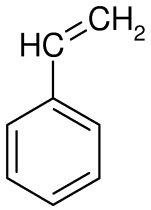
Interim: 02/2008

Styrene
(CAS Reg. No. 100-42-5)



INTERIM ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

PREFACE

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

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

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

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

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

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

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EXECUTIVE SUMMARY

Styrene is a colorless or slightly yellow, viscous liquid, soluble in ethanol, benzene and petroluem ether and slightly soluble in water. Owing to its volatility, low flash point, and the range of explosive limits in air (lower: 1.1 %, upper: 6.3 % v/v), styrene poses an acute fire and explosion hazard. Due to its tendency to polymerize at room temperature in the presence of oxygen and to oxidize on exposure to light and air, styrene is normally stabilized by the addition of < 0.006 - 0.01% w/w tertiary butylcatechol (4-tert-butylbenzene-1,2-diol) as an inhibitor. Styrene is one of the most important monomers in industry worldwide. It is predominantly used for the production of polymers (polystyrene and copolymers of styrene with acrylonitrile and/or butadiene). Worldwide production reached 17,945 thousand tonnes in 1998.

In humans, the observed effects associated with acute exposure to styrene are irritation of eyes and mucous membranes and central nervous system (CNS) depression. Limited data in humans provide no evidence that (occupational) styrene exposure causes lesions of the nasal epithelia or decrements in olfactory function (Dalton et al. 2003; Ödkvist et al. 1985). No data were available indicating reproductive or developmental effects of styrene in humans following acute exposure. Epidemiological studies revealed no sound evidence for an association between repeated occupational exposure to styrene and reproductive or developmental effects. Genotoxicity was observed in human cells *in vitro; in vivo,* no data were available with respect to genotoxicity following acute exposure of humans. In epidemiological studies, evidence for an association of occupational exposure to styrene and genotoxic effects were observed. According to the evaluation of the IARC (2002), the results reported for chromosomal aberrations, micronuclei and sister chromatid exchange in approximately 30 studies of workers exposed to styrene in various industries have been inconsistent. Induction of chromosomal aberrations was reported in 12 of 25 studies, sister chromatid exchange in 6 of 16 and micronuclei in 3 of 14 studies. With respect to carcinogenicity in humans, in its latest evaluation IARC (2002) concluded that there is *"limited evidence* in humans for the carcinogenicity of styrene*"* and, taking into account the results from animal carcinogenicity studies, that styrene is *"possibly carcinogenic to humans (Group 2B)".* Styrene has been assessed under the IRIS Program of the US-EPA, no quantitative carcinogenicity assessment for lifetime exposure is currently proposed.

Animal studies were mostly carried out with rats and mice, limited data are available for guinea pigs, hamsters and an unspecified species of monkeys. As in humans, irritation and CNS effects are also observed in animals following acute inhalation exposure. In mice, RD_{50} values for sensory irritation of 156 ppm - 980 ppm were reported. Signs indicating irritation were also reported in toxicity studies with rats at concentrations as low as 200 ppm, immediate irritation in rats was noted at 1300 ppm. CNS-depression in rats and mice was observed at higher concentrations. Rats lost consciousness at 2000 ppm after 5 hours of exposure and showed reduced attention at 6-hour exposures to 1500 ppm. In mice, signs of CNS depression during a 4-hour exposure were staggered gait at 1420 ppm and apathy and finally narcosis at higher concentrations of 2983 and 3766 ppm. In rats, death was observed when animals were exposed for 4 hours to 4814 ppm and higher concentrations (BASF 1979b). Death was mostly rapid due to CNS depression but some delayed deaths with signs of pulmonary lesions were observed in rats at concentrations also causing severe CNS effects. Mice were much more sensitive than rats (and, based on a limited number of data, guinea pigs and monkeys). Death of mice was observed following a single 6-hour exposure to 250 ppm or 500 ppm. Also, at these concentrations, respiratory toxicity with lesions of the nasal epithelia and of the bronchioles were observed in mice but not in rats.

With respect to developmental or reproductive effects, no embryo-/fetotoxicity or malformations were observed in rats after a single oral treatment on the 11th or 17th day of gestation, respectively. In mice, decreased postnatal survival was observed after signe oral administration of a maternally toxic dose on day 17 of gestation, while no effect was noted at a lower dose. Following

repeated exposure of rats through gestation day 6 – 20 to 300 ppm, an increased neonatal death rate and delayed postnatal development was observed compared to pair-fed controls. Fetotoxicity was also seen in hamsters exposed to 1000 ppm 6 hours/day from gestation day 6 -18, but not at 750 ppm. In other studies with repeated oral or inhalation exposure of rats, mice, and rabbits, no significant developmental effects were observed. Styrene is genotoxic *in vitro,* provided there is sufficient activation to styrene oxide (SO), and *in vivo.* Data from laboratory animals indicate that styrene exposure may lead to the formation of DNA-adducts, sister chromatid exchange, and chromosomal aberrations (1 of 7 studies). With respect to carcinogenicity, no clear effect was observed in rats. In mice, an increase of lung tumors was observed.

Styrene has a pungent, slightly sweetish odor. The derivation of the level of distinct odor awareness (LOA) was based on results from human studies presented in the report of Van Doorn et al. (2002) and follows the guidance as described in the same report. The LOA represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, while about 10 % of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odor perception. For styrene, the calculated level of distinct odor awareness (LOA) is 0.54 ppm.

The AEGL-1 derivation is based on irritating effects of styrene in humans. In a study on psychological reactions related to chemosensory irritation, ratings for odor and annoyance increased similarily with increasing styrene concentrations ranging from 0.5 – 40 ppm, while there was only a marginal increase for irritation. Effects sizes comparing the ratings between exposure to 20 ppm and pre-exposure were higher for odor, irritation, and annoyance. Effects sizes were also higher compared to "clean air only"-exposure. However, the ratings for irritation indicated only marginal effects in this respect (Seeber et al. 2002). No increase in irritation or headaches compared to control was noted at 20 ppm in a further study (Hake et al. 1983). Subjective signs and symptoms of irritation and CNS effects were not negatively influenced during a 6-hour exposure at 25 ppm or 50 ppm or at 50 ppm with 4 peak exposures of 15 minutes at 100 ppm (Ska et al. 2003). At 50 ppm, a further study indicated a slight increase in subjective symptoms ratings for eye and nose irritation, headache, and fatigue (Oltramare et al. 1974). At 100 ppm, Oltramare et al. (1974) further reported that signs of irritation and of mild subjective CNS effects (headaches, fatigue, poor concentration, sleepiness) were felt more often than at 50 ppm. Complaints of mild eye and throat irritation at 99 ppm in one test but not in another at 116 ppm were reported by Stewart et al. (1968). Complaints of eye and nose irritation were frequent at about 200 ppm (Oltramare et al. 1974; Stewart et al. 1968).

A concentration of 20 ppm (Seeber et al. 2003) was selected to derive AEGL-1. Because this concentration represents a NOAEL for local (as well as CNS) effects and in other studies effects at 50 ppm and 100 ppm were only weak or absent, an intraspecies factor of 1 is applied. The value of 20 ppm was used for all timepoints since slight irritation and subjective discomfort that were reported at higher concentrations did not increase within several hours of exposure.

The derivation of AEGL-2 is based on human studies. Irritation and CNS effects have to be considered for the derivation of AEGL-2. Nasal and mild eye irritation were reported by volunteers exposed to 376 ppm (Stewart et al. 1968). In their study of styrene exposed workers, Götell et al. (1972) reported that they themselves suffered from immediate lacrymation and irritation of the nasopharynx when exposed to 300 – 400 ppm, and concentrations of 500 – 800 ppm caused irritation intolerable to the investigators within 1 or 2 minutes. Strong eye and nasal irritation was also reported by volunteers exposed to concentrations ≥ 600 ppm (Carpenter et al. 1944; Wolf et al. 1956).

With respect to effects on the CNS, a 6-hour exposure at 50 ppm with 4 repeated 15-minute peaks at 100 ppm had no negative influence on performance to neuropsychological tests (Ska et al. 2003). At 99 ppm, intermittent difficulties in performing a modified Romberg test were observed in 3/6 subjects

exposed for 7 hours with a 30-minute break in between. Other tests on coordination and on manual dexterity were normal, and no effects were noted at the end of exposure. No CNS effects were seen in another experiment with 116 ppm exposure for 2 hours or 216 ppm for 1 hour in the same study (Stewart et al. 1968). Headaches, but no effects on equilibrium and cognitive function tests were noted in male and female volunteers at repeated exposures to 100 and 125 ppm for at least one hour (Hake et al. 1983). Oltramare et al. (1974) noted slight difficulties in balance performance at 50 – 200 ppm (1.5 hours), but there was no concentration-response, and slight difficulties in balance performance at 200 ppm (1 hour), but the variation of data was large. No effects on simple and choice reaction time was seen following exposure to 250 ppm for 30 minutes. However, when the concentration was raised to 350 ppm for 30 minutes directly afterwards, both simple and choice reaction time were increased (Gamberale and Hultengren 1974). More pronounced effects were observed during exposure to 376 ppm for one hour: One subject complained of nausea that persisted one hour after the end of exposure, 2 subjects had a feeling of being inebriated, 3 of 5 subjects exposed were unable to normally perform a modified Romberg test, and also 3 subjects had significant decrements in other tests of coordination and manual dexterity (Stewart et al. 1968). In a toxicokinetic study, 2 subjects were exposed to 386 ppm styrene for 2 hours while performing light physical exercise of 50 W (Löf and Johanson 1993). In that study, no information was presented as to the presence or absence of subjective or objective signs of intoxication or irritation. However, it may reasonably be assumed that no severe CNS effects will have occurred in such a study. At higher concentrations, the irritation becomes very strong (see above), and only one controlled study was located that was conducted at this level (Carpenter et al. 1944). In this study, 2 subjects exposed to 800 ppm for 4 hours suffered from listlessness, drowsiness, impairment of balance, and, after cessation of exposure, muscular weakness and unsteadiness with inertia and depression. A “steadiness test” measuring manual dexterity indicated a marked decreased of performance compared to pre-exposure level. Besides CNS-depression, the subjects complained of eye and throat irritation.

The AEGL-2 is based on the CNS effects observed in humans following exposure to 376 ppm for 1 hour: nausea in one subject; feeling of being inebriated in two, and unability to normally perform the modified Romberg test and significant decrements in other tests of coordination and manual dexterity in three of five subjects (Stewart et al. 1968). The effects described address a level of CNS depression that seems still below a level for an impairment of the ability to escape and therefore a concentration of 376 ppm is considered a NOAEL. However, this level is close to concentrations causing intolerable irritation in humans that may limit the ability to escape and thus are above AEGL-2.

Generally, for volatile substances with CNS-depressant effects an intraspecies factor of 3 is applied to account for sensitive individuals because the effective concentration range does not differ more than 2-3-fold between individuals. In case of styrene, it must be taken into account that physical activity has a marked effect on the uptake of styrene and its level in blood. In the studies used to derive AEGL-2, the subjects were at rest. In controlled studies, the observed increase of styrene in arterial blood at exposure to about 150 ppm styrene was approximately 3-fold when the physical activity was increased from rest to light exercise (50 W), 5-fold at moderate exercise (100 W), and 10-fold at heavy exercise (150 W) (Astrand 1975). Therefore, it could be argued that an intraspecies uncertainty factor of 10 to account for sensitive subgroups would be necessary to protect individuals at heavy physical exercise. Application of a factor of 10 would lead to a 1-hour AEGL-2 of 38 ppm and similar values at longer time periods. On the other hand, the following two points which indicate that a factor of 3 is justified, are believed to outweigh the above rationale: Firstly, due to physiological limitations, heavy physical exercise (150 W) cannot be performed continuously for longer periods of time. Therefore, it is unrealistic to consider an exposure scenario with heavy exercise for one or several hours. In contrast, light exercise (50 W) may be performed over a longer period of time. In this case, the increase of the styrene concentration in blood will be about 3-fold which is within the range of an uncertainty factor of three. Secondly, an AEGL-2 value in the range of 38 ppm as mentioned above would be in conflict with styrene exposure data at occupational

workplaces. At workplaces, such concentrations are or were frequently observed (IARC 2002) without workers showing signs of CNS depression that would have limited their ability to escape.

Therefore, an intraspecies uncertainty factor of 3 is considered adequate to protect sensitive subgroups including groups exposed to styrene during longer periods of light exercise. This leads to a value of 130 ppm as AEGL-2 for 1 hour.

