very slightly soluble in water. Butadiene is released from biomass combustion with annual total global emissions of about 770,000 tonnes. Production of 1,3-butadiene nowadays is predominantly by recovery from the C_4 coproduct stream from the steam-cracking process used to manufacture ethylene. The worldwide production of butadiene has increased from 1983 (3570 kilo tonnes) through 1989 (6620 kilo tonnes). In 1996, the largest producing country was the USA, followed by Japan, Germany, the Republic of Korea, and France. Butadiene is primarily used in the production of synthetic rubbers.

No human case reports are available. Adequate human data is limited to one study in which two human volunteers were exposed to 1,3-butadiene concentrations of 2000 ppm for 7 hours, 4000 ppm for 6 hours, or to 8000 ppm for 8 hours (Carpenter *et al*. 1944).

Several large epidemiological studies are available. Predominantly on the basis of one large cohort study, several international organizations have concluded that 1,3-butadiene should be regarded as carcinogenic to humans. As to genotoxicity, data were considered to be less conclusive for humans, but the evidence for mutagenic effects *in vitro* and *in vivo* was concluded to be sufficient. Animal carcinogenicity studies show that 1,3-butadiene is clearly carcinogenic in animals.

Lethality data are available for several animal species although most data are very limitedly reported. An unknown number of guinea pigs survived a 2-hour exposure to 89,000 ppm 1,3-butadiene but 100% mortality occurred at a 10-hour exposure to the same concentration. No mortality was reported for rabbits and guinea pigs exposed to 200,000 ppm for 25 and 30 min, respectively, but 2/5 rats died at 30 min exposure to 200,000 ppm. A 4-hour LC_{50} of 128,000 ppm was reported for rats and a 2-h LC_{50} of 122,000 ppm was found for mice.

Very limited data are available addressing nonlethal toxicity following a single exposure. Studies on dogs and rabbits are too poorly reported and no clear conclusions can be drawn. The acute toxicity of 1,3-butadiene is rather low. In a study focused on carcinogenicity Bucher *et al.* (1993) exposed groups of 60 male and 60 female B6C3F$_1$ mice, 8-10-weeks old, for a single 2-hour period to target 1,3-butadiene concentrations of 0, 1000, 5000, or 10,000 ppm. The animals were held for two years. Survival, body weight gain, and the incidence of neoplastic and nonneoplastic lesions were not affected by 1,3-butadiene exposure. No compound-related histopathological effects were observed in male and female mice exposed to 1,3-butadiene concentrations of up to 8000 ppm for 14 weeks. In the latter study an increased mortality and growth retardation were observed at the higher concentrations, but these effects are due to repeated exposure (NTP 1984). No signs of toxicity were observed in rats exposed to 1,3-butadiene concentrations up to 8000 ppm for 6 h/d, 5 d/w for up to 3 months (Crouch *et al*. 1979). Detailed histopathological and hematological examinations were performed.

Two teratogenicity studies with rats and one with mice reported some fetal effects in the presence of maternal toxicity. The rat studies were not consistent regarding the 1,3-butadiene concentrations at which adverse effects might occur. The effects were attributed to be due to maternal toxicity and/or probably caused by repeated exposure and are unlikely to occur from a single exposure at the same dose.

Clear species differences exist in susceptibility in 1,3-butadiene toxicity. The differences between mice and rats are mainly attributed to a higher formation rate of the epoxides and a higher uptake due to a higher ventilation rate in mice. Blood levels of the epoxides are much higher in mice than in rats. Humans have approximately a four times lower ventilation rate than rats and the limited *in vitro* data obtained with human tissue samples show that overall the bioformation rate in human liver will be lower than in mice

and more comparable to that in rats. It is, therefore, concluded that humans will be more comparable to rats with respect to 1,3-butadiene toxicity than to mice.

The derivation of AEGL-1 values is based on the study by Carpenter *et al*. (1944). Two human subjects were exposed to 1,3-butadiene concentrations of 2000 ppm for 7 hours, 4000 ppm for 6 hours, or to 8000 ppm for 8 hours; all exposures were interrupted for a one-hour lunch break in the middle of the exposure period (Carpenter *et al*. 1944). Subjective symptoms reported at 2000 and 4000 ppm included slight smarting of the eyes and difficulty in focusing. No subjective complaints were reported at 8000 ppm. Results of a tapping test and a steadiness test revealed no differences in performance between the exposures. Point of departure is the 7-hour exposure to 2000 ppm. An intraspecies uncertainty factor of 3 is considered sufficient. Because the type of effect (local eye effects) is considered to be concentration-related the AEGL-1 values are set equal for all exposure periods from 10-min to 8-hours.

No studies adequately addressing the level of effect defined by AEGL-2 were available. Two studies were considered relevant for AEGL-2, the study with human volunteers by Carpenter *et al*. (1944) and a 3-month exposure study in rats by Crouch *et al*. (1979). No effects defined by AEGL-2 were observed in either study. The highest exposure for humans was 8000 ppm for 8-hours, the exposure regimen in rats was 6 h/d, 5 d/w for 3 months to 1,3-butadiene concentrations of up to 8000 ppm. Although both studies lead to approximately similar AEGL-2 values the use of human data is preferable to the rat data. Because the point of departure is conservative an intraspecies uncertainty factor of 3 is considered sufficient. Time-scaling to shorter time periods is performed with the default value of n=1; the 10-min AEGL-2 value is set equal to the 30-min value because the point of departure is an 8-hour exposure concentration.

AEGL-3 is based on the acute lethality study by Shugaev (1969). Rats were exposed for 4 hours and a 4-hour LC_{50} of 128,000 ppm was reported. This study allowed the calculation of a 4-hour LC_{01} of 41,000 ppm for rats. An overall uncertainty factor of 3 was considered sufficient for the inter- and intraspecies extrapolation. Using a higher factor would result in AEGL-3 values that would conflict with the human data reported by Carpenter *et al*. (1944). Further, the *in vitro* data obtained with human tissue samples show that overall the bioformation rate in human liver is rather comparable to that in rats. Because of this and since humans have an approximately four times lower ventilation rate than rats, a higher factor is not warranted. Default values of n=1 and n=3 were used for time-scaling to longer and shorter exposure periods, respectively, with the 10-min AEGL-3 values set equal to the 30-min AEGL-3 value.

Summary of AEGL Values for 1,3-butadiene[§]

Classification	10-minute	30-minute	1-hour	4-hour	8-hour	Endpoint (Reference)
AEGLB1 (Nondisabling)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	Difficulty in focusing in humans (Carpenter *et al*. 1944)
AEGLB2 (Disabling)	6700 ppm¶ (15,000 mg/m3)	6700 ppm¶ (15,000 mg/m3)	5300 ppm¶ (12,000 mg/m3)	3400 ppm¶ (7500 mg/m3)	2700 ppm¶ (6000 mg/m3)	No effects in humans (Carpenter *et al*. 1944)
AEGLB3 (Lethal)	See below*	See below*	See below*	See below*	6800 ppm¶ (15,000 mg/m3)	Lethality in rats (Shugaev 1969)

§ It is noted that the derivation of the respective AEGL-values excludes potential mutagenic or carcinogenic effects after single exposure, which may occur at lower concentrations (see Appendix C).

* The calculated AEGL-3 values for 10-min, 30-min, and 1-hour are higher than the lower explosive limit of butadiene in air (LEL = 2 % (20,000 ppm)). The calculated AEGL-3 value for 4-hours is higher than 50% of the lower explosive limit of butadiene in air. Therefore, extreme safety considerations against hazard of explosion must be taken into account.

The respective calculated AEGL-3 values for 10-min, 30-min, 1-hour, and 4-hours are: 27,000 ppm (60,000 mg/m^3), 27,000 ppm (60,000 mg/m^3), 22,000 ppm (49,000 mg/m^3), and 14,000 ppm (31,000 mg/m^3).

¶ The proposed value is higher than 10% of the lower explosive limit of butadiene in air (LEL = 2 % (20,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

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1. INTRODUCTION

This chapter is based on IARC 1999, WHO 2001, EC 2002, and EPA 2002a, unless otherwise stated.

1,3-Butadiene (butadiene) is a highly volatile, colorless gas with a mildly aromatic odor. ERPG (1997) reports detection and recognition thresholds of 0.45 and 1.1 ppm, respectively. Ruth (1986) reports an odor threshold of 0.16 ppm (0.3520 mg/m^3). Nagata (2002) reports a threshold for odor perception at 0.23 ppm (0.51 mg/m^3), a value obtained using the Japanese triangle method (a method which is known to produce results that agree well with the standard method CEN13725). The main chemical and physical properties of butadiene are summarized in Table 1. It is soluble in ethanol, diethyl ether, and organic solvents, and very slightly soluble in water.

Butadiene is released from biomass combustion, especially forest fires. Annual total global emissions of butadiene from biomass combustion were estimated to be 770,000 tonnes. Releases from forest fires in Canada were estimated to constitute about 50% of the total annual releases of butadiene. Butadiene concentrations in ambient air in Canada were up to about 1 μg/m^3 with incidental maximal values of up to 28 μg/m^3 in industrial areas.

Butadiene is available commercially as a liquefied gas under pressure. Butadiene is generally more than 99.5% pure; hydroquinone, catechol, and aliphatic mercaptans can be added as stabilizer. Besides the stabilizer, main impurities are dimers and water.

Butadiene was first produced in the late nineteenth century by pyrolysis of peroleum hydrocarbons; commercial production started in the 1930s and has involved three processes: catalytic dehydrogenation of n-butane and n-butene, oxidative dehydrogenation of n-butene, and recovery from the C_4 coproduct stream from the steam-cracking process used to manufacture ethylene. The latter process is nowadays the most predominant (85% worldwide) in the USA, Western Europe, and Japan, although in other parts of the world it is still produced from ethanol. Steam cracking is a complex, highly endothermic pyrolysis reaction. A hydrocarbon feedstock is heated to approximately 800 °C and 34 kPa for less than one second through which a mixture of olefins, aromatics, tar, and gases is formed. Subsequent cooling and separation reveals specific boiling-range cuts of C_1, C_2, C_3, and C_4 compounds, the latter containing, among others, butadiene. Butadiene is separated and purified by an extractive distillation process.

The worldwide production of butadiene has increased from 1983 (3570 kilo tonnes) through 1989 (6620 kilo tonnes). In 1996, the largest producing country was the USA (1744 kilo tonnes in 1996), followed by Japan (1025 kilo tonnes), Germany (673 kilo tonnes), the Republic of Korea (601 kilo tonnes), and France (344 kilo tonnes). A total production capacity of between 1.2 and 5 million tonnes/year in the EC is reported in the IUCLID database.

Butadiene is primarily used in the production of synthetic rubbers, including styrene-butadiene rubber (SBR), polybutadiene rubber (BR), styrene-butadiene latex (SBL), chloroprene rubber (CR), and nitrile rubber (NR). Important plastics containing butadiene as a monomeric component are shock-resistant polystyrene, ABS polymers consisting of acrylonitrile, butadiene, and styrene, and a copolymer of methyl methacrylate, butadiene and styrene (MBS). In 1981 the worldwide use pattern (%) was SBR + SBL (56), BR (22), CR (6), NR (4), and ABS (4). The use pattern (main uses) for the USA in 1995 was SBR (31), BR (24), SBL (13), production of adiponitrile (12), ABS (5), and CR (4).

SBR and SBL are used for a variety of products, including automobile tires, textiles, paper, and adhesives. Polybutadiene is used in tire manufacturing and in the high-impact resin industry. Neoprene rubber is primarily used in the automotive industry for belts, cables, hoses, and wires. ABS-resins are used to make plastic components such as automotive parts, pipes and fittings, appliances, telephones, and

business machines, among many other uses. Nitrile elastomer or nitrile-butyl rubber is a specialty elastomer known for its resistance to oil solvents and chemicals. Some uses include the manufacture of hoses, belts, cables, seals, and gaskets. Adiponitrile (hexanedinitrile) is primarily an intermediate used in the production of nylon 6,6.

Table 1. Chemical and Physical Properties

Parameter	Value	Reference
Synonyms	buta-1,3-diene, α,β-butadiene, bivinyl, divinyl, biethylene, vinylethylene, erythrene, pyrrolylene	WHO 2001
Chemical formula	$CH_2CHCHCH_2$	
Molecular weight	54.09	WHO 2001
CAS Reg. No.	106-99-0	
Physical state	gas	WHO 2001
Color	colorless	WHO 2001
Solubility in water	0.735 g/L at 25˚C	WHO 2001
Vapor pressure	240.0 kPa at 20˚C 281 kPa at 25˚C	EC 2002 WHO 2001
Vapor density (air = 1)	1.9	WHO 2001
Liquid density (water = 1)	0.62	EC 2002
Melting point	-108.9˚C	EC 2002
Boiling point	-4.4 ˚C	WHO 2001
Odor	Mild aromatic	WHO 2001
Flammability	Flash point: -85˚C	EC 2002
Explosive*	LEL: 1.4% v/v 2.0% v/v UEL: 16.3% v/v	EC 2002
Conversion factors	1 ppm = 2.21 mg/m^3 at 25˚C 1 mg/m^3 = 0.45 ppm	EC 2002

* Two values for the Lower Explosive Limit are reported by EC (2002). The most frequently reported and used by other organizations is the 2% value. This value is also used in the present document.

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

2.1.1. Case Reports

No case reports were located regarding acute lethality of 1,3-butadiene in humans.

2.2. Nonlethal Toxicity

2.2.1. Case Reports

No case reports were located regarding acute toxicity of 1,3-butadiene in humans.

2.2.2. Experimental Studies

Ripp (1967) reported a threshold of 4.0 mg/m^3 (1.8 ppm) for olfactory perception in 16 persons (age: 18-36 y; unspecified sex). In separate studies with 4 volunteers per test (age: 20-35 y; unspecified sex) sensitivity of the eye to light was reported to be altered at 3.8 mg/m^3 (1.7 ppm). A NOAEL of 3 mg/m^3 (1.4 ppm) was reported for the occurrence of an electrocortical conditioned reflex (light-stimulated

desynchronization of α-rhythm of the brain) in a group of 4 volunteers (age: 18-30 y; 2 males, 2 females). All concentrations were determined with a spectrophotometer. This study is poorly reported and is considered of doubtful significance (also considering the observations by Carpenter *et al*. (1944) described below).

Larionov *et al*. (1934) reported a slight increase in pulse rate in human beings (no details on number and sex) exposed to a 1,3-butadiene concentration of 1% (10,000 ppm) for 5 min. Concentrations were regularly monitored. No effects were observed on blood pressure or respiration. Subjective complaints consisted of a tingling sensation and dryness of the nose and throat.

Two males were exposed to 2000 ppm 1,3-butadiene for 7 hours, 4000 ppm for 6 hours, and 8000 ppm for 8 hours (nominal concentrations, regularly monitored); all exposures were interrupted for a one-hour lunch break in the middle of the exposure period (Carpenter *et al*. 1944). Vapor concentrations were regularly monitored and controlled. Subjective symptoms reported included slight smarting of the eyes and difficulty in focusing. No subjective complaints were reported at 8000 ppm, according to the authors probably because of slight anxiety concerning the possibility of an explosion. Both subjects felt particularly alert. Results of a tapping test and a steadiness test revealed no differences in performance between the exposures.

2.2.3. Occupational / Epidemiological Studies

Several epidemiological studies with occupational cohorts are available, conducted both in 1,3-butadiene producing plants (monomer production) and in 1,3-butadiene using plants (polymer production). Since several publications are updates of the same cohort the most recent update has been focused on. This section is based on the recent evaluations by US EPA (2002a) and EC (2002) who have extensively evaluated these studies.

EPA (2002a) evaluated three cohorts in monomer production plants, the Texaco cohort (2795 workers with a total of 85,581 person-years), the Shell Oil Refinery cohort (614 subjects, 7232 person-years), and the Union Carbide cohort (364 workers). The second study failed to show any excess mortality or morbidity but statistically significant excess for lymphosarcoma was observed in the other two studies. Precise exposure estimates were not available but nearly all cases had started employment before 1950. In the last follow-up of the Texaco cohort lymphosarcomas were classified as lymphomas and included in Non Hodgkin Lymphoma (NHL). It was considered that peak exposures rather than cumulative exposures may be associated with the observed increase in NHL, although no real data on the occurrence of peak exposures were available.