This experimentally derived exposure value was scaled to shorter periods of time using the equation $c^n \times t = k$ (Ten Berge et al. 1986). In accordance with NRC (2001), a default of n = 3 for shorter periods of time (30 minutes and 10 minutes) was applied, due to the lack of suitable experimental data for deriving the concentration exponent. The "n" value of 1.2 used for calculations of AEGL-3 (see below) was not used for AEGL-2 for following reasons: Firstly, the exponent was derived from lethality studies in which delayed mortality was observed that was not related to narcotic effects on the CNS (which are relevant for AEGL-2) but probably to pulmonary lesions observed at these very high concentrations (in addition to CNS effects which are the major cause of death). Secondly, toxicokinetics at high exposure concentrations over several hours of exposures (as in the lethality studies) is different from that at lower concentrations for shorter time periods.

Toxicokinetic studies with humans exposed to styrene concentrations at 70 – 200 ppm show that most of the increase of the styrene concentration in blood is seen during the first 30 minutes of exposure and that there is no or very little increase at 1 – 3 hours at these concentrations. Therefore, no additional extrapolation is necessary and the AEGL-2 of 130 ppm derived for 1 hour is applied to longer periods of time.

The AEGL-3 values are derived from a lethality study with rats (BASF 1979b). In rats, exposure to high concentrations of styrene leads to progressive CNS depression with narcosis and, finally, death. At concentrations leading to severe CNS effects, delayed deaths with pulmonary lesions were also described in these studies. In humans, the acute effects on the CNS are well described. However, no reports of lethal intoxications following styrene exposure were identified in the literature; therefore, it is not known if the pulmonary lesions observed in rats may also occur in humans exposed to life-threatening or potentially lethal concentrations of styrene.

For a conservative approach, data from studies with rats taking into account delayed deaths with pulmonary lesions were used to derive AEGL-3. From the data of the 4-hour exposure study of BASF (1979b), a benchmark calculation was performed with the lethality data using different models. A $BMDL_{05}$ for female rats of 3409 ppm (rounded to 3400 ppm) was used as a starting point to derive AEGL-3.

A total uncertainty factor of 10 was applied. This total factor may formally be split up into an interspecies factor of 3 and an intraspecies factor also of 3. For volatile solvents like styrene with a CNS-depressant effect, an interspecies uncertainty factor of 3 has been applied in the derivation of AEGL for several substances. This is based on the similarity of effects manifested in rodents compared to humans. In case of styrene, limited data indicate no gross differences in the concentration of styrene in blood between rats and humans. According to a toxicokinetic model, at concentrations exceeding 200 ppm styrene in air, the non-steady-state concentration of styrene in blood of humans (calculated for 6 hours of exposure) will always be lower than that in blood of rats (Ramsey and Andersen 1984). Styrene levels in human blood were in accordance with this model up to 376 ppm in air, however, no experimental human data are available for validation of the model at higher concentrations.

An intraspecies uncertainty factor of 3 was applied to account for sensitive individuals since the threshold for CNS impairment is not expected to vary much among individuals. As in case of the

derivation of AEGL-2, an intraspecies uncertainty factor of 3 is considered adequate to protect sensitive subgroups including groups exposed to styrene during longer periods of light exercise.

Extrapolation was made to the relevant AEGL time points of 30 minutes and 1 hour using the relationship $C^n \times t = k$ with a value of n = 1.2 which was derived from extrapolation of the LC_{50} in rats for 4- and 6 hours (BASF 1979b; Bonnet et al. 1982a). The 10-minutes AEGL-3 was assigned the same value as that for the 30-minutes AEGL-3 as it was considered inappropriate to extrapolate from an experimental period of 4 hours to 10 minutes. The 8-hour AEGL-3 was assigned the same value as that for the 4-hour AEGL-3 as toxicokinetic data indicate that there is at most little increase of internal dose after 4 hours of exposure; moreover, lower values which would be derived by default calculations are not supported by toxicological data for humans.

Individual cases of respiratory sensitization to styrene were described. Taking into account the wide use of styrene both in industry and in do-it-yourself products, sensitization seems to be an exceptionally rare event. Although the risk of sensitization following a single exposure at AEGL is considered negligible, individuals already sensitized to styrene may not be able to tolerate styrene concentrations that are without effect in non-sensitized individuals and may not be protected by the AEGL developed for styrene in this TSD.

SUMMARY TABLE OF AEGL VALUES FOR STYRENE [a]

Classification	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour	Endpoint (Reference)
AEGL-1 (Nondisabling)	20 ppm (85 mg/m³)	20 ppm (85 mg/m³)	20 ppm (85 mg/m³)	20 ppm (85 mg/m³)	20 ppm (85 mg/m³)	NOAEL for slight irritation (Seeber et al. 2002)
AEGL-2 (Disabling)	230 ppm (980 mg/m³)	160 ppm (680 mg/m³)	130 ppm (550 mg/m³)	130 ppm (550 mg/m³)	130 ppm (550 mg/m³)	CNS effects in humans (Gamberale and Hultengren 1974; Stewart et al. 1968)
AEGL-3 (Lethality)	1900 ppm * (8090 mg/m³)	1900 ppm * (8090 mg/m³)	1100* ppm (4690 mg/m³)	340 ppm (1450 mg/m³)	340 ppm (1450 mg/m³)	No lethality in rats (BASF 1979b)

a: Since liquid styrene is an eye irritant, eye contact must be avoided.
*: The lower explosive limit (LEL) of styrene in air is 1.1 % (11,000 ppm). The AEGL-3 value of 1900 ppm (8090 mg/m³) for 10 minutes and 30 minutes are higher than 1/10 of the LEL. Therefore, safety considerations against hazard of explosion must be taken into account.

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1 INTRODUCTION

Styrene is a colorless or slightly yellow, viscous liquid. It is slightly soluble in water, soluble in ethanol and very soluble in benzene and petroleum ether. Due to its tendency to polymerize at room temperature in the presence of oxygen and to oxidize on exposure to light and air, styrene is normally stabilized by the addition of < 0.006 - 0.01% w/w tertiary butylcatechol (4-tert-butylbenzene-1,2-diol) as an inhibitor (WHO 1983).

Pure styrene has a pungent, slightly sweetish odor. However, oxidation may lead to the formation of peroxides, certain aldehydes and ketones giving a sharp, penetrating, disagreeable odor. When emitted into the air, its half-time is estimated to be about 2 hours, and chemical transformation products include benzaldehyde and formaldehyde, both of which are odorous air pollutants (WHO 2000).

Styrene is one of the most important monomers in industry worldwide. The first step in its industrial production is the catalytic alkylation of benzene with ethylene leading to ethylbenzene. In the second step, ethylbenzene is dehydrogenated to styrene. In an alternative process, styrene is formed as a co-product in the synthesis of propylene oxide from ethylbenzene and propene via ethylbenzene hydro-peroxide and 1-phenylethanol (WHO 1983). Purified products typically are 99.7% to greater than 99.9% w/w styrene with less than 0.1 % ethylbenzene, cumene, phenylpropene, phenyl acetate and p-xylene.

Styrene is predominantly used for the production of polymers (polystyrene, copolymers of styrene with acrylonitrile and/or butadiene) that find wide application in latex paints and coatings, synthetic rubbers, polyesters and styrene-alkyd coatings. Styrene is a HPV (high production volume) chemical with a worldwide production of 17,945 thousand tonnes in 1998. Small amounts of styrene can be found in gum exudate from the damaged trunk of certain trees, probably being produced by decomposition of cinnamic acid derivatives that are present in such exudates in large quantities. Styrene also occurs in many agricultural products and foods, however, it is not clear whether styrene is naturally produced within plants (IARC 2002).

Owing to its volatility, low flash point, and the range of explosive limits in air (lower: 1.1 %, upper: 6.3 % v/v), styrene poses an acute fire and explosion hazard. Chemical and physical properties of styrene are presented in Table 1.

TABLE 1: CHEMICAL AND PHYSICAL PROPERTIES		
Parameter	**Data**	**Reference**
Synonyms	Vinylbenzene, phenylethene, ethenylbenzene, cinnamene	WHO 1983
Chemical formula	C_8H_8	
Molecular weight	104.14 g/mol	WHO 1983
CAS Reg. No.	100-42-5	ATSDR 1992
Physical state	Liquid at room temperature	Weast 1973
Solubility	300 mg/l in water (at 20 °C), soluble in alcohol, ether, acetone, miscible with benzene and petrol ether	Weast 1973; WHO 1983
Vapor pressure	3.1 hPa (at 10 °C), 6.67 hPa (at 20 °C), 8.67 hPa (at 25 °C), 13.3 hPa (at 35 °C)	ATSDR 1992; NIOSH 1983; WHO 1983
Vapor density (air = 1)	3.6	
Liquid density (g/cm³)	0.9060 (at 20 °C)	Weast 1973
Melting point	-30.63 °C	Weast 1973
Boiling point	145.2 °C (at 1013 hPa)	Weast 1973
Explosive limits in air	1.1 – 6.3 %	ATSDR 1992
Flash point (closed cup)	31 °C	ATSDR 1992
Autoignition temperature	490 °C	ATSDR 1992
Conversion factors (at 25 °C)	1 ppm = 4.26 mg/m³ 1 mg/m³ = 0.234 ppm	Calculated according to NRC 2001

2 HUMAN TOXICITY DATA

2.1 Acute Lethality

No reports of lethal intoxication following styrene exposure were located in the literature.

2.2 Nonlethal Toxicity

2.2.1 Case Reports

The investigators of a field study on styrene exposure of workers noted that they could not withstand styrene concentrations of 500 – 800 ppm for more than 1 – 2 minutes, whereas the workers exposed to this level complained of only minor to moderate irritation of eyes and nasopharynx. The authors further report that they themselves (five unadapted persons) suffered from lacrymation and irritation of the nasopharynx at about 300 – 400 ppm (Götell et al. 1972).

By degassing the tank of a ship on a river, styrene was blown into the surrounding air without sufficient dilution. 15 employees of a nearby power plant and 3 river police men who were exposed to an unknown concentration of styrene complained of immediate eye irritation and tickle in the throat, dizziness, headache and nausea (Hahn et al. 2000).

After using a polyester resin canoe building kit, a 36-year old man twice suffered from neurologic symptoms (MacFarlane et al. 1984). The work had been carried out in an unventilated shed for about 4 – 5 hours during which styrene evaporated from the construction kit. The man developed severe postural hypotension, neurological signs (slurred speech, nystagmus, limb ataxia) and conjunctivitis.

Moscato et al. (1987) described two cases of workers employed in plastics factories that had bronchial asthma or runny nose, dry irritating cough and chest tightness. They were exposed to styrene and ethyl benzene and one of them to polyester resin. However, specific inhalation challenges revealed an immediate bronchospastic response only after provoked inhalation exposure to styrene (15 ppm for 15 minutes). In both subjects, symptoms completely disappeared after changing their job. A further case of asthma in a subject occupationally exposed to styrene and showing a positive reaction to styrene in a provoked exposure test was reported by Hayes et al. (1991). A case of skin dermatitis following dermal exposure to styrene was reported by Sjöborg et al. (1982), skin patch tests revealed a strong reaction to styrene and a cross-reaction to vinyl toluene, but a weak one to benzoyl peroxide (used in hardeners for styrene-based plastics) and no reaction to styrene polymerization inhibitors and typical styrene impurities.

Non-inhalation exposure

Repare of a water tank led to contamination of tap water with styrene and subsequent oral and inhalation exposure (Arnedo-Pena et al. 2003). Residents of 27 apartments in two buildings using the contaminated water were contacted. A questionnaire on subjective symptoms was administered to 84 out of 93 persons living in affected apartments at the time of the accident. Styrene measured in samples of water collected two days after the accident reached concentrations up to 900 µg/L. Symptoms were reported by 46 persons, most frequently irritation of the throat (26%), nose (19%), eyes (18%) and skin (14%). General gastrointestinal symptoms were observed with 11% reporting abdominal pain and 7% diarrhea. The factors most strongly associated with symptoms were drinking tap water, exposure to vapors from the basement and eating foods prepared with tap water. All residents in the ground floor reported symptoms.

2.2.2 Occupational exposure

A great number of studies on workers with occupational exposure to styrene in different workplaces have been carried out. These studies have been repeatedly reviewed and summarized (ACGIH 1997; ATSDR 1992; Cohen et al. 2002; DFG 1987; Government Canada 1993; IARC 2002; OEHHA 1999; Sherrington and Routledge 2001; US EPA 1998; WHO 1983; WHO 2000). Workers are exposed to styrene in a number of industries, e.g. in the production of styrene and styrene polymers. In the fabrication of reinforced-polyester plastics composites, 8-hour average samples in breathing zones often exceed styrene concentrations of 100 ppm (IARC 2002). Here, the highest exposure concentrations were observed in chopper gun operators where 8-hour mean concentrations in personal breathing zone of 564 mg/m³ (range 307 – 938 mg/m³) (132 ppm; range 72 – 219 ppm) were measured (Truchon et al. 1992). In previous studies on workers in the manufacture of reinforced plastics, 8-hours TWA concentrations in the breathing zone of up to 292 ppm were reported, with peaks of about 1500 ppm during shorter periods of work for about 5 – 10 minutes (Götell et al. 1972).

In workers exposed to styrene, central and peripheral nervous systems effects have been observed. Especially, reversible decrease in color discrimination has been described in many studies. Decrements of auditory function (threshold for hearing at high frequencies, hearing acuity) was also observed in several smaller cross-sectional studies, however, in the largest study on workers in the glass fibre-reinforced plastics industry, no evidence was observed that exposure to styrene had an effect on hearing acuity when both lifetime styrene exposure and noise were taken into account. Studies of effects on the immune and hematopoetic system, liver, and kidney did not reveal consistent changes (IARC 2002). Generally, in these studies effects on workers with long-term exposure to styrene were investigated. A detailed description of the findings from these studies is beyond the scope of this document because they do not provide data that can be used for the derivation of AEGL. Therefore, only studies are described here in which effects following acute occupational exposure to styrene were investigated.

Acute behavioral effects and symptoms of exposure to styrene were investigated in a cross-sectional study (Edling and Ekberg 1985). 12 workers (mean age 30 years) with a mean exposure to styrene of 2.5 years took part in the study. Neuropsychiatric symptoms (questionnaire) and a reaction time test were conducted after an exposure free intervall of at least 24 hours before and after the morning and the afternoon shift. A reference group of 10 non-exposed men was available for the morning shift. The mean 8-hour TWA of breathing zone personal samples was 43 ± 28 mg/m³ (10 ± 6.5 ppm) in the morning shift and 54 ± 37 mg/m³ (13 ± 9 ppm) in the afternoon shift. No significant differences in neuropsychiatric symptoms and reaction time were observed between pre- and postshift evaluations and between exposed and controls.

Acute (and chronic) effects of styrene on the nervous system were investigated in a further cross-sectional study (Triebig et al. 1989). A total of 36 workers from companies handling polyester resin materials for 1 – 16 (median: 7) years and two control groups were each examined on a Monday. One control group formed to compare acute effects consisted of 20 men from two companies with no exposure to neurotoxic chemicals. To compare chronic effects, a second control group was formed by "one to one matching" with respect to age, socio-economic status, and pre-exposure intelligence level. Ambient air monitoring using active sampling (short time) and passive samplers (long time) showed styrene in air of 3 – 251 ppm (median: 18 ppm) and 140 – 600 ppm during lamination of the inside of boats. Clinical examination revealed no signs or symptoms of peripheral neuropathy or encephalopathy. Acute eye irritation was noted after exposure to about 200 ppm or more. Neurobehavioural tests showed neither significant differences in acute effects between the two groups nor between pre- and postshift testing nor significant differences in relevant neurobehavioural variables between the styrene workers and controls.

Limited data on the effects of styrene vapors on the nasal mucosa are available from a cross-sectional study (Ödkvist et al. 1985). 11 ship builders (mean age 39 years, range 26 – 57 years) exposed to styrene for a mean of 7 years (range 1 – 16 years) took part in the study. Air levels in the plant were in the range of 200 – 250 mg/m³ (47 – 59 ppm) (no details reported). 25 men matched for age and smoking habits and without industrial exposure served as controls. Nasal biopsies were taken from the mucosa of the inferior turbinates, and morphological findings were graded according to a scoring system evaluating histological characteristics. No statistically significant differences between the mean scores of both groups were found.

2.2.3 Experimental Studies

Two male subjects were exposed to 800 ppm styrene for 4 hours in a 4000 cubic ft. room (about 110 m³) in which fans were arranged to produce rapid and thorough mixing of the air (Carpenter et al. 1944). Styrene was evaporated at room temperature from large wicks in air stream and the vapor concentration was monitored (using an "interferometer" developed for the iodometric determination of organic vapors) and controlled manually. Psycho-motor response was followed by means of a "steadiness" test. The test was performed by the subject holding at arm´s length a small wire in a hole drilled in a copper strip. The number of contacts and the time the wire was in contact with the periphery of the hole was recorded during a 3-minute period. Exposure to styrene caused immediate eye and throat irritation, increased nasal mucous secretion, pronounced and persistent metallic taste, and CNS depression with listlessness, drowsiness, impairment of balance, and, after termination of exposure, muscular weakness and unsteadiness that were accompanied by inertia and depression. In the steadiness test, the contact time was 630 % of the day´s normal value. There was apparently no control without exposure so it cannot completely be ruled out that some of the effects described might not be related to the styrene exposure but to the experimental conditions. However, this seems unlikely since the styrene concentration was very high and the effects noted were very pronounced. Furthermore, during exposure to lower concentrations of other chemicals (butadiene, toluene) in the same experiment, weaker or no effects were observed.

An unspecified number of humans were exposed to a range of analytically determined concentrations of styrene in an enclosed, tightly sealed room (Wolf et al. 1956). The subjects qickly entered the room and noted their reactions with respect to odor, eye irritation, and nasal irritation. Probably, there was no unexposed control, but experimental details (esp., number of subjects, duration of exposure) were not reported by the authors. At 60 ppm, there was a "detectable odor but no irritation". 100 ppm were "tolerated without excessive discomfort" though the odor was "strong". An "objectionably strong odor" was felt between 200 and 400 ppm, while 600 ppm or more caused strong eye and nasal irritation.

In a toxicokinetic study, two volunteers were exposed to styrene at concentrations up to 386 ppm for 2 hours while performing light physical exercise of 50 W (Löf and Johanson 1993, see section 4.1). No information was presented with respect to subjective or objective signs of intoxication or irritation, but it may reasonably be assumed that no severe effects will have occurred in such a study.

Local irritation and effects on the nervous system were studied by Stewart et al. (1968). The study was conducted with a group of 9 healthy male technical employees (32 – 55 years old) with no known exposure to styrene for at least a year. In 5 experiments, a number of 1 – 5 subjects were exposed for 1, 2 or (with a 30-minute break at half-time) 7 hours in an exposure chamber of about 50 m³ to analytically (infrared analysis and gas chromatography) confirmed styrene concentrations of 51.4 ppm (1 hour), 99.4 ppm (7 hours), 116.7 ppm (2 hours), 216.1 ppm (1 hour), and 376 ppm (1 hour). During exposure, subjective and objective responses of each individual were recorded every 15 minutes. A neurological examination was performed every 15 minutes during exposures lasting up to 2 hours and every hour at longer lasting exposures. This examination included a modified Romberg test (balancing on one foot with eyes closed and both arms at a side), heel and toe, and finger to nose test. Additionally a

manual dexterity and a Flannigan coordination test were performed the morning and afternoon during the 7-hour exposure and after 30 minutes during the 1-hour exposure to 216 and 376 ppm.

No untoward subjective symptoms or objective signs of illness were recorded during a 1-hour exposure to 51 ppm (3 subjects) or during a 2-hour exposure to 117 ppm (1 subject). The odor was strong, but not judged to be objectionable. At 216 ppm (3 subjects), the odor was initially strong, and one subject noted nasal irritation after 20 minutes. No signs of CNS effects were observed during the 1-hour exposure.

Effects were seen at 376 ppm (5 subjects). Three of the subjects previously exposed to 216 ppm reported they were able to discern that they were now exposed to a higher concentration. “Mild” eye irritation occurred within 3 minutes. All subjects complained of nasal irritation within 15 minutes and one of them of a burning sensation of the skin of his face. Neurological alterations also were seen at this concentration. After 25 minutes, one subject, after 60 minutes two subjects were unable to normally perform the modified Romberg test. After 50 minutes, significant decrements were found in 3 of 5 subjects in other tests of coordination and manual dexterity. Furthermore, nausea after 45 minutes in one subject (persisting one hour post exposure), and feeling of being inebriated (2 subjects) and headache (one subject) after one hour were reported.

Exposure to 99 ppm for 2 x 3.5 hours caused complaints of mild eye and throat irritation in 3 of 6 subjects after 20 – 30 minutes which later subsided. 3 of 6 subjects reported intermittent difficulties in performing the Romberg test one or two times at the eight trials of this test during exposure. Tests of coordination and manual dexterity were normal. At the end of exposure, there were no reports of subjective symptoms. Throughout the study, clinical and laboratory data were normal and not altered compared to preexposure (Stewart et al. 1968).

Effects of styrene on psychological functions were studied in 12 healthy male volunteers (age 21 – 31 years) (Gamberale and Hultengren 1974). They were exposed in groups of six to either air (control) or to nominal but analytically (gas chromatography) monitored concentrations of 50, 150, 250 and 350 ppm styrene via mouthpiece in four continuous 30-minute periods. After each 30-minute period, the concentration of styrene was raised to the next higher level without interruption of exposure. In a second set of experiments, the control group was exposed to styrene and vice versa. Performance tests were carried out during each period of eposure. Care was taken that the volunteers were unaware of the exposure status by introducing menthol into the inhaled air, breathing through a mouthpiece only, and use of nose clips. Additionally, control experiments were initiated with a a relatively strong smell of styrene in the mouthpiece and ended with a short exposure to styrene after completion of the final test. All subjects believed that they had been exposed on both trial days. Local irritation was almost completely absent because the subjects were exposed via mouthpiece so that eyes and nose were spared from direct exposure. Nevertheless, compared to control exposure, subjects felt slight discomfort (feeling of tension and being affected) after exposure to styrene. In the performance tests, the performance level in the two perceptual tests (Identical Numbers and Spokes), was affected by training, both under control and exposure conditions. In both tests, the training effect in exposure to styrene was somewhat less pronounced than in control conditions, especially at the two higher concentrations. This could indicate that training was less effective under styrene exposure, however, the differences between the mean performance values for control and styrene exposure were not significantly different in any case. There was a clear effect of styrene on reaction time. The reaction time was significantly impaired in two tests (simple and choice reaction time) at 350 ppm but not at lower concentrations.

In a further study, 6 volunteers were exposed to analytically (Beckman hydrocarbon analyzer) monitored concentrations of styrene in room of about 15 m^3 (Oltramare et al. 1974). 3 of the 6 volunteers had been exposed occpationally to styrene but not during the last 1 days prior to the experiment. Altogether, 42 exposure sessions were held each lasting 1 – 3 hours and usually exposing one or two

subjects at a time. 2 subjects were exposed to styrene once at 300 ppm, all were exposed once or twice to 100 and 200 ppm, and most were exposed at 3 – 5 ppm ("odor-blinded" control) and 50 ppm.

Psychomotor functions of the three subjects with previous occupational exposed to styrene were studied in sessions each lasting 90 minutes. Volunteers were individually exposed. All were exposed to 3 – 5 ppm first, then to 50 (only two subjects), 100, or 200 pm in random order, and finally to 3 – 5 ppm again. Reaction time was determined before, 1 hour after start, and 30 minutes after termination of exposure. Simple reaction time was about the same as pre-exposure at 3 – 5 ppm but was lengthened by 12 – 37 % at 50, 100, and 200 ppm during exposure. Similar results were obtained in an audiovisual reaction test. However, there was no concentration response trend. In a multiple stimulus reaction test, no effect on performance was seen at 50 ppm. As the ability to perform this test improved with repeated trials, both during each session and from session to session, the authors comment that an effect of styrene at 50 ppm might have been masked. A decrement of about 2 % at 100 ppm and of 10 % at 200 ppm during and after exposure were seen (Oltramare et al. 1974).

Difficulties in balance performance were also studied by Oltramare et al. (1974) in 3 of 6 subjects. Statistically significant differences in a modified Romberg-test on a swaying platform were observed after 1-hour exposure to 200 ppm compared to control. No difference was seen when results from control and the 100-ppm group were compared. The authors noted that – due to the small sample size and the large variation of data – the results should be confirmed before definite conclusions are drawn.

Also, the 6 volunteers were asked to note the occurrence of 12 subjective symptoms (irritation: lips, nose, eyes; gastralgia, CNS effects: nausea, dizziness, headaches, sleepiness, poor concentration, intoxication, fatigue, malaise) during and after the exposure (**FIGURE 1**). A total of 55 individual responses were available for analysis from all of the exposure sessions. For each of the 12 symptoms, the number of positive responses was presented as numerator and the total number of exposures at this concentration as the denominator of a ratio. Since a given subject could have been exposed more than once at a given concentration, it is not evident if multiple positive responses for each individual symptom mean that several subjects experienced a symptom or that one subject experienced that symptom at several occasions. Also, it cannot be deduced from the data which symptoms were reported by the 3 previously exposed workers. However, the authors state that the workers reported irritation at 3 – 5 ppm, and the authors considered that this may have been due to chronic inflammation from working with styrene. On the other hand, the symptoms noted for CNS effects were consistently fewer for the subjects with previous exposure. For the parameters indicating CNS effects and also for gastralgia, there was a clear increase in positive symptom reports at 100 ppm and higher concentrations. The authors reported that at 50 ppm about half of the subjects experienced what was described as a prenarcotic discomfort. For irritation, an increase in symptom reports seems evident only for eye irritation at 200 ppm, and, less so, for irritation of the lips at 200 ppm (Oltramare et al. 1974).