EPA (2002a) discussed two cohort studies in styrene-butadiene rubber (SBR) polymer production plants. The studied plants in the original cohorts nearly fully overlap. In addition, several nested case-control studies were performed in this population. Although exposure estimations were very crude in the earlier studies and not substantiated by monitoring data quantitative exposure estimates based on process analysis, job analysis, and exposure estimations for specific tasks based e.g. on monitoring data were used in later updates or reanalysis. Statistically significant excesses of lymphohematopoietic cancers (leukemias) were reported for polymer production workers. Although co-exposure to styrene and benzene was also present analyses showed that the occurrence of leukemia was predominantly associated with 1,3-butadiene exposure.

Applying a set of criteria that define a causal relationship between exposure and health outcome EPA (2002a) concluded that the criteria (of temporality, strength of association, consistency, specificity, and biological gradient) were satisfactorily met. The criterion of biological plausibility was considered to be also fulfilled because 1,3-butadiene is metabolized by humans and other species to genotoxic metabolites and is carcinogenic in rats and mice. EPA therefore concluded that human evidence for carcinogenicity of 1,3-butadiene is sufficient. To estimate the cancer incidence a linear rate model, as

developed by Health Canada (RR = 1+ 0.0099*X*, where *X* represents cumulative 1,3-butadiene exposure in ppm-years), and age-specific leukemia incidence rates for 1994-1998 from SEER (Surveillance, Epidemiology and End Results program of the National Cancer Institute) were used (EPA 2002b). An LEC_{01} (i.e., the 95% lower confidence limit of the exposure concentration associated with a 1% increased risk) of 0.254 ppm was calculated. Using this LEC_{01} as point of departure and extrapolating linearly to 0 increased risk at 0 exposure, a unit risk estimate of 0.04/ppm was obtained for leukemia incidence. However, rat and mouse experiments showed that females are more sensitive to 1,3-butadiene-induced carcinogenicity than males, with mammary gland tumors as the only tumor site common to both species. Therefore, an adjustment factor of 2 was applied to cover the combined risks for leukemia and mammary cancer and also to provide additional protection to account for the fact that small increases in risk at other sites, particularly the lung, cannot be ruled out. This resulted in a risk estimate of 0.08/ppm. Using this cancer potency estimate, the chronic exposure level resulting in an increased cancer risk of 10^{-6} was estimated as follows (EPA 2002a; see Appendix C for more details):

$$(10^{-6})/0.08/\text{ppm}) = 1 * 10^{-5} \text{ ppm} = 0.01 \text{ ppb}.$$

The EC (2002) evaluated nearly the same studies as discussed by EPA. It was concluded that the largest epidemiological study in SBR workers demonstrated a clear excess of mortality from leukemia, which was associated with exposure to the 1,3-butadiene monomer. It was further concluded that in the 1,3-butadiene production industry the pattern of results does not clearly indicate an association between 1,3-butadiene exposure and excess mortality. It was concluded that 1,3-butadiene should be regarded as carcinogenic to humans.

IARC (1999) concluded that there is limited evidence for the carcinogenicity of 1,3-butadiene. IARC stated that the evaluation of the carcinogenicity of 1,3-butadiene in humans hinges on an increased risk of leukemia found in one large and well conducted study in SBR-producing plants. The smaller studies were concluded to neither support nor contradict this evidence. A role of occupational exposure to other chemicals than 1,3-butadiene in the SBR-producing industry could not be ruled out.

2.3. Neurotoxicity

No relevant data are available (EPA 2002a; EC 2002).

2.4. Developmental / Reproductive toxicity

No data are available (EPA 2002a; EC 2002).

2.5. Genotoxicity

The genotoxicity of inhaled 1,3-butadiene in humans has been evaluated by several organizations (IARC 1999; WHO 2001; EC 2002; EPA 2002a). Human data are mainly obtained from occupational exposure in which there is repeated exposure and often also co-exposure to other chemicals.

The results of some studies suggest that an average 8-hour TWA concentration of about 0.3-3.5 ppm may lead to an increase in the mutation frequency at the *hprt* locus but this has not been confirmed by other studies. The influence of styrene and smoking on this butadiene effect was not clear in the positive studies. Cytogenetic analysis of peripheral blood lymphocytes of workers in three butadiene production facilities in the United States, one in Portugal, and one in the Czech Republic did not show chromosomal aberrations, micronuclei, sister chromatid exchanges, DNA strand breaks or alkali-labile sites (Comet assay). Irradiation with γ-rays of lymphocytes from two of these study groups showed that butadiene exposure reduced DNA repair competence of the cells. Analyses of lymphocytes from 1994 subjects of the butadiene production plant in the Czech Republic indicated that the percentage of aberrant cells was slightly, but significantly, enhanced in exposed subjects compared with the controls (3.11 ± 1.33 and 2.03 ± 1.53, respectively, $p<0.01$). These results were very similar to those from an earlier study

conducted in the same factory, which did not provide evidence for a clastogenic effect (2.9 ± 1.5 and 2.1 ± 1.4, respectively).

Human data regarding mutagenicity of the main metabolites were not available.

The EC (2002) states that the data are inconsistent and not reproducible but that given the clear evidence for mutagenicity in mice the positive findings in humans cannot be dismissed. The WHO (2001) concluded that there is limited evidence from occupationally exposed populations that 1,3-butadiene is genotoxic in humans, inducing mutagenic and clastogenic damage in somatic cells. IARC (1999) considered the increase in *hprt* mutations in lymphocytes conflicting.

2.6. Carcinogenicity

See section 2.2.3, "Occupational / Epidemiological Studies".

2.7. Summary of human data

In a very old and limited reported study a slight increase in pulse rate and some slight irritation of nose and throat was found in humans exposed to 1% (10,000 ppm) 1,3-butadiene for 5 min (Larionov *et al*. 1934). No subjective complaints were reported by two human subjects exposed to 1,3-butadiene concentrations of up to at 8000 ppm for 8 hours (with a 1-hour break in the middle), both subjects felt particularly alert at exposure to 8000 ppm. Results of a tapping test and a steadiness test revealed no differences in performance between the exposures (Carpenter *et al*. 1944).

No relevant human data were available on neurotoxicity and developmental/reproductive toxicity.

Several large epidemiological studies are available. Predominantly on the basis of one large cohort study, EC (2002) and US EPA (2002a) concluded that 1,3-butadiene should be regarded as carcinogenic to humans. EPA (2002a; 2002b) calculated a unit risk of 0.08/ppm for leukemia incidence. IARC (1999) concluded that there is limited evidence for the carcinogenicity of 1,3-butadiene.

As to genotoxicity, the EC (2002) states that the data are inconsistent and not reproducible but that given the clear evidence for mutagenicity in mice the positive findings in humans cannot be dismissed. The WHO (2001) concluded that there is limited evidence from occupationally exposed populations that 1,3-butadiene is genotoxic in humans, inducing mutagenic and clastogenic damage in somatic cells. IARC (1999) considered the increase in *hprt* mutations in lymphocytes conflicting.

3. ANIMAL TOXICITY DATA

3.1. Acute lethality

3.1.1. Rabbits / Guinea Pigs

A target 1,3-butadiene concentration of 25% (250,000 ppm) for an unknown exposure period caused deaths in rabbits (unspecified strain, number, and sex) but no deaths occurred at exposure to 15% (150,000 ppm) for 25 min. The concentrations were monitored (Larionov *et al*. 1934).

It was reported that unpublished Dow data dated from 1941 had shown that 3/5 guinea pigs died when exposed to 50,000 ppm for 12 hours (ERPG 1997). No deaths were observed at exposure to 89,000 ppm for 2 hours but all animals died after 10 hours of exposure to this concentration. No mortality was observed in guinea pigs exposed to 200,000 ppm for 30 min whereas 1/5 guinea pigs died in a one-hour exposure. It was remarked that marked irritation of the respiratory tract and lung edema was noticed, however it was not clear whether this applied to the guinea pig that died during the 1-h exposure to

200,000 ppm or that these effects were general findings in most or all animals that died in these experiments.

3.1.2. Rats

It was reported that unpublished Dow data dated from 1941 had shown that no deaths occurred in rats exposed to 50,000 ppm for 24 hours or to 89,000 ppm for 6 hours. Exposure to 89,000 ppm resulted in 5/7 deaths after 18 hours of exposure. Further, 2/5 rats died when exposed to 200,000 ppm for 0.5 hour (ERPG 1997). No further details were given.

Shugaev (1969) exposed rats (unspecified sex and strain) to varying 1,3-butadiene concentrations for 4 hours. The number of animals was not given but the description of the results suggests that exposure groups may have consisted of 6 animals. No information about the concentrations used was given; exposure concentrations were controlled by gas chromatography, the postexposure observation period is unknown. Deaths appeared to be preceded by deep narcosis. The experimental data were analyzed by probit-analysis. A 4-hour LC_{50} of 128,000 ppm (285 g/m^3) was reported, with 95% confidence limits of 99,000 – 167,000 ppm. The calculated LC_{16} was 79,000 ppm (175 g/m^3) and the calculated LC_{84} was 207,000 ppm (460 g/m^3). Mean 1,3-butadiene concentrations in organs at the LC_{50} were 5.1 µg/g in brain, 5.1 µg/g in liver, 3.6 µg/g in kidney, 4.5 µg/g in spleen, and 15.2 µg/g in perinephric fat.

In an experiment on the kinetics of 1,3-butadiene 2 rats were exposed in a closed chamber (6.4 L) for up to 15 hours. 1,3-Butadiene was added every 2-3 hours in order to maintain the concentration between 2000 and 4000 ppm (Kreiling *et al*. 1987). It was stated that no toxicity was observed, in contrast to mice exposed under similar conditions.

3.1.3. Mice

Larionov *et al*. (1934) exposed white mice (unspecified strain, number, and sex) to 1,3-butadiene. The results were poorly reported. The minimum lethal concentration was reported to be 9 and 14% (90,000 to 140,000 ppm) (exposure duration not reported). Concentrations were monitored.

Killian (1930) exposed groups of 3 white mice (sex unknown) to different mixtures of 1,3-butadiene/oxygen for 20 to 30 min. The results are summarized in Table 2. The study description suggests that the concentrations are initial concentrations in a closed system with a volume of 3 to 5 L. It is noted by Killian that fresh mixtures were frequently added but not in which situations. Significant changes in the mixture composition will have been minimal at relatively short exposure times.

Shugaev (1969) exposed mice (unspecified sex and strain) to varying 1,3-butadiene concentrations for 2 hours. The number of animals was not given but the description of the results suggests that exposure groups may have consisted of 6 animals. No information about the concentrations used was given; exposure concentrations were controlled by gas chromatography, the postexposure observation period is unknown. Deaths appeared to be preceded by deep narcosis. The experimental data were analyzed by probit-analysis. A 2-hour LC_{50} of 122,000 ppm (270 g/m^3) was reported, with 95% confidence limits of 113,000 – 131,000 ppm. The calculated LC_{16} was 91,000 ppm (203 g/m^3) and the calculated LC_{84} was 169,000 ppm (375 g/m^3). The mean 1,3-butadiene concentration in the brain of dead mice exposed at the LC_{50} was 5.4 µg/g.

In a study focused on carcinogenicity Bucher *et al.* (1993) exposed groups of 60 male and 60 female B6C3F$_1$ mice, 8-10-weeks old, for a single 2-hour period to target 1,3-butadiene concentrations of 0, 1000, 5000, or 10,000 ppm (0, 2200, 11,000, or 22,000 mg/m^3, respectively). The animals were held for two years, at which time all survivors were killed and tissues and organs were examined microscopically. Survival, body weight gain, and the incidence of neoplastic and nonneoplastic lesions were not affected by 1,3-butadiene exposure.

TABLE 2. Narcosis and mortality in mice exposed to different 1,3-butadiene/O_2 mixtures (Killian 1930)

Butadiene/O_2 (%)	Excitation	Spontaneous lateral position	Narcosis	Remarks
10/90	Imbalance after 5 min	Drowsy after 21 min	--	--
15/85	60 s	7 min	No true narcosis	Hyperventilation; marked spontaneous spasms
20/80	30-40 s	50-60 s	6-10 min	Extraordinary marked hyperventilation, labored respiration
25/75	Not marked	50-60 s	2-3 min	Hyperventilation; spontaneous spasms; deep sleep
30/70	20-30 s	40-50 s	1-1.2 min	Similar but stronger effects
40/60	Not marked	20-30 s	40-60 s	All dead in 11-14 min; respiratory paralysis

In an experiment on the kinetics of 1,3-butadiene 6 mice were exposed in a closed chamber (6.4 L) for up to 15 hours. 1,3-Butadiene was added every 2-3 hours in order to maintain the concentration between 2000 and 4000 ppm (Kreiling *et al*. 1987). It was reported that mice showed signs of acute toxicity after about 12 hours and lethality occurred when the exposure was prolonged over 15 hours.

TABLE 3. Summary of Acute Lethal Inhalation Data in Laboratory Animals

Species	Concentration (ppm)	Exposure Time	Effect[a]	Reference
Rabbit	150,000 250,000	25 min unknown	No mortality Mortality	Larionov *et al*. (1934)
Guinea pig	50,000 89,000 89,000 200,000 200,000	12 h 2 h 10 h 30 min 1 h	3/5 deaths 100% survival 100% mortality 100% survival 1/5 deaths	ERPG (1997)
Rat	50,000 89,000 89,000 200,000	24 h 6 h 18 h 30 min	100% survival 100% survival 5/7 deaths 2/5 deaths	ERPG (1997)
Rat	79,000 128,000 207,000	4 h	LC_{16} LC_{50} LC_{84}	Shugaev (1969)
Rat	2000-4000	15 h	0/2 deaths	Kreiling *et al*. (1987)
Mouse	10,000	2 h	100% survival	Bucher *et al*. (1993)

TABLE 3. Summary of Acute Lethal Inhalation Data in Laboratory Animals				
Mouse	91,000 122,000 169,000	2 h	LC_{16} LC_{50} LC_{84}	Shugaev (1969)
Mouse	2000-4000	15 h	lethality	Kreiling *et al*. (1987)

3.2. Nonlethal toxicity

3.2.1. Dogs

Ophthalmoscopic examination of the eyes of female dogs (one per concentration) exposed to target concentrations of 0, 600, 2300 or 6700 ppm 1,3-butadiene for 7.5 h/d for up to 8 months revealed no signs of injury (Carpenter *et al.* 1944).

3.2.2. Rabbits / Guinea Pigs

Carpenter *et al.* (1944) exposed 29 rabbits to a nominal concentration of 25% (250,000 ppm) 1,3-butadiene. Concentrations were regularly monitored. Light anesthesia was seen after 1.6 min on average, excitation and tremors after 4.6 min, involuntarily blinking of the pupil after 7.4 min, and death after 23 min of exposure. Six further rabbits received exposure to 20 to 25% (200,000 to 250,000 ppm) 1,3-butadiene daily for several days, long enough to induce deep anesthesia (achieved after approximately 10 min). Recovery was rapid, within two minutes; no pathological effects were noted in these animals.

Ophthalmoscopic examination of the eyes of rabbits exposed to nominal 1,3-butadiene concentrations up to 6700 ppm for 7.5 h/d for up to 8 months revealed no signs of injury (Carpenter *et al.* 1944).

Pokrovski and Volchovka (1968) studied the effects of 1,3-butadiene on hematopoiesis. The study is poorly reported. Rabbits (unspecified strain, number, and sex) exposed to 200 mg/L (90,000 ppm) for 2 hours showed mild leucocytosis on the 3rd to 10th day after exposure. Further, neutrophilia, lymphopenia, and monocytosis were reported to occur. Bone marrow cell proliferation was observed after 10 to 20 days postexposure.

A nominal 1,3-butadiene concentration of 25% (250,000 ppm) for an unknown exposure period caused narcosis followed by death in rabbits (unspecified strain, number, and sex) but no narcosis occurred at exposure to 15% (150,000 ppm) for 25 min. The concentrations were monitored (Larionov *et al*. (1934). Irritation of conjunctiva and the nose and lachrymation were the first signs of toxicity at these concentrations.

3.2.3. Rats

Female rats exposed to an actual 1,3-butadiene concentration up to 7647 ppm for 6 h/d, from day 6-15 of gestation did not show any respiratory distress (Irvine 1981) (see section 3.4 for further details).

No signs of toxicity were reported to occur in a study on DNA-adduct formation in male Sprague-Dawley rats nose-only exposed to an analytical 1,3-butadiene concentration of 201 ppm for 6 hours followed by a 42-hour observation period (Boogaard *et al*. 2004).

Repeated exposure

Groups of 20 male and 20 female Sprague-Dawley rats were exposed to target 1,3-butadiene concentrations of 0, 1000, 2000, 4000, or 8000 ppm for 6 h/d, 5 d/w for 3 months. Additionally groups of 10 rats per sex were included for interim kills at 2 and 6 weeks of exposure (Crouch *et al*. 1979). Animals

were observed daily and detailed histopathological, hematological, and blood biochemical studies were performed. No effects attributed to exposure were found.

3.2.4. Mice

In a study focused on carcinogenicity Bucher *et al.* (1993) exposed groups of 60 male and 60 female $B6C3F_1$ mice, 8-10-weeks old, for a single 2-hour period to target 1,3-butadiene concentrations of 0, 1000, 5000, or 10,000 ppm (0, 2200, 11,000, or 22,000 mg/m^3, respectively). The animals were held for two years, at which time all survivors were killed and tissues and organs were examined microscopically. Survival, body weight gain, and the incidence of neoplastic and nonneoplastic lesions were not affected by 1,3-butadiene exposure.

Larionov *et al*. (1934) exposed white mice (unspecified number and sex) 1,3-butadiene. The results were poorly reported. The minimum concentration for narcosis was reported to be 9 to 14% (90,000 to 140,000 ppm), but death was also reported at these concentrations (exposure duration not reported). Concentrations were continuously monitored. The first signs of toxicity included irritation of the conjunctiva and nose.

Pacchierotti *et al* (1998) exposed male mice to 0 (n=36), 130 (n=28), 500 (n=24), or 1300 (n=28) ppm 1,3-butadiene for 6 h/d for 5 successive days. Male mice were killed immediately, or one or two weeks after the mating period. Testis weight was statistically significantly decreased in mice immediately killed after mating; the decrease was concentration-related. A progressive response was seen at the other two time points with a smaller but still statistically significantly decreased testis weight in the 1300 ppm exposure group at two weeks after the mating period (5 weeks postexposure).

No signs of toxicity were reported to occur in a study on DNA-adduct formation in male $B6C3F_1$ mice nose-only exposed to an analytical 1,3-butadiene concentration of 201 ppm for 6 hours followed by a 42-hour observation period (Boogaard *et al*. 2004).

Groups of 20 adult male $B6C3F_1$ mice were exposed to actual 1,3-butadiene concentrations (SD) of 0, 199 (6.1), 999 (22.6), or 4980 (130) ppm 1,3-butadiene for 6 h/d for 5 successive days (Hackett *et al.* in 1988 as summarized by Morrisey *et al*. (1990), EPA (2002a), and EC (2002). The mice were killed 5 weeks after exposure. No clinical signs of toxicity were observed apart from piloerection and dyspnea during the first 20-30 min of the exposure in the 4980 ppm exposure group.

Repeated exposure

Male and female $B6C3F_1$ mice were exposed to target 1,3-butadiene concentrations of 625, 1250, 2500, 5000, or 8000 ppm for 6 h/d, for 5 d/w for 2 weeks (groups of 5 animals per sex) or 14 weeks (groups of 10 animals per sex) (NTP 1984). In the 2-week study, growth retardation was observed in males at concentrations of 1250 ppm and higher and in females at 5000 ppm and above. Exposure for up to 14 weeks induced increased mortality in the 5000 and 8000 ppm exposure groups, one death occurred in males exposed to 1250 ppm as well as in males exposed to 2500 ppm. Body weight gain was decreased in male mice at 2500 ppm and above and in female mice at 5000 ppm and above. No compound-related histopathological effects were observed.

3.3. Neurotoxicity

No relevant data are available. It has been described by some authors that narcosis precedes death in rats, mice and rabbits (Shugaev 1969; Larionov *et al*. 1934).

Repeated exposure

Groups of 20 male and 20 female Sprague-Dawley rats were exposed to target 1,3-butadiene concentrations of 0, 1000, 2000, 4000, or 8000 ppm for 6 h/d, 5 d/w for 3 months. Additionally groups of 10 rats per sex were included for interim kills at 2 and 6 weeks of exposure (Crouch *et al*. 1979). No

effects of exposure on cholinesterase activity in brain or erythrocytes were observed. Neuromuscular function tests using a modified rotating cone showed no consistent dose-related effect.

3.4. Developmental / Reproductive toxicity

Several studies have been published indicating that chronic exposure may cause testicular and ovarian atrophy in mice (EC 2002; EPA 2002a). However, despite the fact that the results were not always consistent and these studies mainly focused on the carcinogenic properties of 1,3-butadiene, the exposure regimens used are considered of no relevance for AEGL derivation and are therefore not evaluated.

Anderson *et al*. (1996) exposed male CD-1 mice to 0 (n=25) 1250 (n=25) or 6250 ppm (n=50) 1,3-butadiene for 6 hours. Males were mated after 5 days with two untreated females. One female of each pair was sacrificed at day 17 of gestation while the other female was allowed to deliver and rear her litter. No dominant lethal effects were found. The mean number of implantations was reduced in both exposure groups but was statistically significant in the low exposure group only (11.42 (SD: 2.59) in controls versus 9.67 (3.83) in exposed animals). Since there were no accompanying changes in the number of postimplantation deaths or fetal abnormalities this reduction was not considered to represent a genetic effect.

Irvine (1981) exposed groups of 24 mated female Sprague-Dawley rats to mean actual concentrations (SD) of 202 (14), 990 (24), or 7647 (375) ppm 1,3-butadiene for 6 hours/day on days 6-15 of gestation. Exposure concentrations were regularly monitored. A negative (40 animals) and a positive control group (26 animals; treated orally with 250 mg/kg/day acetyl salicylic acid) were included. No deaths occurred. Respiratory distress was observed in the positive control group, but not in the negative control group and the 1,3-butadiene exposure groups. A dose-related decreased body weight gain was observed predominantly during the first three exposure days, with an actual slight body weight loss in the 7647 ppm exposure group in this period. The overall body weight gain during the exposure period (days 6-15) was statistically significantly lower than controls in all 1,3-butadiene exposure groups. Due to a higher body weight gain than in controls during gestation days 15-20, the decrease in body weight gain over the entire gestation period was statistically significant in the highest exposure group only. No differences in gravid uterine weight were observed. No effects were found on pregnancy or implantation. Mean fetal body weight and CR-length were marginally lower in all 1,3-butadiene exposure groups but this difference was statistically significant only in the 7647 ppm exposure group. Mean CR-lengths were 37.8, 37.2, 37.2, and 35.9 mm for controls and the three respective exposure groups. This was attributed to the decreased body weight gain of the dams. There was a dose-related increase in the incidence of skeletal effects (mainly consisting of wavy ribs): 0.6, 2.2, 2.8, and 5.9% for the control and the three subsequent exposure groups, respectively. The incidences in the mid- and high-dose groups were statistically significantly increased over the control incidence. Additionally, there was an increased incidence of bipartite thoracic centra in all exposure groups, but this effect was not dose-related. Statistically significantly increased incidences of marked and severe forms of wavy ribs, irregular rib ossification, and incomplete ossification were noted at 7647 ppm. The effects observed at the low- and mid-dose groups are very minor. The effects noted are mainly the consequence of the maternal toxicity, *i.e.* the growth retardation during the first three days of exposure.

Hackett *et al*. (1987a, 1987b) exposed groups of 31-33 pregnant Swiss CD-1 mice and 30 pregnant Sprague-Dawley rats to target 1,3-butadiene concentrations of 0, 40, 200, or 1000 ppm for 6 hours/day on days 6-15 of gestation. Exposure concentrations were regularly monitored; the relative standard deviation was below 2%. Mice were sacrificed on day 18 of gestation and rats on day 20. Maternal toxicity (reduction in body weight gain only during gestation days 6 to 11) was elicited at the highest exposure level in rats. In contrast to the findings by Irvine *et al*. (1981) the body weight gain in the 200 ppm exposure group was approximately 6% higher than in controls during the exposure period (days 6-16). At gestation day 20, no differences in body weight between the study groups were present.

No differences in gravid uterine weight were observed. No effects on developmental parameters were found at any exposure concentration in rats; the major skeletal effects and the marginally decreased fetal length and weight observed by Irvine at exposure concentrations of 200 and 1000 ppm could not be confirmed by Hackett *et al*. In the mouse study, a statistically significant reduction in maternal body weight gain during gestation was seen at 200 ppm and 1000 ppm. Fetal weight was also statistically significantly lower at these concentrations (16% and 22% less than controls, respectively). There were no statistically significant increases in resorptions or malformations per litter although there was a slight, statistically significant increase in minor skeletal abnormalities at 200 and/or 1000 ppm, indicative of growth retardation.

A sperm-head morphology study as conducted by Hackett *et al.* in 1988 was summarized by Morrisey *et al*. (1990), EPA (2002a), and EC (2002). Groups of 20 adult male $B6C3F_1$ mice were exposed to actual 1,3-butadiene concentrations (SD) of 0, 199 (6.1), 999 (22.6), or 4980 (130) ppm 1,3-butadiene for 6 h/d for 5 successive days. The mice were killed 5 weeks after exposure. No clinical signs of toxicity were observed apart from piloerection and dyspnea during the first 20-30 min of the exposure in the 4980 ppm exposure group. A concentration-related increase in the percentage of abnormal sperm was observed. The percentages of abnormal sperm were 1.92% at 199 ppm, 2.77% at 999 ppm, and 3.66% at 4980 ppm compared with 1.60% in controls; the percentage at the mid- and high-exposure groups were statistically significantly different from controls.

Pacchierotti *et al* (1998) studied reproductive effects in male mice by flow cytometric analysis of spermatogonial cells. Male mice were exposed to 0 (n=36), 130 (n=28), 500 (n=24), or 1300 (n=28) ppm 1,3-butadiene for 6 h/d for 5 successive days. Air concentrations were regularly monitored. Immediately following exposure the mice were mated for three weeks with untreated $B6C3F_1$ female mice. Mating performance was analyzed per week. No effects of 1,3-butadiene exposure on mating performance or on the percentage unfertilized oocytes were observed. Cytogenetic analyses of first cleavage embryos revealed a dose-related statistically significant increase in the percentage zygotes with aberrations in the first week of mating in the 500 and 1300 ppm exposure groups. The effects were less pronounced in the following weeks. Male mice were killed immediately, or one or two weeks after the mating period. Testis weight was statistically significantly decreased in mice immediately exposed after mating, the decrease was concentration-related decrease. A progressive response was seen at the other two time points with a smaller but still statistically significantly decreased testis weight in the 1300 ppm exposure group at two weeks after the mating period (5 weeks postexposure). Flow cytometric analysis of spermatogonial cells revealed a statistically significant decrease depletion of the round and elongated spermatid compartments which paralleled the effects on testis weight.

Summary and conclusions on developmental/reproductive toxicity

Three teratogenicity studies with 1,3-butadiene are available, two performed with rats and one with mice. The two studies with rats are not consistent regarding the 1,3-butadiene concentrations at which adverse effects might occur. Hackett *et al*. (1987a) did not find any effect in pregnant rats exposed to target 1,3-butadiene concentrations up to 1000 ppm and no effects on their offspring were observed. Maternal growth retardation was observed in the second rat study (Irvine 1981) and in the mouse study (Hackett *et al*. 1987b). In both rats and mice the predominant effect on the fetuses consisted of a diminished fetal growth, accompanied by skeletal effects like wavy ribs and delayed ossification. These kind of fetal effects were only observed in the presence of maternal toxicity. This pattern of effects can be typically considered to be the consequence of non-specific growth retardation due to maternal toxicity. Recently the relevance of developmental toxicity endpoints for acute limit setting was evaluated (Van Raaij *et al*. 2003). In this study, the results of repeated exposure studies on these endpoints were compared with those from single exposure studies for a number of chemicals. It was concluded that effects like diminished fetal growth and skeletal effects (wavy ribs, delayed ossification) that can be attributed to maternal toxicity are probably caused by repeated exposure and are unlikely to occur from a

single exposure at the same dose. Further, the NOAEL for maternal toxicity after single exposure will generally be several-fold higher than the NOAEL after repeated exposure.

In two fertility studies in which male mice were exposed for 5 days effects were reported on sperm quality and on offspring (Hackett *et al*. 1988; Pacchierotti *et al*. 1998). However, the relevance of these effects after single exposures is uncertain. No fetal abnormalities were observed in a single exposure study by Anderson *et al*. (1986). Male mice were exposed to 1,3-butadiene up to a target concentration of 1250 or 6250 ppm. Although a reduced number of implantations was reported, this was statistically significant only at the lower exposure concentration while the statistical power at the higher concentration was higher (twice as much animals were exposed).

3.5. Genotoxicity

The genotoxicity of 1,3-butadiene has been evaluated by several organizations (IARC, 1999; WHO, 2001; EC 2002; EPA, 2002a). The EPA (2002a) reported that the genetic toxicology literature on 1,3-butadiene and its epoxymetabolites epoxybutene and diepoxybutane consists of more than 600 publications. A third genotoxic metabolite epoxybutanediol have been less intensively studied, but recent evidence suggests that most of the trihydroxybutyl guanine adducts in mice and rats exposed to 1,3-butadiene are derived from this metabolite. The metabolism of 1,3-butadiene is qualitatively similar among species, although quantitative differences in the metabolic rates for various pathways between different species exist. In addition, 1,3-butadiene is structurally related to other (rodent) carcinogens, such as isoprene and chloroprene.

Below the main results as presented in IARC, 1999 are summarized with special attention to *in vitro* results and *in vivo* effects following acute inhalation exposure to 1,3-butadiene. The HID (highest ineffective dose) and LED (lowest effective dose) for single exposures are given in Table 4 (see IARC (1999) for more details).

In vitro:

Gaseous 1,3-Butadiene was found to be mutagenic in *in vitro* gene mutation assays with *S. typhimurium* strains TA100, TA1530, and TA1535, only in the presence of induced and/or uninduced rat and mouse liver S9. In the presence of uninduced human S9 the assay was negative. Furthermore, a weak positive response was reported for induction of SCE in Chinese Hamster Ovary (CHO) cells (with S9 mix) and human whole blood lymphocytes (both with and without S9 mix).

In vivo:

The mutagenic potential of 1,3-butadiene *in vitro* was confirmed by *in vivo* inhalation studies in mice and rats. In general, genotoxic effects were more pronounced after repeated exposure than after single exposure. Furthermore, mice showed to be more sensitive for mutagenicity and other genetic effects than rats. Single *in vivo* inhalation experiments are given in Table 4 and will be briefly discussed here.

Single i*n vivo* exposure to 1,3-butadiene did induce SCEs, micronuclei, DNA-DNA cross-links, and binding to DNA at N7 of guanine in mice but not in rats (although in the Wistar rat binding to a not specified DNA binding site was reported). Dominant lethal mutations were especially found in mice although only after repeated exposure. In addition, in mice chromosomal aberrations, DNA single-strand breaks, and DNA damage were induced by single exposure. No aneuploidy was found. In both mouse and rat 1,3-butadiene binds to protein.