Symptom		Styrene ppm 3-5	50	100	200	300
Irritation						
Lips	D	0/10	0/6	1/13	2/12	0/2
	P	0/10	0/6	0/13	1/12	0/2
Eyes	D	1/10	4/6	4/13	7/12	2/2
	P	0/10	0/6	1/13	2/12	0/2
Nose	D	4/10	3/6	7/13	5/12	1/2
	P	2/10	1/6	3/13	2/12	0/2
Gastralgia	D	0/10	0/6	3/13	5/12	1/2
	P	0/10	0/10	1/13	2/12	0/2
Nausea	D	0/10	0/6	5/13	4/12	2/2
	P	0/10	0/6	1/13	2/12	0/2
Dizziness	D	1/10	1/6	0/13	3/12	0/2
	P	0/10	1/6	0/13	2/12	0/2
Headaches	D	1/10	3/6	10/13	10/12	2/2
	P	0/10	2/6	8/13	9/12	0/2
Sleepiness	D	3/10	2/6	12/13	12/12	2/2
	P	1/10	1/6	4/13	11/12	2/2
Poor concentration	D	1/10	4/6	9/13	11/12	2/2
	P	0/10	2/6	4/13	9/12	2/2
Intoxication	D	0/10	1/6	2/13	6/12	1/2
	P	0/10	1/6	1/13	3/12	0/2
Fatigue	D	2/10	4/6	10/13	9/12	2/2
	P	2/10	4/6	9/13	9/12	2/2
Malaise	D	0/10	1/6	7/13	7/12	2/2
	P	0/10	0/6	1/13	0/12	0/2

D = occurrences during exposure
P = persistence after exposure

FIGURE 1: SYMPTOM RATINGS AT OR AFTER ACUTE EXPOSURE OF HUMANS TO STYRENE

(Table adopted from Oltramare et al. 1974)

Vestibulo-oculomotor disturbances were studied by Ödkvist et al.(1982). 10 healthy non-smoking volunteers (5 man, 5 women, age 20 – 30 years) inhaled styrene via mouth-tube at an analytically confirmed concentration between 87 and 139 ppm (fluctuating < 2 % during each individual exposure) during light exercise (50 W) for one hour. Vestibulo-oculomotor tests (swing test, optovestibular test, visual suppression test, optokinetic test, saccade test, slow pursuit moving test) were performed before, during and 1 hour after exposure. Each individual served as its own control. There were no effects on any test except the saccade test in which 8 of 10 subjects showed an enhanced maximum speed of the saccade during exposure. The authors conclude that the results suggest an effect of styrene on the vestibulo-ocular system by blocking inhibitory mechanisms in the CNS.

Pierce et al. (1998) exposed 4 healthy male non-smoking volunteers (26 – 30 years old, no known history of solvent exposure) in a 13.8 m^3 chamber to analytically (infrared spectrophotometry) confirmed concentrations of 15 – 99 ppm styrene in different exposure scenarios. No changes were

observed in a digit recognition test performed after 35 minutes of exposures and in electroencephalogram performed after each 100-minute exposure.

TABLE 2: SUMMARY OF ACUTE NON-LETHAL EFFECTS IN CONTROLLED HUMAN STUDIES FOLLOWING INHALATION OF STYRENE

Exposure duration	Concentration ppm (mg/m³)	Effects and remarks	Reference
1 – 2 minutes	500 – 800 ppm 300 – 400 ppm	Intolerable irritation of previously non-exposed subjects; lacrymation, irritation of nasopharynx	Götell et al. 1972
4 hours	800	Immediate eye and throat irritation, CNS depression with listlessness, drowsiness, impairment of balance, and, after termination of exposure, muscular weakness and unsteadiness with inertia and depression	Carpenter et al. 1944
Not reported	 60 ppm 100 ppm 200 – 400 ppm ≥ 600 pm	Rapid onset of effects: "detectable odor but no irritation" "tolerated without excessive discomfort", "strong" odor "objectionably strong odor" strong eye and nasal irritation	Wolf et al. 1956
4 x 30 minutes with stepwise increasing concentration	50 ppm 150 ppm 250 ppm 350 ppm	Exposure via mouthpiece (avoiding eye irritation); slight increase of simple and choice reaction time at 350 ppm	Gamberale and Hultengren 1974
1 hour	87 – 139 ppm	No effect on vestibulo-oculomotor parameters except saccade test where 8 of 10 subjects showed an enhanced maximum speed of the saccade	Ödkvist et al. 1982
35 minutes 100 minutes	15 – 99 ppm 15 – 99 ppm	No changes in digit recognition test No changes in electroencephalogram	Pierce et al. 1998
1 hour 2 hours 20 minutes 1 hour 3 minutes 15 minutes 25 min. – 1 hour 2 x 3.5 hours (with 30 minutes break)	51 ppm 117 ppm 216 ppm 216 ppm 376 ppm 376 ppm 376 ppm 99 ppm	No subjective symptoms or objective signs of illness; strong, but not objectionable odor Odor initially strong, nasal irritation No signs of CNS effects "Mild" eye irritation Nasal irritation CNS effects: difficulties in balance performance tests, decrements in manual dexterity test, nausea, inebriation, headaches Complaints of mild eye and throat irritation after 20 – 30 minutes, subsiding later; intermittent difficulties in performing Romberg test in 3/6 subjects. No subjective symptoms or signs of CNS effects at the end of exposure. Clinical and laboratory data normal.	Stewart et al. 1968

TABLE 2: SUMMARY OF ACUTE NON-LETHAL EFFECTS IN CONTROLLED HUMAN STUDIES FOLLOWING INHALATION OF STYRENE

Exposure duration	Concentration ppm (mg/m³)	Effects and remarks	Reference
1 – 3 hours	50, 100, 200 ppm	Rating scores for subjective symptoms of CNS effects (headaches, sleepiness, nausea, fatigue, poor concentration) ↑ in rating score at ≥ 100 ppm Scores for irritation at 50 ppm also ↑, but no evident concentration response	Oltramare et al. 1974
1 hour	200 ppm	Slight difficulties in balance performance, but large variation of data	
1.5 hours	50, 100, 200 ppm	Possibly slight increase in reaction time, but no dose-response	
6 hours	25 – 50 (with/ without 4 peaks 15 min 100 ppm)	Neither performance to neuropsychological tests nor subjective signs and symptoms of irritation or CNS effects negatively influenced	Ska et al. 2003; Vyskocil et al. 2002a
1 - 7.5 hours		Men: 3 days at 20 ppm, 4 days at 100 ppm, 4 days at 75 – 125 ppm (average 100 ppm), 5 days at 125 ppm, 7 days at 0 ppm Women: 4 days at 100 ppm, 2 days at 0 ppm No CNS effects in equilibrium and cognitive testing Subjective symptoms (exposure times not reported):	Hake et al. 1983
		Irritation (eyes, nose, throat) Men/ Women / Headache: Men/ Women	
	0 ppm	13 %/ 8 % / 3 %/ 0 %	
	20 ppm	17 % / 0 %	
	75 – 125 ppm*	20 % / 0 %	
	100 ppm	33 %/ 32 % / 13 %/ 35 %	
	125 ppm	45 % / 12 %	
3 – 4 hours	20 ppm 0.5 – 40 ppm (peak)	Ratings for odor, annoyance and, marginally, for irritation increase with concentration; ratings of irritation verbally labelled as "hardly at all"	Seeber et al. 2002

*: fluctuating exposure concentration, average 100 ppm.

Acute effects of styrene on the CNS were also studied in a total group of healthy male volunteers (20 – 50 years old, smokers and non-smokers) not previously exposed to styrene and with no documented exposure to neurotoxicants during the study (Ska et al. 2003; Vyskocil et al. 2002a; 2002b). The volunteers were exposed to styrene at rest to 5 different scenarios that lasted 6 hours each: a) continuously to 106 mg/m³ (25 ppm), b) variable exposure with a mean of 25 ppm and four 15-minutes peak exposures up to 213 mg/m³ (50 ppm), c) exposure to 1 ppm, control), d) exposure to 50 ppm, e) mean exposure to 50 ppm with four 15-minutes peaks up to 426 mg/m³ (100 ppm). The sequence of exposures was c-a-b-c-d-e. Exposure was carried out in an 18 m³ chamber and the styrene concentration was monitored by gas chromatography and infrared analysis. Before and after exposure, the volunteers were submitted to a battery of test proposed by the World Health Organization to detect neurotoxic effects of chemicals: sensory tests (visual: Lanthony D-15 and vision contrast test, olfactory: smell test), neuropsychological tests (reaction time, attention, memory, psychomotricity), and self-evaluation questionnaires for mood (seven-category response scale) and symptoms (four-point scale for 17 items regarding irritation and CNS effects) in a test-retest design. The testings were performed before exposure (Base-line) and within 1 hour after the end of exposure. Initially, 42 subjects took part in the study. However, only data from subjects who had taken part in all scenarios were retained for further analyses.

Missing data were due to absence of subjects at a given scenario or to factual problems during testing. Therefore, complete data were available for 24 subjects. The different exposure scenarios negatively influenced neither the performance to any test nor the subjective signs and symptoms.

Psychological reactions related to chemosensory irritation during exposure to a number of chemicals including styrene were investigated by Seeber et al. (2002). Exposure studies were conducted in a ventilated room of 28 m³ (air exchange about 250 m³/h) with continuous control of the concentration of the test substance (deviations < 3 %). In all experiments, 4 young healthy male volunteers who had no knowledge of the experimental conditions, were investigated simultaneously. The concentrations of styrene were 20 ppm for 3 hours or 0,5 ppm periods for 50 minutes followed by 40 ppm peaks for 30 minutes during a total 4 hours. At control and at 20 ppm each, a total number of 16 volunteers were exposed, at 0.5/40 ppm, the total number of volunteers was 24. Ratings for irritation, odor and annoyance were assessed and mean values were calculated from 2 – 5 repeated ratings for a given exposure level (total observations 16 – 246). For odor and annoyance, ratings increased similarly with increasing styrene concentration while there was only a marginal report for irritation. Thus, annoyance was more closely associated with odor than with irritation. Effect sizes comparing the ratings during exposure to 20 ppm and during the pre-exposure test were higher for odor, irritation and annoyance. Effect sizes were also higher compared to "clean air only"-exposure. However, the ratings for irritation (in case of styrene and all other solvents investigated) reached only levels verbally labelled "hardly at all".

Studies with repeated inhalation exposure

In a study conducted for and summarized by NIOSH (1983), 10 men were exposed in groups of 2 – 4 for 1, 3, or 7.5 hours/day to 0, 20, 100, or 125 ppm styrene (Hake et al. 1983). 8 women were exposed in groups of 1 – 4 at 0 or 100 ppm. For men, there were 3 days of exposure at 20 ppm, 4 days at 100 ppm, 4 days at 100 ppm with concentrations fluctuating between 75 and 125 ppm, 5 days at 125 ppm, and 7 days at 0 ppm. For women, there were 4 days at 100 ppm and 2 days at 0 ppm. In control exposures, the chamber was odorized with 10 ppm styrene upon entry of the subjects after which exposure was reduced to 0 ppm within 10 minutes. Each subject was exposed to more than one concentration, non-exposure weekends or control exposures were interspersed with exposure to styrene.

There were no deleterious effects on equilibrium as measured by Romberg- and heel-to-toe tests. Some changes in visual evoked response and amplitude of electroencephalogram (EEC) were observed in 3 of 6 subjects studied that – according to the authors – were consistent with CNS-depression. However, the changes were neither consistent between subjects nor in magnitude within subjects. Furthermore, there was no significant variance in cognitive testing scores related to styrene exposure. Respiratory parameters generally showed no effects of styrene exposure; however, the authors observed decrements in maximal expiration values in subjects repeatedly exposed to 100 ppm for 7.5 hours (no details reported).

With respect to subjective symptoms noted on a checklist during exposure, the overall data indicated some dose-response for irritation (eyes, nose, and throat) and headaches. For men, the reported incidences of irritation were 13 % (0 ppm), 17 % (20 ppm); 20 % (100 ppm), 33 % (100 ppm fluctuating), 45 % (125 ppm); for headaches, incidences were 3 %, 0 %, 0 %, 13 %, 12 %. For women, the incidence of irritation was 8 % (0 ppm) ad 32 % (100 ppm), for headaches, incidences were 0 % and 35 %. There was no specific indication as to which exposure time the various subjective responses were elicited at a given exposure concentration (Hake et al. 1983).

Odor perception

The odor of styrene has been described as solventy, rubbery, and plasticy (Leonardos et al. 1969; Ruth 1986) and also as strongly metallic (Gamberale and Hultengren 1974). A wide range of odor thresholds is reported in the literature. This wide range may be due to different degrees of purities of the test substances used, the presence or absence of polymerization inhibitors, different methodology used, different bases used (median, mean, range), individual variability or an adaptation to odor perception following repetitive exposure.