Table 4. Genetic effects of 1,3–butadiene after single *in vivo* respiratory exposure (summarized from IARC, 1999)

Endpoint[a]	Species	Tissue	Exposure (HID or LED)[b]	Results[c]
SCE	$B6C3F_1$ mouse	bone marrow	116 ppm, 6h	+
	Sprague-Dawley rat	bone marrow	4000 ppm, 6h	-
MN	$B6C3F_1$ mouse	bone marrow	116 ppm, 6h	+
CA	$B6C3F_1$ mouse	bone marrow	1500 ppm, 6h	+
	NIH mouse	bone marrow	1500 ppm, 6h	+
DLT	CD-1 mouse		6250 ppm, 6h	-
AP	$B6C3F_1$ mouse	bone marrow	1500 ppm, 6h	-
	NIH mouse	bone marrow	1500 ppm, 6h	-
DNA X	$B6C3F_1$ mouse	liver and lung	250 ppm, 7h	+
		liver	450 ppm, 7h	+
	Sprague-Dawley rat	liver and lung	2000 ppm, 7h	-
		liver	550 ppm, 7h	-
DNA ss	NMRI mouse	liver and lung	200 ppm, 16h	+
DNA damage	CD-1 mouse	testicular cells	125 ppm, 6h	+
BBD, BS not specified	$B6C3F_1$ mouse[d]	liver	13 ppm, 4-6.6h	+
	Wistar rat[d]	liver	13 ppm, 4-6.6h	+
BBD, BS at N7 of guanine	$B6C3F_1$ mouse[d]	liver	450 ppm, 7h	+
	Wistar rat[d]	liver	550 ppm, 7h	-
BBP	$B6C3F_1$ mouse[d]	liver	13 ppm, 4-6.6h	+
	Wistar rat[d]	liver	13 ppm, 4-6.6h	+

[a]SCE, sister chromatid exchanges; MN, micronucleus test; CA, chromosomal aberrations; DLT, dominant lethal test; AP, aneuploidy; DNA X, DNA ss: DNA single-strand breaks; BBD, binding of 1,3-butadiene to DNA; BS, binding site; BBP, binding of 1,3-butadiene to protein.

[b]HID, highest ineffective dose; LED, lowest effective dose.

[c]+, positive results; -, negative results

[d]Male

The genotoxic potential of the main metabolites, epoxybutene, epoxybutanediol, and diepoxybutane has been tested in several assays. Epoxybutene was mutagenic in the absence of S9 mix. It did not induce single strand breaks or unscheduled DNA synthesis and did not induce SCE or chromosomal aberrations in rat and mouse splenocytes. Gene mutations at the *tk* and *hprt* loci were observed in human TK6 cells and SCEs were induced in human lymphocyte cultures. Epoxybutene was positive in several *in vivo* micronucleus assays in mice and rats after intraperitoneal administration. Epoxybutanediol was positive in the Ames test. Variable results were observed *in vivo* micronucleus assays in rats and mice after intraperitoneal administration. Diepoxybutane has been extensively tested in a large number of *in vitro* and *in vivo* assays. Diepoxybutane is predominantly positive in *in vitro* assays without S9 mix, inducing among others reverse mutations, gene mutations, SCEs, chromosomal aberrations, and unscheduled DNA synthesis. *In vivo* diepoxybutane induced DNA-single strand breaks, gene mutations at the *hprt* locus, SCEs, and was positive in the micronucleus test Most studies were performed with intraperitoneal administration although a few inhalation experiments were carried out.

The WHO (2001) concluded that 1,3-butadiene is mutagenic in somatic cells of both mice and rats, although the mutagenic potency was greater in mice than in rats. Similarly, 1,3-butadiene induced other genetic damage in somatic cells of mice, but not in those of rats. 1,3-Butadiene was also consistently genotoxic in germ cells of mice, but not in the single assay in rats identified. However, there were no apparent differences in species sensitivity to genetic effects induced by epoxide metabolites of

1,3-butadiene. IARC (1999) concluded that 1,3-butadiene was mutagenic in virtually all *in vitro* and *in vivo* test systems. Where a direct comparison could be made between rats and mice 1,3-butadiene positive effects were observed primarily in mice. The EC (2002) also concluded that 1,3-butadiene is genotoxic to mammalian cells *in vivo* and that it is a germ cell mutagen in mice. The epoxide metabolites of 1,3-butadiene have been shown to be genotoxic to bacterial and mammalian cells *in vitro*, to somatic cells of the mouse, rat and/or hamster *in vivo* and to the germ cells of mouse and rat *in vivo*. The EPA (2002a) concluded that there is ample evidence of a mutagenic and clastogenic potential of 1,3-butadiene to a variety of biological systems ranging form bacteria to human beings. It was also clear to EPA that the mutagenic and genotoxic responses require metabolic activation to several DNA-reactive intermediates, especially epoxybutene and diepoxybutane. Epoxybutene required higher concentrations for mutagenic responses than diepoxybutane.

More recently, DNA adduct formation was studied in liver, lung, and testis of mice and rats exposed to 1,3-[2,3-^{14}C]-butadiene concentrations of 1, 5, or 20 ppm for 6 h/d for 5 days. DNA adduct formation was higher in mouse tissues compared to rats and increased with exposure concentration. Following a single 6-hour exposure to 20 ppm resulted in detectable adduct levels in all three tissues in both rats and mice (Booth *et al* 2004a).

3.6. Carcinogenicity

Several carcinogenicity studies have been performed with rats and mice. These studies have recently been summarized by IARC 1999, EC 2002, and EPA 2002a. This section is predominantly based on these documents; the original publications of only the most important studies are studied. For clarity the original references are given.

Rats

Groups of 110 male and 110 female Sprague-Dawley rats, 5 weeks of age, were exposed to 0, 1000, or 8000 ppm butadiene for 6 h/d, 5 d/w for 111 weeks (males) or 105 weeks (females) (Owen *et al.* 1987). Interim kills of 10 animals per group were scheduled after one-year of exposure. Survival was reduced in all exposure groups. Statistically significantly increased tumor incidences were only observed in the high exposure groups and included pancreatic exocrine adenomas and carcinomas and interstitial-cell tumors of the testis in males, and follicular-cell adenomas and carcinomas of the thyroid gland in females. In addition, positive trends were observed in female rats for sarcomas of the uterus, carcinomas of the Zymbal gland, and benign and malignant mammary tumors.

Mice

Four carcinogenicity studies with B6C3F$_1$ mice have been reported, of which one (Bucher *et al.* 1993) is of specific interest for derivation of AEGL-values. Groups of 60 male and 60 female mice, 8-10-weeks old, were exposed for a single 2-hour period to target 1,3-butadiene concentrations of 0, 1000, 5000, or 10,000 ppm (0, 2200, 11,000, or 22,000 mg/m^3, respectively). The animals were held for two years, at which time all survivors were killed and tissues and organs were examined microscopically. Survival, body weight gain, and the incidence of neoplastic and nonneoplastic lesions were not affected by 1,3-butadiene exposure.

Groups of 50 male and 50 female B6C3F$_1$ mice were exposed to 625 or 1300 ppm (1380 or 2760 mg/m^3) butadiene for 6h/d, 5 d/w, for 60-61 weeks (NTP 1984; Huff *et al.* 1985). The study was terminated after 61 weeks of exposure because of a high incidence of lethal neoplasms. Increased incidences of tumors in both sexes were hemangiosarcomas of the heart (with metastasis to other organs), malignant lymphomas, alveolar-bronchiolar adenoma or carcinoma of the lung, and papillomas or carcinomas of the forestomach. Further, tumors that occurred with statistically increased incidence in females only included hepatocellular adenoma or carcinoma of the liver, acinar-cell carcinoma of the mammary gland, and granulosa-cell tumors of the ovary.

In an additional study groups of 60 male B6C3F$_1$ mice and 60 male NIH Swiss mice, 4-6 weeks of age, were exposed to 0 or 1250 ppm (2760 mg/m^3) butadiene for 6 h/d, 5 d/w for 52 weeks (Irons *et al.* 1989). An additional group of 50 male B6C3F$_1$ mice was exposed similarly to 1,3-butadiene for 12 weeks and held for the remainder of the study. All animals were killed after 52 weeks.

Groups of 70-90 male and 70-90 female B6C3F$_1$ mice, 6.5 weeks of age, were exposed to butadiene concentrations of 0, 6.25, 20, 62.5, 200, or 625 ppm (0, 14, 44, 138, 440, or 1380 mg/m^3, respectively) for 6 h/d, 5 d/w for up to 2 years (NTP 1993; Melnick *et al.* 1990). Ten animals per group were killed and evaluated after 40 and 65 weeks of exposure. Survival was significantly reduced at exposure levels of 20 ppm and higher. Exposure to butadiene increased the incidences in both sexes of lymphomas, hemangiosarcomas of the heart, lung alveolar/bronchiolar adenomas and carcinomas, forestomach papillomas and carcinomas, Harderian gland adenomas and adenocarcinomas, and hepatocellular adenomas and carcinomas. Additionally increases in the incidences of mammary gland adenocarcinomas and ovarian granulosa-cell tumors were observed in females. Females appeared to be more susceptible than males with lung tumors already observed at 6.25 ppm and lymphomas and liver tumors at 20 ppm. The lowest concentration at which statistically significant increases in tumor incidences were observed in males was 62.5 ppm. The NTP concluded that there was clear evidence of carcinogenicity of butadiene in male and female B6C3F$_1$ mice (NTP 1993).

In the same series of experiments (NTP 1993; Melnick *et al.* 1990) also stop-exposure studies were performed. Groups of 50 male B6C3F$_1$ mice, 6.5 weeks of age, were exposed for 6 h/d, 5 d/w to 200 ppm (440 mg/m^3) for 40 weeks, 625 ppm (1380 mg/m^3) for 13 weeks, 312 ppm (690 mg/m^3) for 52 weeks, or 625 ppm (1380 mg/m^3) for 26 weeks. The first two groups received a total exposure of about 8000 ppm-weeks while the latter two groups received a total exposure of approximately 16,000 ppm-weeks. A group of 70 mice served as controls. The animals were held for the remainder of the 103-week study. Survival was reduced in all exposure groups. Tumor sites were similar to those in the two-year exposure study with the exception that no liver tumors were observed and an increased incidence in perpetual gland adenomas and carcinomas was observed. In addition, renal tubular adenomas were observed in the two groups exposed to 625 ppm. Overall, considering the mortality-adjusted tumor rates, the incidences appeared to be more determined by daily exposure concentration than by cumulative exposure.

Based on the data described EPA (2002a) concluded that 1,3-butadiene is carcinogenic in mice and rats, inducing tumors at multiple organ sites. Since all tested exposure concentrations induced tumors it was considered likely that concentration below 6.25 ppm would also induce tumors in mice.

Based on the same studies, IARC (1999) concluded that there was sufficient evidence in experimental animals for the carcinogenicity of 1,3-butadiene.

IARC concluded that there is sufficient evidence in experimental animals for the carcinogenicity of butadiene as well as for its metabolite diepoxybutane.

EC (2002) noted that there appeared to be a marked species difference in the susceptibility of rodents to the carcinogenic properties of 1,3-butadiene. The evidence in mice showed that 1,3-butadiene is a potent, multi-organ carcinogen, with tumor development occurring at relatively low exposure concentrations. All the evidence in mice was concluded to indicate that a genotoxic mechanism is involved. In contrast, the available rat study showed a lower tumor frequency, fewer tumor types, with effects seen at exposure concentrations 2-3 orders of magnitude higher than in the mouse. EC stated that the tumor type in rats suggested that hormonal influences may play a role in the carcinogenic response, and that thus a non-genotoxic mechanism may underlie the tumor formation in this species.

3.7. Summary of animal data

The acute mortality data are summarized in Table 3. Lethality data are presented for several species although most data are very limited reported. An unknown number of guinea pigs survived a 2-hour exposure to 89,000 ppm 1,3-butadiene but 100% mortality occurred at a 10-hour exposure to the same concentration. No mortality was reported for rabbits and guinea pigs exposed to 200,000 ppm for 25 and 30 min, respectively, but 2/5 rats died at 30 min exposure to 200,000 ppm (Larionov *et al*. 1934; ERPG 1997). A 4-hour LC_{50} of 128,000 ppm was reported for rats and a 2-h LC_{50} of 122,000 ppm was found for mice (Shugaev 1969).

As to nonlethal toxicity very limited data are available. The acute toxicity of 1,3-butadiene is rather low. Studies on dogs and rabbits are too poorly reported and no clear conclusions can be drawn. A nominal 1,3-butadiene concentration of 25% (250,000 ppm) for an unknown exposure period caused narcosis followed by death in rabbits (unspecified strain, number, and sex) but no narcosis occurred at exposure to 15% (150,000 ppm) for 25 min (Larionov *et al*. (1934). Irritation of conjunctiva and the nose and lachrymation were the first signs of toxicity at these concentrations. No clinical signs of toxicity were reported to occur in male Sprague-Dawley rats nose-only exposed to a 1,3-butadiene concentration of 201 ppm for 6 hours followed by a 42-hour observation period (Boogaard *et al*. 2004). In a study focused on carcinogenicity Bucher *et al.* (1993) exposed groups of 60 male and 60 female B6C3F_1 mice, 8-10-weeks old, for a single 2-hour period to target 1,3-butadiene concentrations of 0, 1000, 5000, or 10,000 ppm. The animals were held for two years. Survival, body weight gain, and the incidence of neoplastic and nonneoplastic lesions were not affected by 1,3-butadiene exposure.

No signs of toxicity were observed in rats who were exposed to 1,3-butadiene concentrations up to 8000 ppm for 6 h/d, 5 d/w for up to 3 months (Crouch *et al*. 1979). Detailed histopathological and hematological examinations were performed. Apart from piloerection and transient dyspnea no clinical signs were reported for male mice exposed to 4980 ppm for 6/d for 5 days (Hackett *et al*. 1988). No compound-related histopathological effects were observed in male and female mice exposed to 1,3-butadiene concentrations of up to 8000 ppm for 14 weeks. In the latter study an increased mortality and growth retardation were observed at the higher concentrations, but these effects are due to repeated exposure (NTP 1984).

Two teratogenicity studies with rats were not consistent regarding the 1,3-butadiene concentrations at which adverse effects might occur. Hackett *et al*. (1987a) did not find any effect in pregnant rats exposed to target 1,3-butadiene concentrations up to 1000 ppm and no effects on their offspring were observed. Maternal growth retardation and fetal effects (diminished growth accompanied by non-specific skeletal effects like wavy ribs and delayed ossification) were observed in the second rat study (Irvine 1981) and in a mouse study (Hackett *et al*. 1987b). These effects were only observed in the presence of maternal toxicity. This pattern of effects is probably caused by repeated exposure and is unlikely to occur from a single exposure at the same dose (Van Raaij *et al*. 2003). Fertility studies with male mice did not point to clear adverse effects of single exposure to 1,3-butadiene (Anderson *et al*. 1986; Hackett *et al*. 1988; Pacchierotti *et al*. 1998).

The WHO (2001) concluded that 1,3-butadiene is mutagenic in somatic cells of both mice and rats, although the mutagenic potency was greater in mice than in rats. 1,3-Butadiene was also consistently genotoxic in germ cells of mice, but not in the single assay in rats identified. IARC (1999) concluded that 1,3-butadiene was mutagenic in virtually all *in vitro* and *in vivo* test systems. Where a direct comparison could be made between rats and mice 1,3-butadiene positive effects were observed primarily in mice. The EC (2002) also concluded that 1,3-butadiene is genotoxic to mammalian cells *in vivo* and that it is a germ cell mutagen in mice. The epoxide metabolites of 1,3-butadiene have been shown to be genotoxic to bacterial and mammalian cells *in vitro*, to somatic cells of the mouse, rat and/or hamster *in vivo* and to the germ cells of mouse and rat *in vivo*. US EPA (2002a) concluded that there is ample evidence of a mutagenic and clastogenic potential of 1,3-butadiene to a variety of biological systems ranging form

bacteria to human beings. It was also clear to EPA that the mutagenic and genotoxic responses require metabolic activation to several DNA-reactive intermediates, especially epoxybutene and diepoxybutane. Epoxybutene required higher concentrations for mutagenic responses than diepoxybutane.

Based on the data described EPA (2002a) concluded that 1,3-butadiene is carcinogenic in mice and rats, inducing tumors at multiple organ sites. Since all tested exposure concentrations induced tumors it was considered likely that concentration below 6.25 ppm would also induce tumors in mice. Based on the same studies, IARC (1999) concluded that there was sufficient evidence in experimental animals for the carcinogenicity of 1,3-butadiene. EC (2002) concluded that the evidence in mice showed that 1,3-butadiene is a potent, multi-organ carcinogen, with tumor development occurring at relatively low exposure concentrations. All the evidence in mice was concluded to indicate that a genotoxic mechanism is involved. In contrast, the available rat study showed a lower tumor frequency, fewer tumor types, with effects seen at exposure concentrations 2-3 orders of magnitude higher than in the mouse. EC stated that the tumor type in rats suggested that hormonal influences may play a role in the carcinogenic response, and that thus a non-genotoxic mechanism may underlie the tumor formation in this species.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

This section is predominantly based on the reviews by US EPA (2002a) and EC (2002) supplemented with more recent information. In addition, Himmelstein *et al* (1997) provide a detailed description of the metabolic pathways and enzymes involved and a comprehensive overview of the experimental evidence for the individual biotransformation steps. Original publications are described into more detail where relevant. It is noted that most of the experiments focused on metabolites considered to be of importance for the carcinogenic potential of 1,3-butadiene.

In vivo experiments

Absorption

A number of uptake and metabolism experiments with rats and mice have been performed in closed chamber systems. 1,3-Butadiene is moderately soluble in blood, with a blood:air partition coefficient of about 1. These experiments showed that 1,3-butadiene is readily taken up. The uptake appeared to be linear in rats and mice exposed to 1,3-butadiene concentrations up to 1000 ppm, above which concentration saturation occurs of this process. Uptake and metabolism appear to be faster in mice compared to rats.

Bond et al. (1986) performed the most detailed animal experiment. Male Sprague-Dawley rats and male $B6C3F_1$ mice were exposed nose-only to target 1,3-[1-^{14}C] butadiene concentrations of 0.08, 0.8, 7, 70, 1000, or 7100 (rats only) ppm for 6 hours. Groups of three animals per species were withdrawn from exposure at 2, 4, and 6 hours of exposure for analyses of metabolites in blood. Immediately after exposure groups of four animals per species were placed in metabolism cages and excreta were collected up to 65 hours postexposure. The animals in the metabolism studies were exposed to 7 ppm 1,3-butadiene and higher. No differences were found in breathing frequencies, minute volumes, and tidal volumes between exposure concentrations. The percentage of inhaled [^{14}C] 1,3-butadiene retained at 6 hours in rats ranged from 17% at the lowest concentrations to 1.5% at the highest concentration; these values were 16 and 4%, respectively, for mice. These data are indicative of saturable metabolism. Urine and exhaled air were the main routes of postexposure excretion. With increasing concentration the major excretion route changed from urine to exhaled air (predominantly as CO_2. Greater than 90% of the ^{14}C in the blood of rats and mice consisted of (mainly nonvolatile) butadiene metabolites. Quantities of metabolites in blood increased with increasing exposure concentrations, but not proportional. Mice had especially higher blood

levels of 1,2-epoxy-3-butene, blood concentrations of butadiene and butadiene diepoxide were similar or slightly higher in mice.

Dahl *et al*. (1991) exposed three male cynomolgus monkeys nose-only to actual exposure concentrations of 10.1, 310, and 7760 ppm [1-^{14}C] 1,3-butadiene for 2 hours. Each animal was exposed to the three concentrations under anesthesia, with three-month intervals. Actual concentrations were within 3% of the target concentrations. Excreta and blood were sampled during and for 96 hours after exposure. The total inhaled volume of air was decreased by 30% at the highest concentration. At the lowest concentration slightly more ^{14}C was excreted as CO_2 than via urine, while at higher concentrations urinary excretion became the predominant excretion route. The majority of exhaled ^{14}C consisted of not further identified compounds.

Distribution

Following absorption 1,3-butadiene is widely distributed throughout the body. Higher concentrations of reactive metabolites in target tissues were found in mice compared to rats because of differences in rates of metabolism of 1,3-butadiene and its metabolites.

Bond *et al*. (1987) exposed 39 male Sprague-Dawley rats and 39 male B6C3F$_1$ mice to ^{14}C-1,3-butadiene concentrations of 550 ppm and 54 ppm, respectively, for 3.4 hours. Concentrations were regularly monitored. Groups of three animals per species were killed at 1, 2, 4, 8, 18, 27, 43, 51, and 67 hours and 6, 8, 10, and 13 days after termination of exposure. Radioactivity was distributed widely in tissues immediately following exposure, highest concentrations were found at 1 hour postexposure. Tissue concentrations of ^{14}C were generally twofold higher in rats Tissue elimination appeared to be biphasic with $T_{½}$ for most tissues between 6 to 8 hours. Already at 1 hour postexposure the percentage of nonvolatile ^{14}C material was higher than 60 to 70% for all tissues except fat.

The concentration of 1,3-butadiene in the blood of rats and mice exposed to 1,3-butadiene concentrations up to 1250 ppm for 6 hours reached steady-state by 2 hours of exposure. The steady-state concentration in the blood of mice was about twofold higher considered to be due to the higher alveolar ventilation rate per gram body weight in mice. The 1,3-butadiene concentration in blood was not proportional to the exposure concentration indicating saturable uptake. Postexposure 1,3-butadiene concentration in blood decreased rapidly.

Epoxybutene can be detected both in liver and lung tissue of rats and mice exposed to 1,3-butadiene concentrations of 625 ppm and higher. Diepoxybutane was only found in mice lung tissue. In another experiment tissue levels of epoxybutene were about 3-12 fold greater in mice as compared to rats whereas tissue levels of diepoxybutane were 38-163 fold greater. The reported blood levels of diepoxybutane are approximately 40-fold higher in mice than in rats. Both in rats and mice tissue levels of both epoxides generally return to control values within 0.5-1 hour after a 4-hour exposure to 62.5 ppm 1,3-butadiene. Some data indicate that the production of epoxybutene is greatest in the mouse and least in the cynomolgus monkey, with Syrian hamsters and rats in between.

One study reported some gender differences in the distribution of epoxybutene and diepoxybutane between male and female rats exposed to 62.5 ppm 1,3-butadiene for 6 hours. Whereas the epoxybutene concentration in lung was higher in males the concentration of diepoxybutane was higher in females in all tissues (blood, femur, lung and liver) examined. The data are too limited to draw clear conclusions and the few data available for mice do not support these findings in rats.

Metabolism

A schematic presentation of the metabolic pathway of 1,3-butadiene is given in Figure 1. In brief, the first step in the main route is biotransformation to epoxybutene, which can either undergo conjugation with GSH, hydrolysis to butenediol, or further oxidation to diepoxybutane. Butenediol can be conjugated

with GSH or oxidized to epoxybutanediol. Diepoxybutane also can be metabolized to epoxybutanediol and subsequently to erythritol or can be conjugated with GSH. The biotransformation of 1,3-butadiene is qualitatively similar between species but quantitatively large differences have been observed. Mercapturic acids derived from the conjugation of the different metabolites with GSH have been found in urine of mice and rats exposed to 1,3-butadiene. Biotransformation of the mono- and diepoxide (detoxification) is mainly through conjugation with GSH in mice whereas in humans epoxide hydrolase appears to be more of importance.

In comparison with rodents, the total concentration of epoxybutene, diepoxybutane, and nonvolatile metabolites in the blood of cynomolgus monkeys was lower for equivalent exposure concentrations. This was considered to be partly due to difference in uptake rates between the species.

Another biotransformation route that has been suggested is oxidation to 3-butenal, which subsequently can give rise to the formation of crotonaldehyde, acrolein and acrylic acid. CO_2 can be formed during several steps but these steps have not yet been identified (see e.g. Himmelstein *et al*. 1997 for possibilities). Concentration of epoxybutene appears to be 4-8-fold higher in the blood of mice compared to rats. Although diepoxybutane is easily detected in blood of mice it is hardly or not detectable in blood of rats at comparable 1,3-butadiene exposure concentrations. Epoxybutene, epoxybutanediol, and diepoxybutane have been found to form DNA and hemoglobin adducts in rats and mice. Recently, Booth *et al*. (2004a; 2004b) found globin adducts in male $B6C3F_1$ mice and male Sprague-Dawley rats exposed to 1, 5, or 20 ppm 1,3-[2,3-^{14}C]-butadiene for 6 hours. EC (2002) provides data on the use of hemoglobin adducts as biomarkers for 1,3-butadiene exposure. It was found that epoxybutene can bind to hemoglobin. Mice showed consistently higher levels of hemoglobin adducts than rats. Very low but detectable levels of hemoglobin adducts have also been detected in some workers exposed to an 8-hour TWA 1,3-butadiene concentration of 3.5 ppm. These levels were lower than in rats exposed to 2 ppm.

The major group of enzymes involved in the various oxidation steps of 1,3-butadiene and its metabolites are cytochromes P450 (especially 2E1 and 2A6). The oxidation of butenediol to epoxybutanediol can also be catalyzed by alcohol dehydrogenase. Hydrolysis and the conjugation with GSH of the various compounds can either occur chemically or enzymatically by epoxide hydrolase or glutathione transferases, respectively. Oxidation and hydrolysis by epoxide hydrolase occur in microsomes while glutathione conjugation primarily occurs in the cytosolic fraction *in vitro*.

A large number of *in vitro* experiments on interspecies differences in enzymatic activities and kinetic constants have been carried out. US EPA (2002a) provides a detailed overview of the outcome of these studies.

Quantitative differences in the metabolism of 1,3-butadiene were observed using liver and lung tissue from mice, rats and humans. However, these results should be considered with care since some human tissue samples were taken form patients, e.g. lung tissue from lung cancer patients. As to lung tissue, lung microsomes from humans and rats had a lower capacity than liver microsomes to oxidize 1,3-butadiene, whereas lung and liver microsomes from mice showed similar capacities. Only mouse liver microsomes were capable of oxidation of epoxybutene to diepoxybutane.

Mice consistently had the highest enzyme activities compared with rat, human, or monkey. *In vitro* experiments with lung and liver tissue samples from mice, rats, and humans showed quantitative differences between the species. The V_{max} for oxidation of 1,3-butadiene to epoxybutene in mouse liver microsomes was approximately twofold higher compared with that for human liver microsomes, which in turn was twofold higher than in rat liver microsomes. Both human and rat lung microsomes showed a lower capacity than liver microsomes to oxidize 1,3-butadiene, whereas the mouse lung was comparable with liver. (However, it is noted that the human lung samples were obtained from 5 lung cancer patients and the results should be treated with care). In addition, only mouse liver microsomes were capable of

oxidation of epoxybutene to diepoxybutane. Human liver had the highest V_{max} for enzyme-mediated hydrolysis of epoxybutene and the lowest V_{max} for conjugation with GSH as compared with mouse and rat. In all three species, the detoxification mechanisms (conjugation) were kinetically favored over the activation (oxidation) of 1,3-butadiene to the monoepoxide. The activation/detoxification ratio was an order of magnitude higher for mice than for rats with humans in between.

However, another study with human liver microsomes showed a great interindividual variability in the metabolism of 1,3-butadiene to the monoepoxide, in some cases the rate of formation was similar to that in mice. A further study with liver microsomes from 10 Caucasian trauma victims showed a 60-fold variation in the rate of transformation of the monoepoxide to the diepoxide. All samples showed lower transformation rates than that for mice but were comparable or higher than that for rats. However, this study appeared to show some shortcomings in the control of volatilization of epoxybutene and further metabolism by hydrolysis or GSH conjugation.

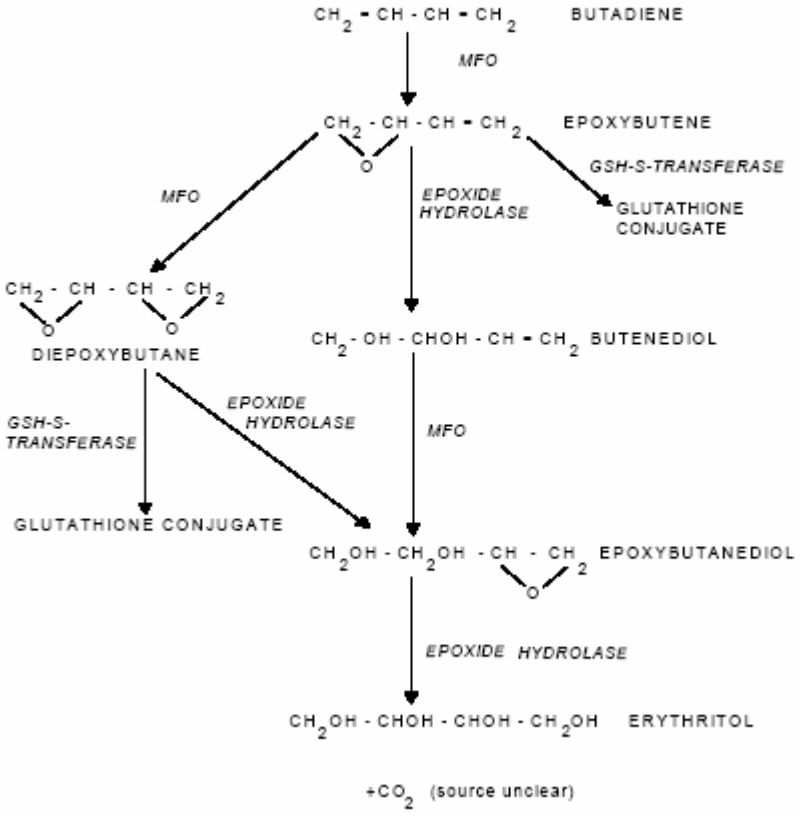

Figure 1. Schematic presentation of the metabolic pathway of 1,3-butadiene (from EC 2002).

Excretion

The major routes for excretion are exhalation and via urine. However, exhalation (mainly as CO_2) is an important route of excretion only at relatively low exposure levels (below 100 ppm). The main urinary metabolites appeared to be mercapturic acids of either epoxybutene or butenediol. The former is the main metabolite in mice and to a lesser extent also in rats and hamsters, while in cynomolgus monkeys the mercapturic acid from butenediol is the predominant form. Urinary excretion in humans

reflects the relatively large contribution of epoxide hydrolase in the biotransformation steps of 1,3-butadiene.

In a study with workers exposed to 8-hour TWA 1,3-butadiene concentrations of up to 3-4 ppm no evidence was found for epoxybutene conjugation with GSH as a detoxification pathway in humans. The mercapturic acid of butenediol was detected in urine. It was suggested that the metabolism of 1,3-butadiene to epoxybutene followed by hydrolysis to butenediol with subsequent conjugation is the predominant detoxification pathway in humans.

Summary and conclusions

1,3-Butadiene is readily taken up through the respiratory tract. The uptake in mice is about twice as high as in rats, probably reflecting the higher ventilation rate and higher rate of metabolism in mice. After absorption 1,3-butadiene is widely distributed throughout the body.

Qualitatively, metabolism of 1,3-butadiene is similar among species, including humans. However, there are quantitative differences in predominant pathways for detoxication and in the rates of metabolism by the various pathways. The rate of oxidation is greatest in mice with humans and rats showing on average approximately equivalent rates.

The first step in the biotransformation of 1,3-butadiene is oxidation to epoxybutene. *In vitro* data show that mice appear to have the highest rate of formation of the monoepoxide. The data obtained with human liver samples are not consistent. On average, the metabolic rate for this step in human liver microsomes is on average more close to that in rats but shows a large variability. However, overall variability in total metabolism and susceptibility is unknown.

Epoxybutene can either be further oxidized to diepoxybutane, hydrolyzed to butenediol, or conjugated with GSH. Diepoxybutane can in its turn be hydrolyzed to epoxybutanediol or conjugated with GSH. Although mice and rats predominantly remove epoxybutene and diepoxybutane through conjugation with GSH, in humans, the main route appears to be enzyme-mediated hydrolysis. Butenediol can be further oxidized to epoxybutanediol, which can be hydrolyzed to erythritol or conjugated with GSH. 1,3-Butadiene and its metabolites can be excreted through exhalation while metabolites can also be excreted through urine (mainly as mercapturic acids) or, to a lesser extent through feces.

Due to the differences in enzymatic rates and detoxication pathways tissue levels of 1,3-butadiene metabolites are much higher in mice than in rats.

In conclusion, the differences between mice and rats are mainly attributed to a higher formation rate of the epoxides and a higher uptake due to a higher ventilation rate in mice. Blood levels of the epoxides are much higher in mice than in rats. These differences are considered to be the cause of the high difference in susceptibility in 1,3-butadiene toxicity in the two species. It is noted that humans have approximately a four times lower ventilation rate than rats and that the limited *in vitro* data obtained with human tissue samples show that overall the bioformation rate in human liver will be lower than in mice and more comparable to that in rats. Although a wide interindividual variation is observed in the some metabolic rates in human liver samples the overall variability is unknown. However, as to the kinetics of 1,3-butadiene it is concluded that humans will be more comparable to rats than to mice.