The olfactory function and the styrene odor detection threshold were compared between a group of workers exposed to styrene at least 4 years (reinforced-plastics industry, current mean personal air sampling concentrations of styrene about 11 – 66 ppm) and a group of age- and gender-matched naïve controls (Dalton et al. 2003). Absolute odor threshold concentration values were not presented in the study. The styrene odor detection threshold for workers was on average 32-fold higher than for controls. Furthermore, when the results were stratified by age, the most pronounced increase in odor threshold was observed in workers who were in their 5^{th} or 6^{th} decade, while duration of exposure was not related to the effect. No differences were found betweeen workers and controls with respect to the odor threshold for an olfactory standard, phenylethyl alcohol, and for the ability to identify a variety of 20 different aroma compounds in an odor identification test. The results do not provide evidence that styrene is an olfactory toxicant in humans.

Van Doorn et al. (2002) present results of odor threshold determinations for styrene that were a) measured by olfactometry methods considered compatible with a precursor of the NVN2820 and EN13725 method or b) were measured by TNO in the Netherlands using a precursor of the NVN2820 and EN 13725 methods, with a mean n-butanol threshold of 25 ppb. Results of both were converted to the reference agreed in EN13725 of 400 ppb n-butanol by using a factor of 40:25 = 1.6. Thereby, odor thresholds of 0.049 ppm and 0.025 ppm, respectively, were obtained. Taking into account the threshold value of 0.033 ppm obtained by the Japanese method (see below Hoshika et al. 1993), Van Doorn et al. (2002) calculated a mean odor threshold of 0.0345 ppm for styrene.

A comparison of odor threshold values determined by different methods in Japan (triangle olfactometer method, odor room, 20 trained male perfumers 30 – 45 years old) and in the Netherlands (olfactometer, 4 men, 4 women 18 – 40 years old), showed that the "barely perceptible or detectable odor thresholds" of 0.033 ppm and 0.016 ppm, respectively, are quite similar (Hoshika et al. 1993). The Japanese "triangle olfactometer method" produces an n-butanol threshold of 38 ppb that is compatible with the value (40 ppb) of the method according to EN13725 (Van Doorn et al. 2002).

In accordance with these data, WHO (1983) reported an odor perception threshold of 0.05 – 0.08 ppm. In other older studies and compilations, substantially higher values were reported. Odor thresholds ranging from 0.1 – 201 ppm (0.43 – 860 mg/m³) for styrene (inhibited) and from 0.047 – 201 ppm (0.2021 – 860 mg/m³) for styrene (uninhibited) were reported by Ruth (1986). Based on 10 original literature references which were not explicitly reported, a geometric mean odor threshold of 0.32 ppm styrene (standard error 2.0 ppm) was calculated (Amoore and Hautala 1983).

The odor recognition threshold was determined for 53 odorant chemicals including styrene under controlled laboratory conditions using a standardized and defined procedure (Leonardos et al. 1969). The odor threshold represents that concentration at which all four trained panelists could positively recognize the odor. Different threshold values were obtained for styrene without inhibitor (0.047 ppm) or with inhibitor (0.10 ppm) and for inhibited styrene additionally purified by gas-liquid chromatography (0.21 ppm). The chemical nature of the inhibitor was not reported.

2.3 Developmental/Reproductive Toxicity

No data regarding developmental or reproductive toxicity in humans following single exposure to styrene have been found in the available literature.

Studies with repeated inhalation exposure

The epidemiological data have been extensively reviewed recently (Brown et al. 2000; IARC 2002). In case reports, malformations in children of styrene-exposed mothers and spontaneous abortion in female workers occupationally exposed to styrene were described. However, these observations could not be confirmed in epidemiological studies. According to the reviews mentioned above, there is no sound evidence for an association between workplace exposure to styrene and spontaneous abortions, malformations or decreased male fecindity.

2.4 Genotoxicity

Genotoxicity studies have been extensively evaluated and summarized in a number of reviews (ATSDR 1992; Bonassi et al. 1996; Cohen et al. 2002; IARC 1994; IARC 2002; Scott and Preston 1994; Vodicka et al. 2002; WHO 1983; WHO 2000). Since a detailed description of the findings from these studies is beyond the scope of this TSD, findings as described in these reviews are summarized.

In *in vitro* systems with human cells, styrene induced chromosomal aberrations (CA), sister chromatid exchanges (SCE), micronuclei, and hypoploidy in whole-blood cultures in the absence of exogenous metabolic activation system were observed. CA and SCE were also observed in lymphocyte cultures in the absence of exogenous metabolic activation system.

In vivo, no data regarding genotoxic effects in humans following single exposure to styrene have been found in the available literature.

Studies with repeated inhalation exposure

A number of cytogenetic studies have been conducted on workers with occupational exposure to styrene, especially in the reinforced plastics industry. Some studies have shown associations between styrene exposure and the frequency of chromosomal abnormalities, but there is less evidence for an association between styrene exposure and the frequency of sister chromatid exchanges and no compelling evidence for micronuclei formation in human studies (Cohen et al. 2002). According to the evaluation of the IARC (2002), the results reported for chromosomal aberrations, micronuclei and sister chromatid exchange in approximately 30 studies of workers exposed to styrene in various industries have been inconsistent. Induction of chromosomal aberrations was reported in 12 of 25 studies, sister chromatid exchange in 6 of 16 and micronuclei in 3 of 14 studies.

Recently, physiological modeling of the relative contributions of styrene-7,8-oxide (SO) derived from direct inhalation and from styrene metabolism to the systemic dose in humans has been performed. From these calculations, it has been suggested that SO which is present in the air at workplaces in the reinforced plastics industry could present a greater hazard of cytogenetic damage than inhalation of styrene (Tornero-Velez and Rappaport 2001). However, this conclusion has been questioned by others, as other studies reported much higher levels of blood styrene oxide when measured directly than the level calculated by these authors from styrene-hemoglobin adducts (Filser et al. 1999, 2002).

2.5 Carcinogenicity

No data regarding the development of cancer in humans following single exposure have been found in the available literature.

Studies with repeated inhalation exposure

The cancer epidemiology data have been reviewed recently (Cohen et al. 2002; IARC 2002).

Retrospective cohort mortality studies and nested case-control studies were conducted in three types of industy: in the production of styrene monomer and polystyrene, of glass-fibre reinforced plastics, and of styrene-butadiene rubber.

Because workers in the reinforced plastics industry have higher styrene exposure and less potential for exposure to other substances than the other cohorts studied, the most informative data with regard to an association between styrene exposure and cancer come from studies of these cohorts. In the three studies in such cohorts of reinforced plastic workers, an excess of lung or respiratory cancer was found. However, the excess occurred in those groups of workers with lower exposure. An excess of lymphatic and hematopoietic (LH) cancers was observed in some epidemiological studies in the reinforced plastics industry, but not in others. Such an association also was found in workers in styrene production, but exposure was poorly documented and may have been also to other chemicals beside styrene. Studies in workers of the styrene-butadiene rubber production also found a small excess of leukemia mortality. However, these findings are difficult to evaluate because of the high correlation between exposure to styrene and butadiene (Cohen et al. 2002).

Reports of increased risks of other cancers (rectal, pancreatic, nervous system) are also reported in some studies. Mostly, the numbers of cases are small, and these findings are not supported from data of larger cohort studies.

2.6 Summary

A great number of studies on workers with occupational exposure to styrene in different workplaces have been carried out. These studies have been repeatedly reviewed and summarized (ACGIH 1997; ATSDR 1992; Cohen et al. 2002; DFG 1987; Government Canada 1993; IARC 2002; OEHHA 1999; Sherrington and Routledge 2001; US EPA 1998; WHO 1983; WHO 2000). The highest exposure occurs in the fabrication of reinforced-polyester plastics composites, where 8-hour average samples in breathing zones often exceeded 100 ppm styrene (IARC 2002). In older studies on workers in the manufacture of reinforced plastics, 8-hours TWA concentrations in the breathing zone of up to 292 ppm were reported, with peaks of about 1500 ppm during shorter periods of work for about 5 – 10 minutes (Götell et al. 1972).

In workers with chronic exposure to styrene, effects on the central and peripheral nervous systems that were described in many studies include a reversible decrease in color discrimination. Decrements of auditory function was also observed, though findings made in several smaller cross-sectional studies could not be confirmed in the largest study. Studies of effects on the immune and hematopoetic system, liver, and kidney did not reveal consistent changes (IARC 2002).

No reports of lethal intoxication following styrene exposure were located in the literature.

Pure styrene has a pungent, slightly sweetish odor. A wide range of odor thresholds has been reported. Van Doorn et al. (2002) presented results of odor threshold determinations for styrene and calculated an n-butanol corrected mean odor threshold of 0.0345 ppm for styrene.

Given that hundreds of thousands of workers have been exposed to styrene vapors and had skin contact with the liquid from the 1940s to the present, the development of asthma or skin allergies does not represent a significant health risk from styrene based on industrial experience.

Styrene is irritating to eyes and the respiratory tract. In a number of controlled studies with human volunteers, irritation and effects on the CNS were investigated.

In a study on psychological reactions related to chemosensory irritation, ratings for odor and annoyance increased similarily with increasing styrene concentrations ranging from 20 – 40 ppm, while there was only a marginal increase for irritation. Effects sizes comparing the ratings between exposure to 20 ppm and pre-exposure were higher for odor, irritation, and annoyance. Effects sizes were also higher compared to "clean air only"-exposure. However, the ratings for irritation reached only levels verbally labelled as "hardly at all" (Seeber et al. 2002). No increase in irritation or headaches compared to control was noted at 20 ppm in a further study (Hake et al. 1983). At 50 ppm, one study indicated a marginal increase in subjective symptoms ratings for eye and nose irritation, headache, and fatigue (Oltramare et al. 1974). In that study, signs of irritation and of mild subjective CNS effects (headaches, fatigue, poor concentration, sleepiness) were reported more often at 100 ppm. Complaints of mild eye and throat irritation at 100 ppm in one test but not in another were reported by Stewart et al. (1968). In a recent study, subjective signs and symptoms during 6-hour exposure to 25 – 50 ppm styrene with 4 peaks of 15 minutes at 100 ppm indicated no irritation (Vyskocil et al. 2002a,b). At about 200 ppm, most subjects noted irritation of eye and nose (Oltramare et al. 1974; Stewart et al. 1968) and the severity increased with a further increase in concentration to 376 ppm. In their study on styrene-exposed workers, Götell et al. (1972) noted that they themselves suffered from immediate lacrymation at 300 – 400 ppm and could not withstand 500 – 800 ppm for more than 1 – 2 minutes although the workers tolerated such concentrations. In two further studies with controlled exposure of volunteers, concentrations $\geq$ 600 ppm caused strong eye, nasal, and throat irritation (Carpenter et al. 1944; Wolf et al. 1956).

No lesions of the nasal mucosa were observed in a cross-sectional study on styrene-exposed workers. Furthermore, the ability to detect and identify different odors in a controlled odor test was not affected in workers with long-term exposure to styrene. These limited data provide some evidence that – in contrast to rats and especially mice – styrene does not seem to be an olfactory or upper respiratory tract toxicant in humans. Support for this conclusion also comes from toxicokinetic studies *in vitro* in which the metabolic capacity of nasal epithelia from humans, rats, and mice was compared (see section 4.1, page 48).

With respect to CNS effects, one study reported higher ratings of headaches, poor concentration, and fatigue at 50 ppm compared to „odor-blinded" control exposed to 3 – 5 ppm (Oltramare et al. 1974). In another study, headaches did not occur when subjects were repeatedly exposed to fluctuating concentrations of 75 – 125 ppm (average: 100 ppm), but were reported at 125 ppm (Hake et al. 1983). Pierce et al. (1998) found no changes in a digit recognition test after 35 minutes of exposure and in electroencephalogram after 100 minutes of exposure to 15 – 99 ppm styrene in different exposure scenarios. At 100 ppm, intermittent difficulties in performing a modified Romberg test were observed in 3/6 subjects exposed for 7 hours with a 30-minute break in between. Other tests on coordination and on manual dexterity were normal, and no effects were noted at the end of exposure. In the same study, no CNS effects were seen in another experiment with 100 ppm exposure for 2 hours or 216 ppm for 1 hour (Stewart et al. 1968). Also, exposure for 6 hours at 50 ppm with 4 peaks of 15 minutes at 100 ppm had no negative influence on performance to neuropsychological tests (Vyskocil et al. 2002a,b). No effects on

equilibrium and cognitive function tests were noted in male and female volunteers at repeated exposures to 100 and 125 ppm for at least one hour (Hake et al. 1983). Oltramare et al. (1974) noted that slight difficulties in balance performance at 50 – 200 ppm (1.5 hours), but there was no concentration-response, and slight difficulties in balance performance at 200 ppm (1 hour), but the variation of data was large. No effects on simple and choice reaction time was seen following exposure to 250 ppm for 30 minutes. However, when the concentration was raised to 350 ppm for 30 minutes directly afterwards, both simple and choice reaction time were increased (Gamberale and Hultengren 1974). More pronounced effects were observed during exposure to 376 ppm for one hour: one subject complained of nausea that persisted one hour after the end of exposure, 2 had a feeling of being inebriated, and 3 of 5 subjects exposed were unable to normally perform the modified Romberg test and also 3 subjects (unclear, if the same 3 subjects) had significant decrements in other tests of coordination and manual dexterity (Stewart et al. 1968). Only one controlled study was located in which CNS effects were followed at a higher concentration than 376 ppm. In that study, two subjects exposed to 800 ppm for 4 hours reported that they suffered from listlessness, impairment of balance, drowsiness, and, after termination of exposure, from muscular weakness and unsteadiness with inertia and depression. CNS-depression was also indicated by a marked decrease in performance in a "steadiness test" (measuring manual dexterity) (Carpenter et al. 1944).