4.2. Physiologically-based pharmacokinetic (PBPK) models

Various models have been developed to simulate the toxicokinetics of 1,3-butadiene and its metabolites. These models mainly focused on explanation of the interspecies differences and the site specificity of the carcinogenic response. These models and their (dis)advantages have been comprehensively described by US EPA (2002a) and in lesser detail by EC (2002). In both reports it is concluded that the present models do not yet provide clear understanding of the basis of the marked

interspecies differences in susceptibility. Furthermore, the models do not appear to sufficiently account for the large intra-individual differences in humans. It was concluded that uncertainties in the model structures and parameter values also prohibit their use in refining risk assessment dosimetry. At present there is no good model that can be used in human risk assessment to 1,3-butadiene.

4.3. Mechanism of Toxicity

The studies on the mechanism of action mainly focus on the carcinogenic potential of 1,3-butadiene and its metabolites. Because conjugation with GSH is an important detoxification route GSH depletion occurs at longer exposure duration or at higher concentrations leading to higher body burdens of epoxybutene and diepoxybutane (Himmelstein *et al*. 1997). Deutschman and Laib (1989) studied the depletion of non-protein sulfhydryl (NPSH) content in lung, heart, and liver tissue of male Sprague-Dawley rats and of male $B6C3F_1$ mice. Groups of 9 animals were exposed for 7 hours to target 1,3-butadiene concentrations of 0, 10, 50, 100, 250, 500, 1000, and 2000 ppm. Actual concentrations were reported to be within 5% of the target concentrations. Significant depletion of NPSH content started at 250 ppm in lung and liver in both animal species. In rats, a maximum depletion of approximately 30 and 60% was reached in these organs, respectively, at the highest concentration. NPSH content in heart remained practically constant over the exposure range. In mice, a reduction of more than 80% was found in liver and heart and almost complete depletion in lungs at the highest concentration. The precise mechanism is of carcinogenicity is unsure, a marked species difference in the susceptibility of rodents to the carcinogenic properties has been noted. In the mouse, 1,3-butadiene is a potent, multi organ carcinogen while in rats hormonal influences may play an important role. Tumors appear in mice at much lower exposure concentrations than in rats (EC 2002; EPA 2002a). No data are available with respect to the mechanism of action with respect to non-carcinogenic end points in acute exposures.

4.4. Other relevant information

4.4.1. Species variability

Mice are much more susceptible to the carcinogenic properties of 1,3-butadiene than rats (EPA 2002a; EC 2002). Many studies, predominantly focused on the toxicokinetics, have been performed to elucidate the basis of this difference. Although the metabolism of 1,3-butadiene is qualitatively similar among species, including humans, large quantitative differences have been observed in predominant pathways for detoxication and in the rates of metabolism by the various pathways. The rate of oxidation is greatest in mice with humans and rats on average approximately equivalent. The *in vitro* data obtained with human liver samples are not very consistent. Although mice and rats predominantly remove epoxybutene and diepoxybutane through conjugation with GSH, in humans, the main route appears to be enzyme-mediated hydrolysis. Butenediol can be further oxidized to epoxybutanediol, which can be hydrolyzed to erythritol or conjugated with GSH.

As to carcinogenicity, it has been argued that mice may be a more relevant model for humans in terms of site specificity, in that 1,3-butadiene induces tumors of the lymphohematopoietic system in both mice and humans (EPA 2002a). However, carcinogenic endpoints are not relevant for AEGL-derivation. Mice also appear to be more susceptible than rats in noncarcinogenic endpoints like developmental toxicity parameters (see section 3.4) and in the formation of hemoglobin adducts (Booth *et al*. 2004a, 2004b). In addition, no toxicity was observed in rats exposed to 1000 or 8000 ppm for 3 months (6 h/d, 5 d/w). A NOAEL of 1000 ppm was derived from a study with a similar exposure regimen for over 100 weeks. In contrast, significant toxicity was observed in mice exposed to 1,3-butadiene concentrations as low as 20 ppm for up to 2 years (EC (2002). Further, the limited acute exposure data may also indicate that mice are more susceptible than rats (Shugaev *et al*. 1969; see Table 3). However, in a study focused on carcinogenicity Bucher *et al.* (1993) observed no increased incidence of neoplastic and nonneoplastic lesions in groups of 60 male and 60 female $B6C3F_1$ mice exposed for a single 2-hour period to target 1,3-butadiene concentrations of 0, 1000, 5000, or 10,000 ppm and observed for two years.

These differences between rats and mice are attributed to a higher uptake per kg body weight in mice compared with rats and to quantitative differences in predominant pathways for detoxication and in the rates of metabolism by the various pathways. For comparison, humans have a lower ventilation rate per kg body weight than both mice and rats. Furthermore, the rate of oxidation is greatest in mice with humans and rats showing on average approximately equivalent rates. Therefore, it can be concluded that mice are extremely susceptible to 1,3-butadiene and humans will be more equal to rats (see also section 4.1).

4.4.2. Intraspecies variability / Susceptible populations

The only data available with respect to differences in susceptibility between humans come from *in vitro* biotransformation studies with human lung and liver microsomes. Large differences in metabolic rates for specific steps in the biotransformation of 1,3-butadiene have been observed, in some cases the rate of formation was similar to that in mice. On average, the metabolic rate for this step in human liver microsomes is on average more close to that in rats.

This variability is not unexpected considering the complexity of the metabolic pathways involved in the biotransformation of 1,3-butadiene. The three principal (groups of) enzymes that are involved are cytochrome P450 (probably P450 2E1), glutathione-*S*-transferases, and epoxide hydrolase. Cytochrome P450 2E1 is easily induced by low molecular weight compounds like ethanol and suggestions have been made of the presence of polymorphism. Genetic polymorphism for specific glutathione-*S*-transferases (GST) has been clearly described but the GST involved in 1,3-butadiene metabolism is unknown. However, overall variability in total metabolism and susceptibility is unknown and cannot be evaluated.

4.4.3. Irritation and Sensitization

Acute exposure to 90,000 – 140,000 ppm butadiene was reported to cause conjunctivitis in mice, and conjunctivitis and lachrymation were observed in rabbits exposed to 150,000-250,000 ppm (Larionov *et al*. 1934). In another study with rabbits, ophthalmoscopy revealed no signs of eye injury following exposure up to 6,700 ppm butadiene 7.5 hours/day, 6 days/week for 8 months; the same result was recorded for dogs, for which only one animal per exposure level was used (Carpenter *et al*. 1944).

Slight irritation and dryness of the nose and mouth were reported by human volunteers exposed to 10,000 ppm butadiene for 5 minutes (Larionov *et al* 1934). In another study, 2 subjects exposed to 2,000 ppm butadiene for 7 hours or 4,000 ppm for 6 hours reported slight smarting of the eyes and difficulty in focussing. No effects were reported at a 6-hour exposure to 8000 ppm (Carpenter et al., 1944).

There are no data available on the sensitization potential of 1,3-butadiene.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of human data relevant to AEGL-1

Limited human data that address the level of effects defined by the AEGL-1 were retrieved. This study reported by Ripp (1967) is poorly reported and is considered of doubtful significance, also considering the observations by Carpenter *et al*. (1944). They exposed two males to 2000 ppm 1,3-butadiene for 7 hours, 4000 ppm for 6 hours, and 8000 ppm for 8 hours (nominal concentrations, regularly monitored). These exposure times are total times of actual exposure with all exposures interrupted for a one-hour lunch break in the middle of the exposure period. Subjective symptoms reported at 2000 and 4000 ppm included slight smarting of the eyes and difficulty in focusing. No subjective complaints were reported at 8000 ppm, according to the authors probably because of slight anxiety concerning the possibility of an explosion. Both subjects felt particularly alert. Results of a tapping test and a steadiness test revealed no differences in performance between the exposures. Larionov

et al. (1934) reported a slight increase in pulse rate in human beings (no details on number and sex) exposed to a 1,3-butadiene concentration of 1% (10,000 ppm) for 5 min. No effects were observed on blood pressure or respiration. Subjective complaints consisted of a tingling sensation and dryness of the nose and throat.

5.2. Summary of animal data relevant to AEGL-1

Ophthalmoscopic examination of the eyes of female dogs and rabbits exposed to 1,3-butadiene concentrations up to 6700 ppm for 7.5 h/d for up to 8 months revealed no signs of injury (Carpenter *et al.* 1944). A nominal 1,3-butadiene concentration of 25% (250,000 ppm) for an unknown exposure period caused narcosis followed by death in rabbits (unspecified strain, number, and sex) but no narcosis occurred at exposure to 15% (150,000 ppm) for 25 min (Larionov *et al*. (1934). Irritation of conjunctiva and the nose and lachrymation were the first signs of toxicity at these concentrations. No clinical signs of toxicity were reported to occur in male Sprague-Dawley rats nose-only exposed to a 1,3-butadiene concentration of 201 ppm for 6 hours followed by a 42-hour observation period (Boogaard *et al*. 2004).

Female rats exposed to a 1,3-butadiene concentration up to 7647 ppm for 6 h/d, from day 6-15 of gestation did not show any respiratory distress (Irvine 1981).

Groups of 20 adult male $B6C3F_1$ mice were exposed to actual 1,3-butadiene concentrations (SD) of 0, 199, 999, or 4980 ppm 1,3-butadiene for 6 h/d for 5 successive days (Hackett *et al.* in 1988 as summarized by Morrisey *et al*. (1990), EPA (2002a), and EC (2002). The mice were killed 5 weeks after exposure. No clinical signs of toxicity were observed apart from piloerection and dyspnea during the first 20-30 min of the exposure in the 4980 ppm exposure group.

5.3. Derivation of AEGL-1

Only one adequate human study is available that addresses AEGL-1 endpoints. Carpenter *et al*. (1944) exposed two males to nominal concentrations of 2000 ppm 1,3-butadiene for 7 hours, 4000 ppm for 6 hours, and 8000 ppm for 8 hours. These exposure times are total times of actual exposure with all exposures interrupted for a one-hour lunch break in the middle of the exposure period. Subjective symptoms reported at 2000 and 4000 ppm included slight smarting of the eyes and difficulty in focusing. No subjective complaints were reported at 8000 ppm, according to the authors probably because of slight anxiety concerning the possibility of an explosion. Both subjects felt particularly alert. Results of a tapping test and a steadiness test revealed no differences in performance between the exposures. It is assumed that the absence of subjective symptoms at 8000 ppm could indeed have been due to an increased awareness. If so, this would indicate that the complaints were of very minor severity, and possibly sub-AEGL-1 effects. The 7-hour exposure to 2000 ppm is therefore considered to be an appropriate point of departure without a further modifying factor. However, since only two humans were exposed an intraspecies factor of 3 is considered appropriate.

Since the type of effect (local eye effects) is considered to be concentration- rather than time-related AEGL-1 values will be set equal for all exposure periods. The following AEGL-1 values were derived:

TABLE 5. AEGL-1 Values for 1,3-butadiene

10-minute	30-minute	1-hour	4-hour	8-hour
670 ppm (1500 mg/m^3)	670 ppm (1500 mg/m^3)	670 ppm (1500 mg/m^3)	670 ppm (1500 mg/m^3)	670 ppm (1500 mg/m^3)

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of human data relevant to AEGL-2

No adequate human data that address the level of effects defined by the AEGL-2 were retrieved.

Carpenter *et al*. (1944) exposed two males to 2000 ppm 1,3-butadiene for 7 hours, 4000 ppm for 6 hours, and 8000 ppm for 8 hours (nominal concentrations, regularly monitored). These exposure times are total times of actual exposure with all exposures interrupted for a one-hour lunch break in the middle of the exposure period. Subjective symptoms reported at 2000 and 4000 ppm included slight smarting of the eyes and difficulty in focusing. No subjective complaints were reported at 8000 ppm, according to the authors probably because of slight anxiety concerning the possibility of an explosion. Both subjects felt particularly alert. Results of a tapping test and a steadiness test revealed no differences in performance between the exposures.

6.2. Summary of animal data relevant to AEGL-2

A nominal 1,3-butadiene concentration of 25% (250,000 ppm) for an unknown exposure period caused narcosis followed by death in rabbits (unspecified strain, number, and sex) but no narcosis occurred at exposure to 15% (150,000 ppm) for 25 min (Larionov *et al*. (1934). Irritation of conjunctiva and the nose and lachrymation were the first signs of toxicity at these concentrations. No clinical signs of toxicity were reported to occur in male Sprague-Dawley rats nose-only exposed to a 1,3-butadiene concentration of 201 ppm for 6 hours followed by a 42-hour observation period (Boogaard *et al*. 2004).

In a study focused on carcinogenicity Bucher *et al.* (1993) exposed groups of 60 male and 60 female B6C3F$_1$ mice, 8-10-weeks old, for a single 2-hour period to target 1,3-butadiene concentrations of 0, 1000, 5000, or 10,000 ppm. The animals were held for two years. Survival, body weight gain, and the incidence of neoplastic and nonneoplastic lesions were not affected by 1,3-butadiene exposure.

Repeated exposure of groups of 20 rats per sex to 1,3-butadiene concentrations 1000, 2000, 4000, or 8000 ppm (6 h/d, 5 d/w for 3 months) induced no histopathological or hematological effects in rats (Crouch *et al*. 1979). Growth retardation was observed in mice exposed under similar conditions for 15 days at concentrations of 1250 ppm. An increased mortality was observed at 5000 and 8000 ppm in mice exposed for 14 weeks for 6 h/d for 5 d/w but no chemical-related histopathological effects were found (NTP 1984).

Two teratogenicity studies with rats were not consistent regarding the 1,3-butadiene concentrations at which adverse effects might occur. Hackett *et al*. (1987a) did not find any effect in pregnant rats exposed to target 1,3-butadiene concentrations up to 1000 ppm and no effects on their offspring were observed. Maternal growth retardation and fetal effects (diminished growth accompanied by skeletal effects like wavy ribs and delayed ossification) were observed in the second rat study (Irvine 1981) and in the mouse study (Hackett *et al*. 1987b). These effects were only observed in the presence of maternal toxicity (growth reduction). Such a pattern of effects can be typically considered to represent non-specific growth retardation due to the maternal condition. It is concluded that such effects are most probably caused by repeated exposure and are unlikely to occur from a single exposure at the same dose (Van Raaij *et al*. 2003). Fertility studies with male mice did not point to clear adverse effects of single exposure to 1,3-butadiene (Anderson *et al*. 1986; Hackett *et al*. 1988; Pacchierotti *et al*. 1998).

6.3. Derivation of AEGL-2

Two studies are considered relevant for the derivation of AEGL-2. The study by Carpenter *et al*. (1944) with two human volunteers showed no AEGL-2 effects during an 8-hour exposure to 8000 ppm. The second study is the 3-month exposure study in rats (Crouch *et al*. 1979). Based on the information presented in Chapter 4, the rat is concluded to be the most appropriate model for humans for nonneoplastic endpoints. The fetal effects observed when female rats were exposed to 1,3-butadiene were concluded to be related to maternal growth inhibition and are probably caused by repeated exposure and unlikely to occur from a single exposure at the same dose (Van Raaij *et al*. 2003). These data do therefore not provide an appropriate point of departure for AEGL-2. Crouch *et al*. (1979) exposed groups of 20 rats per sex were exposed for 6 h/d for 5 d/w for 3 months to 1000, 2000, 4000 or 8000 ppm 1,3-butadiene. The animals were thoroughly examined but no adverse effects due to 1,3-butadiene exposure were found. The 8000 ppm exposure concentration, the highest concentration tested, is a NOAEL in semichronic exposure. This concentration is therefore a very conservative point of departure for AEGL-2.