No data are are available indicating reproductive or developmental toxicity of styrene in humans after acute exposure.

In *in vitro* systems with human cells, styrene induced chromosomal aberrations (CA), sister chromatid exchanges (SCE), micronuclei, and hypoploidy. No data regarding genotoxic effects in humans following single exposure to styrene are available. Epidemiological studies provide some evidence for genotoxic effects (chromosomal aberrations, mutations, DNA-adducts) in occupationally exposed workers. However, the overall results have been regarded as inconsistent by IARC (2002).

With respect to carcinogenicity, IARC (2002) concluded that the increased risks for cancers of the lymphatic and hematopoetic system are small, statistically unstable and often based on subgroup analyses, the findings are not very robust and that it cannot be ruled out that the observations are the results of chance, bias or confounding. Cohen et al. (2002) conclude that, although the balance of epidemiologic studies do not suggest a causal association between styrene and any human cancer, because of the limited power of these studies, the inconclusive results do not rule out the possibility that the observed increase of lung tumors in mice are of relevance to humans.

In its latest evaluation, IARC (2002) concluded that there is *"limited evidence* in humans for the carcinogenicity of styrene*"* and, taking into account the results from animal carcinogenicity studies (see 3.5), that styrene is *"possibly carcinogenic to humans (Group 2B)"* (IARC 2002). Styrene has been assessed under the IRIS Program of the US-EPA, no quantitative carcinogenicity assessment for lifetime exposure is currently proposed.

Based on the body burden of styrene-7,8-epoxide or its adducts with hemoglobin and DNA, and taking into account the results from carcinogenicity studies with styrene in animals, a cancer risk has been estimated in the range of 1.7 – 7.5 per 100,000 persons exposed for 40 years to 20 ppm styrene, 8 hours/day, 5 days/week, 48 weeks/year (Greim 2003).

3 ANIMAL TOXICITY DATA

3.1 Acute Lethality

Data on acute lethality after inhalation exposure to styrene are available for rats, mice, and guinea pigs (**TABLE 3**). Non-lethal effects observed in these studies are described in section 3.2.2.

3.1.1 Rats

Female and male Sprague-Dawley rats (10 of each sex/group; 20 of each sex at the highest concentration) were exposed to analytically (gas chromatography) determinded concentrations of 2983, 3766, 4814, 5911, 6621, 7218 and 8407 ppm styrene in a 180 L dynamic exposure chamber for 4 hours (BASF 1979b). Survival of animals was followed for 14 days after the exposure. No deaths were observed at 2983 and 3766 ppm. At the other conentrations, deaths were observed up to three days after exposure. No differences in LC_{50} between female and male rats were observed. A combined LC_{50} of 6410 ppm (95 % conf. limit 6025 – 6769 ppm) for male and female rats was determined. Necroscopy revealed acute dilation and congestive hyperemia in the heart, enlarged lung, and centrilobular liver changes with fatty degeneration. Other, non-lethal effects are described in section 3.2.2.

The LC_{50} values were determined for a number of benzene derivatives in male Sprague-Dawley rats (Bonnet et al. 1982a). Groups of 12 rats each were exposed (as more precisely described in Gradiski et al.(1978)) in 170 L dynamic exposure chambers to analytically (gas chromatography) confirmed vapor concentrations of styrene for 6 hours. Animals were observed for 14 days after the end of exposure. The 6-hour LC_{50} for styrene was 4618 ppm (95 % confidence interval 4399 – 4894 ppm). Death was preceded by somnolence, tremors, and muscular seizures but no lacrymation was observed. From the figure presented in the publication, it can be estimated that 90 % of the animals died (LC_{90}) at about 5000 ppm and 10 % died (LC_{10}) at about 3300 ppm (**FIGURE 2**) indicating a steep concentration-response curve. The authors also reported the occurrence of delayed deaths (more than 24 hours after the end of exposure) and that surviving animals showed growth retardation betweeen day 7 and 14 post exposure. However, no detailed data were presented.

A total number of 405 rats (sex, strain and number of animals per exposure group not reported) were exposed to styrene concentrations ranging from 1300 ppm to 10,000 ppm for one hour to up to more than 30 hours (Spencer et al. 1942). After the exposure the animals were observed for 2 – 4 weeks. No LC_{50} but only LC_0 and LC_{100} were reported in the study. The highest concentration that could be reached without observed condensation of the chemical out of the atmosphere was 10,000 ppm[1]. At this concentration, no deaths were observed after one hour of exposure, but all rats exposed for 3 hours died. At 5000 ppm, no animal died after exposure for 2 hours but all animals exposed for 8 hours died. At 2500 ppm, all animals survived an 8-hour exposure and death of all animals only was observed when the exposure lasted 21 hours. All animals survived 16 hours of exposure to 2000 ppm. Immediate deaths during or shortly after the end of exposure were due to the effect of styrene on the CNS (see 3.2.2). However, there were also delayed deaths with pulmonary edema and hemorrhages that were considered to develop as a result of the acute irritating effect of styrene on the lung.

[1] [Note: from the vapor pressure data – see **TABLE 1** – a saturated vapor concentration of 6580 ppm at 20 °C and of 8560 ppm at 25 ° C can be calculated].

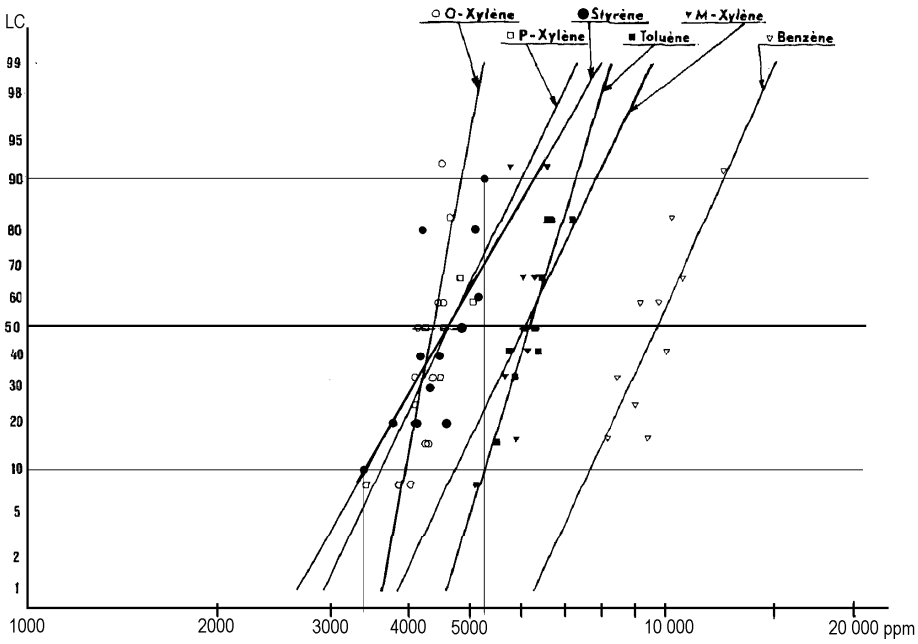

FIGURE 2: CONCENTRATION-RESPONSE CURVE FOR ACUTE LETHALITY FOLLOWING INHALATION OF STYRENE IN RATS

(Figure from Bonnet et al. 1982a)

Shugaev (1969) exposed rats (strain, sex and number of animals not reported) to analytically (gas chromatography) controlled styrene vapor concentrations for 4 hours in dynamic flow exposure chambers. A LC_{50} of 11.8 mg/l (95 % confidence interval 10.3 – 13.5 mg/l) (2761 ppm; 95 % confidence interval 2410 – 3159 ppm) was calculated. It was also reported that animals "often" died after the exposure. It is further reported that these animals were not used for the determination of lethal brain styrene concentrations but it is not clear if data for animals dying after the end of exposure were included in the determination of the LC values.

Groups of ten female Sprague-Dawley rats were exposed to analytically (infrared analysis) confirmed styrene vapor concentrations for 4 or 8 hours, respectively (Lundberg et al. 1986). Deaths were counted 24 hours after the start of exposure. At 33,200 mg/m³ (7769 ppm), a concentration that corresponded to approximately saturated styrene vapor in air at 25 °C (see footnote 1), no deaths were seen after a 4-hour exposure but four of ten animals died within 24 hours after an 8-hour exposure. No LC_{50} could be determined.

TABLE 3: SUMMARY OF LETHAL EFFECTS IN ANIMALS AFTER ACUTE INHALATION EXPOSURE TO STYRENE

Species, sex, strain	Concentration in ppm	Exposure Duration	Effect/Remarks	Reference
Rat, f, m, S-D	6410 6310 6480	4 hours	LC_{50}, female and male LC_{50}, females only LC_{50}, males only	BASF 1979b
Rat, f, m, S-D	8407	4 hours	18/20 f, 20/20 m, 38/40 m + f died	BASF 1979b

TABLE 3: SUMMARY OF LETHAL EFFECTS IN ANIMALS AFTER ACUTE INHALATION EXPOSURE TO STYRENE				
Species, sex, strain	**Concentration in ppm**	**Exposure Duration**	**Effect/Remarks**	**Reference**
	7218 6621 5911 4814 3766 2983		5/10 f, 8/10 m, 13/20 m + f died 6/10 f, 3/10 m, 9/20 m + f died 6/10 f, 1/10 m, 7/20 m + f died 1/10 f, 2/10 m, 3/20 m + f died 0/10 f, 0/10 m, 0/20 m + f died 0/10 f, 0/10 m, 0/20 m + f died	
Rat, nd, nd	1300 2000 2500 5000 10,000	30 hours > 40 hours 16 hours > 30 hours 8 hours 21 hours 2 hours 8 hours 1 hour 3 hours	LC_0 LC_{100} LC_0 LC_{100} LC_0 LC_{100} LC_0 LC_{100} LC_0 LC_{100}	Spencer et al. 1942
Rat (18 f; 15 m)	5100	1 hour	No death during exposure	Niklasson et al. 1993
Rat, nd, nd	2270 (9.7 mg/l) 2761 (11.8 mg/l) 3276 (14.0 mg/l)	4 hours 4 hours 4 hours	LC_{16} LC_{50} LC_{84}	Shugaev 1969
Rat, nd, nd	2700	4 hours	LC_{50}; abstract only	Jaeger et al. 1974
Rat, f, m, CD	1500	6 hours	0/20 died after repeated (subchronic) exposure	Cruzan et al. 1997b
Rat, f, m, CD	1000	6 hours	0/70 died after repeated (chronic) exposure	Cruzan et al. 1998
Rat, m, S-D	~ 5000 4618 ~ 3300	6 hours 6 hours 6 hours	LC_{90} estimated from figure LC_{50} LC_{10} estimated from figure	Bonnet et al. 1982a
Rat, f, S-D	7769 (33.2 mg/l)	4 hours 8 hours	0/10 animals died 4/10 animals died	Lundberg et al. 1986
Mouse, f, m, NMRI	1600 1840 1370	4 hours	LC_{50}, female and male LC_{50}, females only LC_{50}, males only	BASF 1979a
Mouse, f, m, NMRI	3766 2983 1528 1420 864 680	4 hours	10/10 f, 10/10 m died 7/10 f, 9/10 m died 3/10 f, 8/10 m died 4/10 f, 6/10 m died 1/10 f, 0/10 m died 0/10 f, 0/10 m died	BASF 1979a
Mouse, f, OF1	2429	6 hours	LC_{50}	Bonnet et al. 1979b; 1982a