The use of human data (Carpenter *et al*. 1944) is preferable to the rat data as point of departure for AEGL-2. The 8-hour exposure to 8000 ppm is considered to be a conservative point of departure (no effects observed at the highest concentration tested) and an intraspecies factor of 3 is considered sufficient. The value of (8000/3=) 2700 ppm for 8 hours was extrapolated across time periods using $C^n xt = k$ with a default value of *n*=3 for extrapolation to shorter time periods. The relationship between concentration and duration of exposure as related to lethality was examined by Ten Berge et al. (1986) for approximately 20 irritant or systemically-acting vapors and gases. The authors subjected the individual animal data sets to probit analysis with exposure duration and exposure concentration as independent variables. An exponential function ($C^n xt = k$), where the value of n ranged from 0.8 to 3.5 for different chemicals was found to be an accurate quantitative descriptor for the chemicals evaluated. Approximately 90 percent of the values of n range between n=1 and n=3. Consequently, n=3 was selected as the reasonable upper bound of n to use when data are not available to derive a value of n. Because the point of departure for time extrapolation is longer than 4 hours, the AEGL-2 10-minute value is the same as the AEGL-3 30-minute value. The AEGL-2 values are presented in Table 6. These values are supported by the rat study reported by Crouch *et al*. (1979). No effects were observed in rats exposed to 8000 ppm for 6 h/d, 5 d/w for 3 months. Because this study provides a very conservative point of departure (highest concentration tested, no effects observed, 3-month exposure) a total UF of 3 can be considered sufficient. This would lead to AEGL-2 values that are very similar to the proposed values.

TABLE 6. AEGL-2 Values for 1,3-butadiene

10-minute	30-minute	1-hour	4-hour	8-hour
6700 ppm¶ (15,000 mg/m^3)	6700 ppm¶ (15,000 mg/m^3)	5300 ppm¶ (12,000 mg/m^3)	3400 ppm¶ (7500 mg/m^3)	2700 ppm¶ (6000 mg/m^3)

¶ All proposed values are higher than or equal to 10% of the lower explosive limit of butadiene in air (LEL = 2 % (20,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of human data relevant to AEGL-3

No adequate human data that address the level of effects defined by the AEGL-3 were retrieved.

No significant toxicity was observed in two humans exposed to 2000 ppm of 1,3-butadiene for 7 hours, to 4000 ppm for 6 hours, or to 8000 ppm for 8 hours (Carpenter *et al*. 1944).

7.2. Summary of animal data relevant to AEGL-3

Most data on acute lethality of 1,3-butadiene are poorly reported and not available for evaluation. ERPG (1997) referred to mortality data in rats and guinea pigs reported by Dow dated from 1941, but these data were not available for evaluation. Only one adequate study could be retrieved. Shugaev reported a 2-hour LC_{50} of 122,000 ppm (270 g/m^3) in mice and a 4-hour LC_{50} of 128,000 ppm (285 g/m^3) in rats.

Repeated exposure at 1,3-butadiene concentrations of up to 8000 ppm (6 h/d, 5 d/w for 3 months) induced no effects in rats (Crouch *et al*. 1979) and no mortality was observed in mice exposed under similar conditions for 15 days at 1,3-butadiene concentrations of up to 8000 ppm. A clearly increased mortality was observed in mice exposed to 1,3-butadiene concentrations of 5000 and 8000 ppm for 14 weeks (NTP 1984).

7.3. Derivation of AEGL-3

There are no adequate human data for derivation of AEGL-3. Therefore, AEGL-3 will be based on animal data. Based on the information presented in Chapter 4, the rat is concluded to be the most appropriate model for humans for nonneoplastic endpoints. The only study that provides adequate data is the one performed by Shugaev (1969). Rats were exposed to butadiene 4 hours. Since Shugaev does not provide the individual experimental data but only the LC_{16}, LC_{50}, and the LC_{84} as obtained by probit analyses, benchmark dose-response modeling is not possible. However, the LC_{01} can be calculated since the mean is known and the SD of the underlying lognormal distribution can be derived from these data. The calculated 4-hour LC_{01} for rats is then 41,000 ppm.

A total UF of 3 is considered sufficient for toxicokinetic and toxicodynamic differences between individuals and interspecies differences. A higher UF would lead to unrealistically low values for AEGL-3 in comparison with the experiment by Carpenter *et al*. (1944) who reported that two humans showed no clear signs of toxicity during exposure to 8000 ppm for a total of 8 hours. Using a higher factor would also result in AEGL-3 values that would be very close to the corresponding AEGL-2 values. The *in vitro* data obtained with human tissue samples show that overall the bioformation rate in human liver is rather comparable to that in rats. Because of this and since humans have an approximately four times lower ventilation rate than rats, a higher factor is not warranted.

The value of (41,000/3=) 13,700 ppm for 4 hours was extrapolated across time periods using $C^n xt = k$ with default values n=1 for extrapolation to longer time periods and n=3 for extrapolation to shorter time periods. The relationship between concentration and duration of exposure as related to lethality was examined by Ten Berge et al. (1986) for approximately 20 irritant or systemically-acting vapors and gases. The authors subjected the individual animal data sets to probit analysis with exposure duration and exposure concentration as independent variables. An exponential function ($C^n xt = k$), where the value of n ranged from 0.8 to 3.5 for different chemicals was found to be an accurate quantitative descriptor for the chemicals evaluated. Approximately 90 percent of the values of n range between n=1 and n=3. Consequently, these values were selected as the reasonable lower and upper bounds of n to use when data are not available to derive a value of n. Because the point of departure for time extrapolation is 4 hours, the AEGL-3 10-minute value is the same as the AEGL-3 30-minute value. The AEGL-3 values are presented in Table 7. These values are supported by the human data provided by Carpenter *et al*. (1944). They reported no effects in two humans exposed to 8000 ppm for 8 hours.

TABLE 7. AEGL-3 Values for 1,3-butadiene				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
See below*	See below*	See below*	See below*	6800 ppm¶ (15,000 mg/m^3)

* The calculated AEGL-3 values for 10-min, 30-min, and 1-hour are higher than the lower explosive limit of butadiene in air (LEL = 2 % (20,000 ppm)). The calculated AEGL-3 value for 4-hours is higher than 50% of the lower explosive limit of butadiene in air. Therefore, extreme safety considerations against hazard of explosion must be taken into account.
The respective calculated AEGL-3 values for 10-min, 30-min, 1-hour, and 4-hours are: 27,000 ppm (60,000 mg/m^3), 27,000 ppm (60,000 mg/m^3), 22,000 ppm (49,000 mg/m^3), and 14,000 ppm (31,000 mg/m^3).

¶ The proposed value for the 8-hour exposure period is higher than 10% of the lower explosive limit of butadiene in air (LEL = 2 % (20,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

8. SUMMARY OF AEGLS

8.1. AEGL values and toxicity endpoints

TABLE 8. Summary of AEGL Values§					
Classification	**Exposure Duration**				
	10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
AEGL-1 (Nondisabling)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)
AEGL-2 (Disabling)	6700 ppm¶ (15,000 mg/m3)	6700 ppm¶ (15,000 mg/m3)	5300 ppm¶ (12,000 mg/m3)	3400 ppm¶ (7500 mg/m3)	2700 ppm¶ (6000 mg/m3)
AEGL-3 (Lethal)	See below*	See below*	See below*	See below*	6800 ppm¶ (15,000 mg/m3)

§ It is noted that the derivation of the respective AEGL-values excludes potential mutagenic or carcinogenic effects after single exposure, which may occur at lower concentrations (see Appendix C).

* The calculated AEGL-3 values for 10-min, 30-min, and 1-hour are higher than the lower explosive limit of butadiene in air (LEL = 2 % (20,000 ppm)). The calculated AEGL-3 value for 4-hours is higher than 50% of the lower explosive limit of butadiene in air. Therefore, extreme safety considerations against hazard of explosion must be taken into account.
The respective calculated AEGL-3 values for 10-min, 30-min, 1-hour, and 4-hours are: 27,000 ppm (60,000 mg/m^3), 27,000 ppm (60,000 mg/m^3), 22,000 ppm (49,000 mg/m^3), and 14,000 ppm (31,000 mg/m^3).

¶ The proposed value is higher than 10% of the lower explosive limit of butadiene in air (LEL = 2 % (20,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

8.2. Comparison with other standards and guidelines

Because most standards focus on the carcinogenic properties of 1,3-butadiene they cannot be directly compared to AEGL-values. The IDLH of 2000 ppm is set at 10% LEL (LEL=2% (20,000 ppm)).

The ERPG-1 for 1,3-butadiene is based on its odor, the odor is detectable at this level but is considered an aromatic odor and not objectionable until higher concentrations are reached. Odor is not an AEGL-1 endpoint. The ERPG-2 is set at 200 ppm and is 24 times lower than the 1-h AEGL-2. The ERPG-2 is based on in fetotoxicity data in mice and rats and on comparative metabolism between species. It was concluded that as to metabolism humans were closer to rats than to mice. The ERPG-2 value of

200 ppm was based on the conclusion that fetotoxicity occurred in rats at 1000 ppm but not at 200 ppm. For the derivation of AEGLs these fetal effects were concluded to be related to maternal growth inhibition and are probably caused by repeated exposure and unlikely to occur from a single exposure at the same dose. The ERPG-3 of 5000 ppm is about a factor 4 lower than the 1-h AEGL-3 value (which is above the LEL). It was concluded that the acute toxicity of 1,3-butadiene is of low order. At levels higher than 5000 ppm CNS depression observed in animals studies would be expected in humans.

TABLE 9. Extant Standards and Guidelines for 1,3-butadiene

Guideline	Exposure Duration				
	10 minute	**30 minute**	**1 hour**	**4 hour**	**8 hour**
AEGL-1	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)
AEGL-2	6700 ppm¶ (15,000 mg/m3)	6700 ppm¶ (15,000 mg/m3)	5300 ppm¶ (12,000 mg/m3)	3400 ppm¶ (7500 mg/m3)	2700 ppm¶ (6000 mg/m3)
AEGL-3	See below*	See below*	See below*	See below*	6800 ppm¶ (15,000 mg/m3)
ERPG-1 (AIHA)[a]			10 ppm		
ERPG-2 (AIHA)			200 ppm		
ERPG-3 (AIHA)			5000 ppm		
EEGL (NRC)[b]					
PEL-TWA (OSHA)[c]					1 ppm
PEL-STEL (OSHA)[d]	5 ppm				
IDLH (NIOSH)[e]			2000 ppm		
REL-TWA (NIOSH)[f]					1 ppm
REL-STEL (NIOSH)[g]					
TLV-TWA (ACGIH)[h]					2 ppm (A-2 carcinogen)
TLV-STEL (ACGIH)[i]					
MAK (Germany)[j]					
MAK Peak Limit (Germany)[k]					21 ppm
MAC (The Netherlands)[l]					

It is noted that the derivation of the respective AEGL-values excludes potential mutagenic or carcinogenic effects after single exposure, which may occur at lower concentrations (see Appendix C).

* The calculated AEGL-3 values for 10-min, 30-min, and 1-hour are higher than the lower explosive limit of butadiene in air (LEL = 2 % (20,000 ppm)). The calculated AEGL-3 value for 4-hours is higher than 50% of the lower explosive limit of butadiene in air. Therefore, extreme safety considerations against hazard of explosion must be taken into account. The respective calculated AEGL-3 values for 10-min, 30-min, 1-hour, and 4-hours are: 27,000 ppm (60,000 mg/m^3), 27,000 ppm (60,000 mg/m^3), 22,000 ppm (49,000 mg/m^3), and 14,000 ppm (31,000 mg/m^3).

¶ The proposed value is higher than 10% of the lower explosive limit of butadiene in air (LEL = 2 % (20,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

[a]**ERPG (Emergency Response Planning Guidelines, American Industrial Hygiene Association** (AIHA 1994)

The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor.

The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual=s ability to take protection action.

The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing life-threatening health effects.

[b]**EEGL (Emergency Exposure Guidance Levels, National Research Council** (NRC 1985)

The EEGL is the concentration of contaminants that can cause discomfort or other evidence of irritation or intoxication in or around the workplace, but avoids death, other severe acute effects and long-term or chronic injury.

[c]**OSHA PEL-TWA (Occupational Safety and Health Administration, Permissible Exposure Limits - Time Weighted Average)** (OSHA 1996) is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[d]**OSHA PEL-STEL (Permissible Exposure Limits - Short Term Exposure Limit)** (OSHA 1996) is defined analogous to the ACGIH-TLV-STEL.

[e]**IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)** (NIOSH 1994) represents the maximum concentration from which one could escape within 30 minutes without any escape-impairing symptoms, or any irreversible health effects.

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

[g]**NIOSH REL-STEL (Recommended Exposure Limits - Short Term Exposure Limit)** (NIOSH 1992) is defined analogous to the ACGIH TLV-STEL.

[h]**ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average)** (ACGIH 1994) is the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[i]**ACGIH TLV-STEL (Threshold Limit Value - Short Term Exposure Limit)** (ACGIH 1994) is defined as a 15-minute TWA exposure which should not be exceeded at any time during the workday even if the 8-hour TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 minutes and should not occur more than 4 times per day. There should be at least 60 minutes between successive exposures in this range.

[j]**MAK (Maximale Arbeitsplatzkonzentration [Maximum Workplace Concentration])** (Deutsche Forschungsgemeinschaft [German Research Association] 2000) is defined analogous to the ACGIH-TLV-TWA.

[k]**MAK Spitzenbegrenzung (Peak Limit [give category])** (German Research Association 2000) constitutes the maximum average concentration to which workers can be exposed for a period up to 30 minutes with no more than 2 exposure periods per work shift; total exposure may not exceed 8-hour MAK.

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

8.3. Data quality and research needs

The quality of the database for 1,3-butadiene is very poor with exception of toxicokinetic/mechanistic studies. Most studies on 1,3-butadiene focus the carcinogenic properties of 1,3-butadiene and are therefore not relevant for AEGL derivation. The derivation of AEGL-1 is based on very limited data, adequate human data focused on AEGL-1 endpoints are needed. AEGL-2 is based on no effects at the highest exposure concentration in a rat study and may therefore be set rather conservative. The AEGL-3 is based on a sufficiently performed and reported animal study. The available human data is limited, the key study for AEGL-1 dates from 1944 and is only poorly reported. No adequate human data are available for AEGL-2 and -3.

The database on 1,3-butadiene for the derivation of AEGLs is rather limited, however 1,3-butadiene is of low acute toxicity and the explosion properties of 1,3-butadiene may pose a greater danger than its toxicity.