TABLE 3: SUMMARY OF LETHAL EFFECTS IN ANIMALS AFTER ACUTE INHALATION EXPOSURE TO STYRENE				
Species, sex, strain	**Concentration in ppm**	**Exposure Duration**	**Effect/Remarks**	**Reference**
Mouse, nd, nd	4142 (17.7 mg/l) 4914 (21.0 mg/l) 5873 (25.1 mg/l)	2 hours 2 hours 2 hours	LC_{16} LC_{50} LC_{84}	Shugaev 1969
Mouse	2223 (9.5 mg/l)	4 hours	LC_{50} (no details reported)	Izmerov et al. 1982
Mouse, B6C3F1, 65 f, 23-27 m	500 250	6 hours 6 hours	5 m, 0 f died after one exposure no death after one exposure	Morgan et al. 1993c
Mouse, B6C3F1, 5 m	500 250	6 hours 6 hours	4/5 found moribund and sacrificed, no death/moribund after one exposure	Morgan et al. 1993c
Mouse, B6C3F1, 36 f, 36 m	500 250	6 hours 6 hours	6/36 m, 1/36 f died after one exposure no death after one exposure	Morgan et al. 1993a
Mouse, B6-C3F1, 30 m	500	6 hours	2/30 died after one exposure	Mahler et al. 1999
Mouse, B6-C3F1, 20 f, 20 m	500	6 hours	no death after one exposure	Cruzan et al. 1997b
Mouse, CD-1, 20 f, 20 m	500 250	6 hours	1/20 m died after one exposure no death after one exposure	Cruzan et al. 1997b
Mouse, B6-C3F1, 39 m	250	6 hours	4/39 died after one exposure	Sumner et al. 1997
Mouse, CD-1, 30 m	250	6 hours	0/39 died after one exposure	Sumner et al. 1997
Guinea pig, nd, nd	1300 2000 2500 5000 10,000	16 hours 40 hours 7 hours 30 hours 6 hours 14 hours 3 hours 8 hours 1 hour 3 hours	LC_0 LC_{100} LC_0 LC_{100} LC_0 LC_{100} LC_0 LC_{100} LC_0 LC_{100}	Spencer et al. 1942

f: female; m: male; nd: no data; S-D: Sprague-Dawley.

According to an abstract (Jaeger et al. 1974), a 4-hour LC_{50} of 2700 ppm was estimated for fed and fasted rats. It is further reported that styrene caused death by pulmonary irritation and edema, but no further details were presented (strain, no. of animals and dose gropus, treatment with inducers, inhalation exposure conditions, occurrence of CNS effects).

No deaths occurred in CD (Sprague-Dawley) rats exposed 6 hours/day, 5 days/week to 1500 ppm for 13 weeks (Cruzan et al. 2001a) or to 1000 ppm for 104 weeks (Cruzan et al. 1998). Acute non-lethal (irritation) effects observed in these studies are described in section 3.2.2.

Studies with non-inhalation exposure

The oral toxicity of styrene was determined in a total number of 57 young adult white rats raised from a stock obtained from the Wistar institute. Styrene was given by gavage, but it is not clear whether the compound was given undiluted or as an olive-oil or corn-oil solution emulsified with an aqueous soltion of gum arabic. The oral toxicity was low as indicated by an LD_{50} of 5.0 g/kg b.w. (Wolf et al. 1956). In accordance with these data, in another study no death of rats was observed after oral administration of 1600 mg/kg b.w. but all rats died after treatment with 8000 mg/kg b.w. (Spencer et al. 1942).

Groups of six female Sprague-Dawley rats were treated i.p. with styrene (Lundberg et al. 1986). Deaths were counted 24 hours and 14 days after the injection. The reported LD_{50} of 898 mg/kg b.w. and the 95 % confidence limits (768 – 1051 mg/kg b.w.) were identical at both time points indicating that there were no delayed deaths after intraperitoneal administration of styrene.

3.1.2 Mice

Bonnet et al. (1979) studied the toxicity of styrene in female OF1 mice. Groups of at least 20 mice were exposed by whole body exposure in 170 L dynamic exposure chambers (as more precisely described in Gradiski et al. (1978) to analytically (gas chromatography) confirmed vapor concentrations of styrene for 6 hours. Animals were observed for 14 days after the end of exposure. The 6-hour LC_{50} for styrene was 2429 ppm (95 % confidence intervals 2352 – 2530 ppm). The authors reported the occurrence of delayed deaths on the 5^{th} to 10^{th} day after exposure but no detailed data were presented.

Shugaev (1969) exposed mice (strain, sex and number of animals not reported) to analytically controlled styrene vapor concentrations for 2 hours in dynamic flow exposure chambers. A LC_{50} of 21.0 mg/l (95 % confidence interval 17.8 – 24.8 mg/l) (2761 ppm; 95 % confidence interval 4165 – 5803 ppm) was calculated. It was also reported that animals "often" did not die during, but after the exposure.

BASF (1979) conducted an acute inhalation toxicity study with NMRI mice. 10 female and 10 male mice per dose group were exposed "whole body" to analytically (gas chromatography) determined concentrations of 680, 864, 1420, 1528, 2983 or 3766 ppm styrene for 4 hours in a 180 L dynamic exposure chamber. Survival of animals was followed for 14 days after the exposure. No deaths were observed at 680 ppm. At the other conentrations, deaths were observed 1 – 4 days after the exposure. The concentration-response curve was steeper for male mice, and males seemed more sensitive than females. LC_{50} of 1370 ppm (95 % conf. limit 1087 – 1653 ppm) for male mice and of 1840 ppm (1486 – 2359 ppm) for female mice were determined. Necroscopy revealed acute dilation and congestive hyperemia in the heart, enlarged lung, and centrilobular liver changes. Other, non-lethal effects are described in section 3.2.3.

Without presenting further details, a 4-hour LC_{50} of 9500 mg/m³ (2223 ppm) for mice is reported by Izmerov et al. (1982).

In a study to evaluate toxic effects of short-term exposure to B6C3F1 mice, 23 – 27 male and 65 female animals (8 weeks old) per group were exposed to analytically confirmed concentrations of 0, 125, 250, or 500 ppm styrene (99.9 % pure) for 6 hours/day (starting at 7 AM) for up to 14 days (Morgan

et al. 1993c). Each animal was exposed individually in Hazleton 2000 chambers and the styrene concentration was measured every minute by infrared spectophotometry. After one exposure day, 5 males exposed to 500 ppm died. Mortality and morbidity were delayed after exposure and typically animals were found dead or moribund the morning after the exposure day. In an additional experiment in the same study conducted only with male mice, 4 of 5 animals died after one 6-hour exposure at 500 ppm. No deaths were observed after one exposure in male mice at lower concentrations or at any concentration in female mice.

In a further study of the same group (Mahler et al. 1999), 2/30 male, 8-week old B6C3F1 mice were found dead one day after a single 6-hour exposure to 500 ppm styrene. Death was attributed to massive hepatic necrosis. In another study, death of 4/39 male B6C3F1 mice was observed following one exposure to 250 ppm for 6 hours (Sumner et al. 1997).

Sex differences in susceptibility of B6C3F1 mice were further investigated (Morgan et al. 1993a). 36 animals (8 weeks old) per sex and dose were exposed as described above to 0, 125, 250, or 500 ppm styrene. At 500 ppm, six male and one female mice were found dead or were terminated moribund after one exposure. No deaths occurred after one exposure to 250 ppm or 125 ppm. Necropsy of dead or moribund mice revealed that the liver of these animals was engorged with blood, and microscopic examination showed severe congestion and necrosis in the liver of these animals.

In a subacute toxicity study with CD-1 and B6C3F1 mice, one of 20 male CD-1 mice exposed to 500 ppm died after one 6-hour exposure (Cruzan et al. 1997b).

Data on lethality following repeated short-term exposure in Morgan et al. (1993a), Morgan et al. (1993c) and Cruzan et al. (1997) are summarized below *("studies with repeated exposure").*

Studies with repeated inhalation exposure

In a developmental toxicity study, 2 of 6 pregnant BMR/T6T6 mice exposed to 500 ppm 6 hours/day from the 6th day of gestation on died before the intended end of the exposure phase on day 16. At 750 ppm, 3 of 5 mice died. Surviving dams carried a high number of dead and resorbed fetuses (Kankaanpää et al. 1980, see 3.3.2).

B6C3F1 mice were exposed up to 14 consecutive days to styrene as described above (see 3.1.2, Morgan et al. 1993c). No animals died at 0 and 125 ppm styrene. The highest mortality was observed at 250 ppm where 7 males and 2 females died after two exposures and a total of 11 males and 6 females after 14 days. At 500 ppm, no deaths occurred in females, 7 males died after 2 exposures and a total of 8 males died during the 14-day exposure.

In a second study investigating sex-related differences in susceptibility of B6C3F1 mice to styrene, animals were exposed up to 3 consecutive days to styrene as described above (see 3.1.2, Morgan et al. 1993a). No control mice or mice exposed to 125 ppm died. At 250 ppm, 2 males and 3 females died or were terminated moribund after 2 exposures. At 500 ppm, 6 males and one female died after one exposure but no additional deaths occurred after subsequent exposures.

The susceptibility of different strains of mice to styrene inhalation exposure was studied (Morgan et al. 1993b). 8 week old B6C3F1, C57BL/6, Swiss and DBA/2 mice (20 of each sex and strain at each dose group) were exposed to styrene (99.9 % pure) at nominal but analytically confirmed concentrations of 0, 125, 250, or 500 ppm in Hazleton 2000 chambers for 6 hours/day for 4 consecutive days. No animals of any strain died at 0 and 125 ppm. Both strain and sex differences in mortality (or sacrifice in moribund condition) were observed. The highest mortality occurred in Swiss mice and both sexes proved similarly susceptible (male: 10/20 died at 500 ppm, female: 3/20 at 250 ppm, 8/20 at 500

ppm died). Overall mortality in B6C3F1 mice was comparable to that in Swiss mice but there was a clear sex-specific effect with mortality in male B6C3F1 mice (14/20 at 250 ppm, 3/20 at 500 ppm died) being much higher than in females (1/20 at 250 ppm). Mortality in C57/BL6 mice also differed between males (7/20 at 250 ppm, 1/20 at 500 ppm) and females (1/20 at 250 ppm and 500 ppm each). Mortality in male B6C3F1 and C57BL/6 mice at 250 ppm was higher than at 500 ppm. No mortality was observed in male and female DBA/2 mice.

Sex-related differences in mortality were also observed in B6C3F1 and CD-1 mice in another subacute study in which mice (20 per sex and dose group) were exposed in 0.75 m³ inhalation chambers to analytically confirmed concentrations of 0, 15, 60, 250 or 500 ppm styrene for 6 hours/day, 5 days/week for 14 days (Cruzan et al. 1997b). No deaths were observed at 15 and 60 ppm. Remarkably, the concentration-response was non-linear in female mice of both strains as mortality after two weeks clearly was higher at 250 ppm (7 CD-1, 10 B6C3F1) than at 500 ppm (2 CD-1, 0 B6C3F1). This was not observed in male mice of both strains where mortality increased with increasing concentration: One male of each strain died at 250 ppm, 7 male CD-1 and 8 B6C3F1 males died at 500 ppm.

In a further study by Morgan et al. (1995), 8 week old male and female B6C3F1 and Swiss mice were exposed to 0, 150 or 200 ppm styrene as described above for 6 hours/day on 4 consecutive days. One female Swiss mouse died after four exposures to 200 ppm, necropsy revealed centrilobular hepatocellular necrosis in this mouse. No deaths were observed in male Swiss or in B6C3F1 mice of both sexes.

3.1.3 Guinea pigs

A total number of 410 guinea pigs (sex, strain and number of animals per exposure group not reported) were exposed to styrene concentrations ranging from 1300 ppm to 10,000 ppm for one hour to 40 hours (Spencer et al. 1942). After the exposure the animals were observed for 2 – 4 weeks. No LC_{50} but only LC_0 and LC_{100} were reported in the study. The highest concentration that could be reached without condensation of the chemical out of the atmosphere was 10,000 ppm. At this concentration, no deaths were observed after one hour of exposure, but all guinea pigs exposed for 3 hours died. At 5000 ppm, no animal died after exposure for 3 hours but all animals exposed for 8 hours died. At 2500 ppm, all animals survived a 6-hour exposure and death of all animals only was observed when the exposure lasted 14 hours. All animals survived 7 hours of exposure to 2000 ppm but all animals died when exposure at this concentration was extended to 30 hours. Immediate deaths during or shortly after the end of exposure were due to the effect of styrene on the CNS. However, there were also delayed deaths with pulmonary edema and hemorrhages that were considered to develop as a result of the acute irritating effect of styrene on the lung.

3.1.4 Hamsters

Studies with non-inhalation exposure

Groups of 23 male Syrian hamsters were treated with 0, 450 or 600 mg/kg b.w. styrene in corn oil by gavage (Parkki 1978). 3 of the animals that had received 600 mg/kg b.w. styrene died within 24 hours after administration. No deaths occurred at 450 mg/kg b.w.