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APPENDIX A: Derivation of AEGL Values

Derivation of AEGL-1

Key study:	Carpenter *et al*. 1944
Toxicity Endpoint:	Difficulty in focusing in humans during a 7-h exposure to 2000 ppm
Time scaling:	Flatling from 10-min to 8-h (local eye effects: considered to be concentration-related)
Uncertainty factors:	3 (intraspecies)
Calculations:	
10-minute AEGL-1	2000/3 ≈ 670 ppm (=1500 mg/m^3)
30-minute AEGL-1	2000/3 ≈ 670 ppm (=1500 mg/m^3)
1-hour AEGL-1	2000/3 ≈ 670 ppm (=1500 mg/m^3)
4-hour AEGL-1	2000/3 ≈ 670 ppm (=1500 mg/m^3)
8-hour AEGL-1	2000/3 ≈ 670 ppm (=1500 mg/m^3)

Derivation of AEGL-2

Key study:	Carpenter *et al*. 1944
Toxicity Endpoint:	No AEGL-2 effects following a single 8-h exposure to 8000 ppm
Time scaling:	Default value of n=3 is used to extrapolate to shorter time periods. $C^3*t = k$ for extrapolation to 30 min, 1-, 4-, and 8-hour exposure, flatlining from 30-min to 10-min exposure. $k = (8000 \text{ ppm})^3*480 \text{ min} = 245.76 * 10^{12} \text{ ppm}^3 \text{ min}$
Uncertainty factors:	3 (intraspecies)
Calculations:	
10-minute AEGL-3	10-min AEGL-2 = 6700 ppm (= 15,000 mg/m^3) (set equal to 30-min AEGL-2)
30-minute AEGL-3	$C^3*30 \text{ min} = 245.76 * 10^{12} \text{ ppm}^3 \text{ min}$ C= 20,159 ppm 30-min AEGL-2 = 20,159 / 3 ≈ 6700 ppm (= 15,000 mg/m^3)
1-hour AEGL-2	$C^3*60 \text{ min} = 245.76 * 10^{12} \text{ ppm}^3 \text{ min}$ C= 16,000 ppm 1-hour AEGL-2 = 16,000 / 3 ≈ 5300 ppm (= 12,000 mg/m^3)
4-hour AEGL-2	$C^3*240 \text{ min} = 245.76 * 10^{12} \text{ ppm}^3 \text{ min}$ C= 10,079 ppm 4-hour AEGL-2 = 10,079 / 3 ≈ 3400 ppm (= 7500 mg/m^3)
8-hour AEGL-2	8-hour AEGL-2 = 8000 (point of departure) / 3 ≈ 2700 ppm (= 6000 mg/m^3)

All proposed values are higher than 10% of the lower explosive limit of 1,3-butadiene in air (LEL = 2 % (20,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

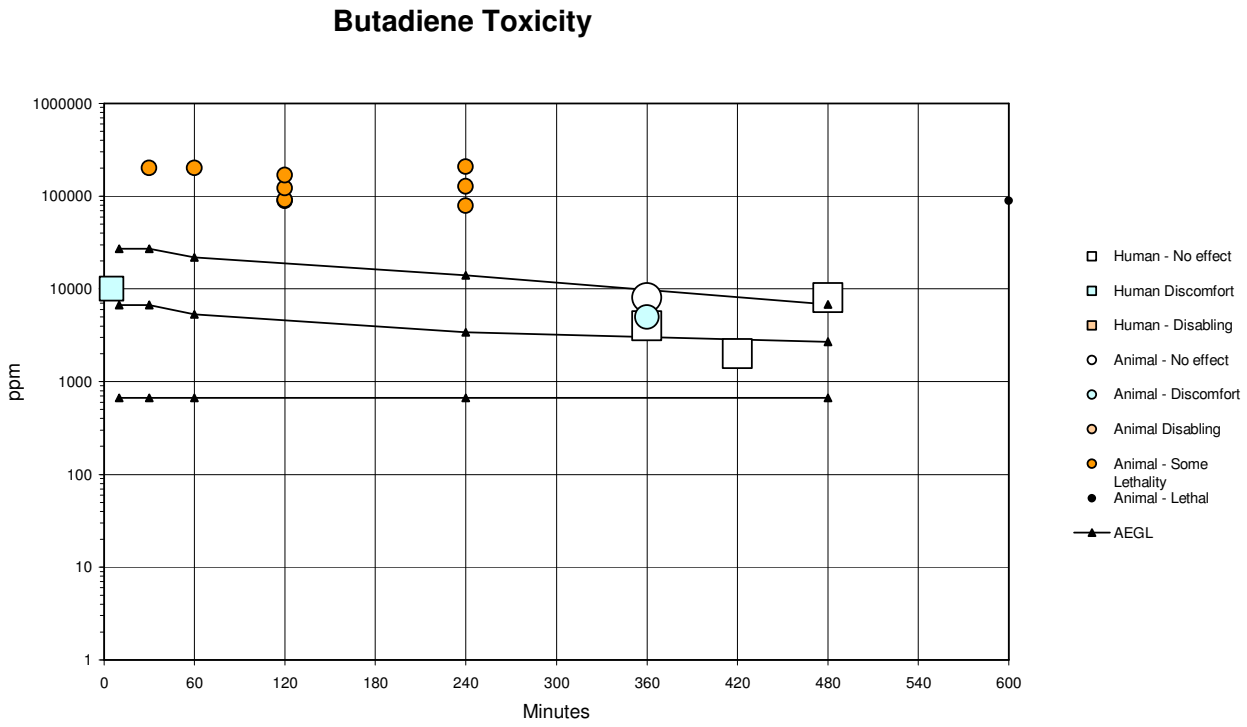
Derivation of AEGL-3

Key study:	Shugaev 1969
Toxicity Endpoint:	Mortality in rats exposed for 4 hours. The calculated LC_{01} is 41,000 ppm.
Time scaling:	Default values of n=1 for extrapolation to 8-hour exposure and of n=3 is for extrapolation to shorter time periods. $C^n*t = k$ for extrapolation to 30 min, 1-, 4-, and 8-hour exposure, flatlining from 30-min to 10-min exposure. $k = (41{,}000 \text{ ppm})^n * 240 \text{ min}$
Uncertainty factors:	3 (total factor accounting for intraspecies and interspecies extrapolation)
Calculations:	
10-minute AEGL-3	10-min AEGL-3 = 27,000 ppm (= 60,000 mg/m^3) (set equal to 30-min AEGL-3)
30-minute AEGL-3	$C^3*30 \text{ min} = 16.541* 10^{15} \text{ ppm}^3 \text{ min}$ C= 82,000 ppm 30-min AEGL-3 = 82,000 / 3 ≈ 27,000 ppm (= 60,000 mg/m^3)
1-hour AEGL-3	$C^3*60 \text{ min} = 16.541* 10^{15} \text{ ppm}^3 \text{ min}$ C= 65,083 ppm 30-min AEGL-3 = 65,083 / 3 ≈ 22,000 ppm (= 49,000 mg/m^3)
4-hour AEGL-3	4-hour AEGL-3 = 41,000 (point of departure) / 3 ≈ 14,000 ppm (= 31,000 mg/m^3)
8-hour AEGL-3	$C^1*480 \text{ min} = 9.84 * 10^6 \text{ ppm min}$ C= 20,500 ppm 480-min AEGL-3 = 20,500 / 3 ≈ 6800 ppm (= 15,000 mg/m^3)

The calculated AEGL-3 values for 10-min, 30-min, and 1-hour are higher than the lower explosive limit of 1,3-butadiene in air (LEL = 2 % (20,000 ppm)). The calculated AEGL-3 value for 4-hours is higher than 50% of the lower explosive limit of 1,3-butadiene in air. Therefore, extreme safety considerations against hazard of explosion must be taken into account.

The calculated AEGL-3 value for 8-hours is higher than 10% of the lower explosive limit of 1,3-butadiene in air (LEL = 2 % (20,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

APPENDIX B: Category Plot


Butadiene Toxicity
ppm
1000000
100000
10000
1000
100
10
1
0
60
120
180
240
300
360
420
480
540
600
Minutes
Human - No effect
Human Discomfort
Human - Disabling
Animal - No effect
Animal - Discomfort
Animal Disabling
Animal - Some Lethality
Animal - Lethal
AEGL

1

2

APPENDIX C: Carcinogenicity Assessment

EPA concluded that 1,3-butadiene is carcinogenic to mice and rats, inducing tumors at multiple organ sites. Since all tested exposure concentrations induced tumors it was considered likely that concentration below 6.25 ppm would also induce tumors in mice. Based on the same studies, IARC (1999) concluded that there was sufficient evidence in experimental animals for the carcinogenicity of 1,3-butadiene. EC (2002) noted that there appeared to be a marked species difference in the susceptibility of rodents to the carcinogenic properties of 1,3-butadiene. The evidence in mice showed that 1,3-butadiene is a potent, multi-organ carcinogen, with tumor development occurring at relatively low exposure concentrations. All the evidence in mice was concluded to indicate that a genotoxic mechanism is involved. In contrast, the available rat study showed a lower tumor frequency, fewer tumor types, with effects seen at exposure concentrations 2-3 orders of magnitude higher than in the mouse. EC stated that the tumor type in rats suggested that hormonal influences may play a role in the carcinogenic response, and that thus a non-genotoxic mechanism may underlie the tumor formation in this species.

Based on the epidemiological data the EC (2002) concluded that 1,3-butadiene should be regarded as carcinogenic to humans. IARC (1999) concluded that there is limited evidence for the carcinogenicity of 1,3-butadiene. Applying a set of criteria that define a causal relationship between exposure and health outcome EPA (2002a) concluded that human evidence for carcinogenicity of 1,3-butadiene is sufficient.

To estimate the cancer incidence EPA used a linear rate model, as developed by Health Canada ($RR = 1 + 0.0099X$, where X represents cumulative 1,3-butadiene exposure in ppm-years), and age-specific leukemia incidence rates for 1994-1998 from SEER (Surveillance, Epidemiology and End Results program of the National Cancer Institute) (EPA 2002b). An LEC_{01} (i.e., the 95% lower confidence limit of the exposure concentration associated with a 1% increased risk) of 0.254 ppm was calculated. Using this LEC_{01} as point of departure and extrapolating linearly to 0 increased risk at 0 exposure, a unit risk estimate of 0.04/ppm was obtained for leukemia incidence. However, rat and mouse experiments showed that females are more sensitive to 1,3-butadiene-induced carcinogenicity than males, with mammary gland tumors as the only tumor site common to both species. Therefore, an adjustment factor of 2 was applied to cover the combined risks for leukemia and mammary cancer and also to provide additional protection to account for the fact that small increases in risk at other sites, particularly the lung, cannot be ruled out. This resulted in a risk estimate of 0.08/ppm (EPA 2002a):

To convert to a level of 1,3-butadiene that would cause a theoretical excess cancer risk of 10^{-4}:

Risk of 1×10^{-4}: $10^{-4} / 0.08\ (\text{ppm})^{-1} = 1.25 \times 10^{-3}$ ppm (round to 1.3×10^{-3} ppm)

To convert a 70 year exposure to a 24 h exposure:

24-hour exposure = C * 25,600 days = 33.3 ppm

To account for uncertainty regarding the variability in the stage of the cancer process at which methylene chloride or its metabolites may act, a multistage factor of 6 is applied (NRC, 2001):

33.3 ppm * 1/6 = 5.5 ppm

Therefore, based upon the potential carcinogenicity of 1,3-butadiene when continuous lifetime exposure takes place, an acceptable 24 h exposure would be 5.5 ppm.

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

24-hour exposure = 5.5 ppm (12 mg/m^3)
8-hour exposure = 17 ppm (36 mg/m^3)
4-hour exposure = 33 ppm (72 mg/m^3)
1-hour exposure = 130 ppm (287 mg/m^3)

30-minute exposure = 260 ppm (575 mg/m^3)
10-minute exposure = 790 ppm (1746 mg/m^3)

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

It is however noted that Bucher *et al.* (1993) did not find any evidence for an increased incidence of neoplastic lesions in mice exposed to 1,3-butadiene for a single 2-hour period. Groups of 60 male and 60 female mice, 8-10-weeks old, were exposed for a single 2-hour period to target 1,3-butadiene concentrations of 0, 1000, 5000, or 10,000 ppm (0, 2200, 11,000, or 22,000 mg/m^3, respectively). The animals were held for two years, at which time all survivors were killed and tissues and organs were examined microscopically. Survival, body weight gain, and the incidence of neoplastic and nonneoplastic lesions were not affected by 1,3-butadiene exposure.

1

APPENDIX D: Derivation Summary for 1,3-butadiene AEGLs

ACUTE EXPOSURE GUIDELINE LEVELS FOR 1,3-BUTADIENE (CAS Reg. No. 106-99-0) DERIVATION SUMMARY

AEGL-1 VALUES

10-minute	30-minute	1-hour	4-hour	8-hour
670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)	670 ppm (1500 mg/m3)

Key Reference: Carpenter *et al*. 1944

Test Species/Strain/Number: humans (n=2)

Exposure Route/Concentrations/Durations: Inhalation exposure for 6-8 hours to 2000, 4000, and 8000 ppm

Effects:
2000 ppm (7 hours): slight smarting of the eyes, difficulty in focusing
4000 ppm (6 hours): slight smarting of the eyes, difficulty in focusing
8000 ppm (8 hours): no subjective symptoms

Endpoint/Concentration/Rationale: Humans exposed to 2000 ppm for 7 hours reported slight eye effects.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1
Intraspecies: 3

Modifying Factor: not applicable

Animal to Human Dosimetric Adjustment: not applicable

Time Scaling:

Flatlining from 10-min to 8-hour of exposure because the effects are considered to be concentration-related.

Data Adequacy:

The database is very poor. 1,3-Butadiene has no high acute toxicity and it is expected that the AEGL-1 values are rather low.

AEGL-2 VALUES				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
6700 ppm¶[(15,000 mg/m3)	6700 ppm¶[(15,000 mg/m3)	5300 ppm¶[(12,000 mg/m3)	3400 ppm¶[(7500 mg/m3)	2700 ppm¶[(6000 mg/m3)

Key Reference: Carpenter *et al*. 1944

Test Species/Strain/Number: humans (n=2)

Exposure Route/Concentrations/Durations: Inhalation exposure for 6-8 hours to 2000, 4000, and 8000 ppm

Effects:
2000 ppm (7 hours): slight smarting of the eyes, difficulty in focusing
4000 ppm (6 hours): slight smarting of the eyes, difficulty in focusing
8000 ppm (8 hours): no subjective symptoms

Endpoint/Concentration/Rationale:

Humans exposed to 8000 ppm for 8 hours reported no effects defined by AEGL-2.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1
Intraspecies: 3

A factor of 3 is considered sufficient because the point of departure is conservative (no effects at the highest concentration tested).

Modifying Factor: not applicable

Animal to Human Dosimetric Adjustment: not applicable

Time Scaling:

Default value of n=3 is used to extrapolate to shorter time periods. The 10-min value is set equal to the 30-min value because the point of departure is an 8-hour exposure.

Data Adequacy:

The database is very poor. 1,3-Butadiene has no high acute toxicity and it is expected that the AEGL-2 values are rather low.

All proposed values are higher than or equal to 10% of the lower explosive limit of 1,3-butadiene in air (LEL = 2 % (20,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

AEGL-3 VALUES				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
See below*	See below*	See below*	See below*	6800 ppm¶ (15,000 mg/m3)
Key Reference: Shugaev (1969)				
Test Species/Strain/Number: rats (unspecified sex and strain, numbers per group unspecified)				
Exposure Route/Concentrations/Durations: Rats were exposed by inhalation for 4 hours.				
Effects (calculated by probit analyses): 4-hour LC_{16}: 79,000 ppm 4-hour LC_{50}: 128,000 ppm 4-hour LC_{84}: 207,000 ppm				
Endpoint/Concentration/Rationale: Calculated LC_{01}: 41,000 ppm				
Uncertainty Factors/Rationale: Total uncertainty factor: 3 (combined factor for inter- and intraspecies extrapolation) A total factor of 3 was considered sufficient because a higher factor would lead to AEGL-3 values that would conflict with the human data obtained from Carpenter *et al.* (1944) (no effects following an 8-hour exposure to 8000 ppm).				
Modifying Factor: not applicable				
Animal to Human Dosimetric Adjustment: not applicable				
Time Scaling: Default value of n=3 is used to extrapolate to shorter time periods; the default value of n=1 is used to extrapolate to 8 hours. The 10-min value is set equal to the 30-min value because the point of departure is a 4-hour exposure. Data Adequacy: Sufficient.				

* The calculated AEGL-3 values for 10-min, 30-min, and 1-hour are higher than the lower explosive limit of butadiene in air (LEL = 2 % (20,000 ppm)). The calculated AEGL-3 value for 4-hours is higher than 50% of the lower explosive limit of butadiene in air. Therefore, extreme safety considerations against hazard of explosion must be taken into account. The respective calculated AEGL-3 values for 10-min, 30-min, 1-hour, and 4-hours are: 27,000 ppm (60,000 mg/m^3), 27,000 ppm (60,000 mg/m^3), 22,000 ppm (49,000 mg/m^3), and 14,000 ppm (31,000 mg/m^3).

¶ The proposed value for the 8-hour exposure period is higher than 10% of the lower explosive limit of 1,3-butadiene in air (LEL = 2% (20,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

APPENDIX E: Derivation of level of distinct odor awareness

For 1,3-butadiene Nagata (2002) reports an odor threshold of 0.23 ppm (0.51 mg/m^3). This value was obtained using the Japanese Triangle Method which has been shown to produce results that agree very well with the standard method CEN13725. The same Japanese source reports an odor threshold of 0.038 ppm for n-butanol. The latter value is very close to the European Reference Odor Mass for n-butanol of 0.040 ppm.

The value reported by Nagata (2002) represents a Level 1 odor threshold as defined in the AEGL document "Guidance for the Use of Odor in the Derivation of AEGL-1".

The standardized odor threshold for acetaldehyde ($C_{0, stand}$) is equal to:

0.23 * 0.040/0.038 = 0.24 ppm

For 1,3-butadiene a Fechner-Weber coefficient for odor intensity (K_w) is not established. The default value of 11.8 is used to derive a distinct odor level. The default adjustment for distraction and peak-to-mean-ratio is 4/3.

The Level of Distinct Odor Awareness (LOA) for 1,3-butadiene can now be calculated according to Ruijten (2004):

LOA = 0.24 ppm * 11.8 * 4/3 = 3.8 ppm.

References

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