3.2 Nonlethal Toxicity

3.2.1 Nonhuman primates

Studies with repeated inhalation exposure

Spencer et al. (1942) exposed 4 monkeys (two of each sex, species not specified) to 1300 ppm styrene 7 – 8 hours/day, 5 days/week. Male monkeys received 142 exposures during 7 months, females 262 – 264 exposures over a period of 12 months. Additionally, there were at least 3 control monkeys. No further experimental details were reported. There were no signs of irritation or intoxication. Furthermore, the animals were reported to be in excellent condition and to show no gross or microscopic pathological lesions (at least lung, liver, kidney, spleen, pancreas, adrenals were examined). Blood examination revealed no differences between the four exposed and three control monkeys.

3.2.2 Rats

Female and male Sprague-Dawley rats (10 of each sex/group; 20 of each sex at the highest concentration) were exposed to 2983, 3766, 4814, 5911, 6621, 7218 and 8407 ppm styrene for 4 hours (BASF 1979b, see 3.1.1). Styrene was irritating to eyes and respiratory tract as indicated by closed eyes, eye and nasal secretion, salivation, and dyspnoe. Signs of CNS impairment were staggered or stalking gait, tremors, lying on the side, and narcosis. Symptoms were not differentiated with respect to the individual exposure concentrations except that it was reported that narcosis was “slight” at the lowest concentration.

Shugaev (1969) reported that rats (strain, sex and number of animals not reported) inhaling styrene for one hour at a concentration corresponding to the 4-hour LC_{50} (reported to be 2761 ppm) were in a state of deep narcosis at the end of the 1-hour exposure.

Effects on the nervous system (somnolence, tremors, and muscular seizures) that preceded death were also reported by Bonnet et al. (1982). Lacrymation was not observed in styrene exposed animals in this study.

Exposure of rats to 1300 ppm led to immediate irritation of eyes and nose with lacrymation, salivation, and nasal discharge (Spencer et al. 1942, see 3.1.1 for further information). At this concentration, no other signs of intoxication were noted until 12 hours of exposure when general weakness and unsteadiness became apparent. These effects were more evident at 2000 ppm but more pronounced signs of effects on the CNS with loss of consciousness were only seen in “some” animals after 24 – 30 hours. At 2500 ppm, rats showed definite CNS depression (weakness, stupor, incoordination, loss of equilibrium, tremor, finally unconsciousness) after 10 – 12 hours. Animals were “usually” completely unconscious within one hour exposure to 5000 ppm and even more rapid at 10,000 ppm. Unconsciousness was preceded by loss of quilibrium, falling on the side, running leg movements, tremors and convulsions. In addition to the immediate irritant action and the effects on the CNS, pulmonary changes were observed. These varied from slight congestion to hemorrhages, edema, exudation, and leucocytic infiltration. Generally, the severity of effects varied with the exposure concentration and duration. Marked pulmonary lesions were seen at all concentrations when the exposure time was so long that at least some of the exposed animals died following exposure. Regarding other organs, liver and kidney changes were seen in “comparatively few animals”; these changes were most often recorded at 2500 ppm but less frequently at higher concentrations.

Irritation during exposure also was observed in a subchronic study in which female and male CD (Sprague-Dawley) rats (10 females, 10 males per group) were exposed to analytically (gas

chromatography) confirmed concentrations of 200, 500, 1000 and 1500 ppm styrene in 0.75 m³ inhalation chambers for 6 hours/day, 5 days/week for 13 weeks (Cruzan et al. 1997b). Irritation was observed at all concentrations during exposure. Signs reached from closed eyes at 200 ppm to salivation and rubbing of paws and chin on the cage at higher concentrations. Similar effects (salivation, restlessness, hunched posture) were also observed in groups of 70 male and female CD rats each during exposure to 500 and 1000 ppm for 6 hours/day, 5 days/week for 104 weeks; generally, effects tended to decrease during each week of exposure (Cruzan et al. 1998).

Salivation and reduced attention were observed in a subacute study in which 10 male Wistar rats/group were exposed 6 hours/day on 5 consecutive days to an analytically confirmed concentration of 1500 ppm. At the beginning of the exposure period, signs of sensory irritation were also observed at 500 ppm but not at 150 ppm (Jarry et al. 2002).

Male Wistar rats were exposed to analytically (infrared spectophotometry) confirmed concentrations of 0, 100, 300, or 600 ppm styrene 12 hours/day, 5 days/week for 4 weeks (Mäkitie et al. 2003). During the exposures, the animals were mostly recumbent, especially at 600 ppm. The authors saw no clear signs of irritation of skin, eye or mucous membranes at the end of the daily exposures.

TABLE 4: SUMMARY OF ACUTE NON-LETHAL EFFECTS IN ANIMALS AFTER INHALATION EXPOSURE TO STYRENE

Species (strain, sex, no./ group)[a]	Concentration (ppm)	Exposure Duration	Effect	Reference
Rat (Wistar, m)	2000 ppm	5 hours	Loss of consciousness in "many of the test animals"	Withey and Collins 1979
Rat (nd; 18 f, 15 m)	1730 ppm	1 hour	Inability to suppress nystagmus	Niklasson et al. 1993
Rat (Wistar, 10 m)	1500 ppm 500 ppm	6 hours	Reduced attention, sensory irritation Sensory irritation at start of exposure	Jarry et al. 2002
Rat (nd)	4-hour LC_{50} (2760 ppm)	1 hour	State of deep narcosis	Shugaev 1969
Mouse (Swiss OF_1, 10 m)	549 ppm	4 hours	50 % decrease in immobility time in behavioral "despair swimming" test	de Ceaurriz et al. 1983
Mouse (Swiss Webster, m)	156 ppm	3 minutes	RD_{50}	Alarie 1973
Mouse (Swiss OF_1, 6 m)	586 ppm	5 minutes	RD_{50}	de Ceaurriz et al. 1981
Mouse (Swiss Webster, m)	980 ppm	10 minutes	RD_{50}	Bos et al. 1992
Mouse (NMRI, 10 f, 10 m)	1420 ppm 2983 ppm 3766 ppm	4 hours	Staggered gait Apathy Narcosis	BASF 1979a

Effects of styrene on the vestibulo- and opto-oculo motor system effects were studied in a total number of 18 female and 15 male "pigmented rats" (Niklasson et al. 1993). Each rat was used for an initial control experiment with no solvent exposure and one or two subsequent experiments with exposure

to one or two styrene concentration levels. Exposure for each animal was separated by at least one week. Animals were exposed in a dynamic exposure chamber to nominal concentrations (fluctuating within about 15 % as confirmed analytically during all exposures) of 3600; 7400; 13,400; 17,300; and 21,800 mg/m³ of styrene (840, 1730, 3140, 4050, 5100 ppm). 10 minutes after initiation of exposure, a few eye saccades were provoked and registered. Then, during continuous exposure, repeated vestibular stimulations in darkness and combined vestibular and optokinetic stimulations were performed (lasting 35 minutes) followed by optokinetic stimulations (lasting 15 minutes). Optokinetic stimulation caused nystagmus with a gain that was reduced by styrene exposure in a concentration-dependent manner. Effects could be observed at the lowest concentration of styrene applied. Repeated vestibular stimulations in darkness showed a prolongation of nystagmus at 4050 and 5100 ppm. The combined vestibular and optokinetic stimulation caused no or little nystagmus in control experiments since the visual input suppressed the vestibular reaction. Styrene exposure caused a concentration-dependent inability to suppress the nystagmus at concentrations ≥ 1730 ppm.

Pulmonary toxicity was studied in Sprague-Dawley rats (Green et al. 2001b). Groups of 5 female and 5 male animals were exposed "whole body" in 3.4 m³ chambers to analytically (gas chromatography) controlled concentrations of 0 or 500 ppm styrene for 6 hours. No treatment-related effects were observed in the lung of animals exposed to styrene for 1, 5, 6, or 10 days.

The serum activity of sorbitol dehydrogenase (SDH) was used as an indicator of liver damage in a study with female Sprague-Dawley rats (Lundberg et al. 1986). Animals were exposed from 1/32 to ½ of the saturation concentration of styrene in air (33,200 mg/m³; 7769 ppm) for 4 hours and sacrificed 20 hours after the end of exposure. No increase in serum SDH-activity was observed at any concentration.

Studies with non-inhalation exposure

In the study of Lundberg et al. (1986), female Sprague-Dawley rats were also treated by i.p. injections of styrene in peanut oil at doses of 1/8, 1/16, and 1/32 of the LD_{50} (898 mg/kg b.w.). The serum activity of sorbitol dehydrogenase (SDH) was used as an indicator of liver damage; no increase was observed at any of the concentrations.

Studies with repeated inhalation exposure

The degeneration and regeneration of respiratory mucosa of the trachea and the nose following subacute exposure of male Sprague-Dawley rats was studied by Ohashi et al. (1986). Groups of 10 animals each were exposed to analytically (gas chromatography) determined concentrations of 171 ± 21.8 ppm or 1108 ± 73.8 ppm of styrene or air in dynamic exposure chambers for 4 hours/day, 5 days/week for 3 weeks. On the first day of the post-exposure period, the ciliary activity of the tracheal mucosa showed some deterioration in the 171-ppm group (80 % of control value). There was also an increased number of dense bodies and small vacuoles in the epithelial cells and small compound cilia were observed but there was no severe degeneration of epithelial cells. In the nasal mucosa, ciliary activity was reduced (41 % of control) and morphological alterations (ballooning of cells, fewer ciliated cells, increase of dense bodies) were seen. 12 weeks after the last exposure, the ciliar activity of the tracheal and nasal mucosa was normal and cells with an increased number of dense bodies were only sporadically found in the trachea. At 1200 ppm, ciliar activity of the tracheal mucosa was poor (18 % of control) the first day after the last exposure, and cells showed morphological changes (vacuolization, increased number of dense bodies, cytoplasm protuberances). In the nasal mucosa, ciliary activity was disabled the first post-exposure day, there were few ciliated cells and severe degeneration of epithelial cells. The effects in the trachea largely resolved within the following 12 weeks but in the nasal mucosa reduced ciliary activity and morphological alterations were still detectable.

In the nasal olfactory epithelium of female and male CD (Sprague-Dawley) rats, histopathological changes were seen in a subchronic study after exposure to 500, 1000 and 1500 ppm for 6 hours/day, 5 days/week for 13 weeks. At 200 ppm, no effects were seen. In the same study, no styrene-related effects were observed in the lungs at any concentration (Cruzan et al. 1997b).

Effects on serum prolactin and dopamine levels and on hypothalamic and striatal catecholamie concentrations were studied in male Wistar rats (Jarry et al. 2002). Groups of 10 animals each were exposed to analytically (gas chromatography) confirmed concentrations of 0, 150, 500, and 1500 ppm styrene for 6 hours/day on 5 consecutive days. Parameters were measured immediately after the end of exposure and after a recovery period of 24 hours. No significant changes in dopamine, dihydroxyphenylacetic acid, noradrenaline, and homovanillic acid levels were observed in hypothalamus and striatum of styrene-exposed rats compared to controls. Also, no change in prolactin level in serum was observed. The dopamine level in peripheral blood was higher at 150 ppm and at 1500 ppm in the 24-hour recovery group at 150 ppm, but no concentration-response was obvious; no change was seen immediate after cessation of exposure.

Ototoxicity of styrene was investigated in several studies. When male Long-Evans rats were exposed to concentrations between 500 ppm and 1500 ppm 6 hours/day, 5 days/week, for 4 weeks, a permanent increase in auditory threshold was observed at mid frequency ranges in animals exposed to 850 and 1000 ppm. At higher concentrations, the threshold was increased in the mid-low, mid- and high frequency (Loquet et al. 1999). In a further study, Long-Evans rats were exposed to 1000 ppm, 6 hours/day, for 5 days. Immediately after the end of exposure, cochlear function as tested by DPOAE (distortion product otoacoustic emissions) showed no decrease in DPOAE amplitude compared to pre-exposure. However, 2 and 4 weeks after the end of exposure, DPOAE indicated a disruption of auditory function (Lataye et al. 2003). Histological lesions of the cochlea and worsening of the electrophysiological results (evoked potentials from the inferior colliculus of the cochlea) after the end of exposure was also described in another publication of the same group in which Long-Evans rats were exposed to 1000 ppm, 6 hours/day, 5 days/week, for up to 4 weeks (Campo et al. 2001). In male Wistrar rats exposed to 100, 300, or 600 ppm styrene for 12 hours/day, 5 days/week, for 4 weeks, 100 and 300 ppm caused no hearing impairment as measured by auditory brain response (ABR). At 600 ppm, a slight hearing loss (~ 3 dB) was observed only at the highest test frequeny of 8 kHz; cytocochleograms showed a substantial loss of the outer hair cells. A synergism with exposure to noise (100 dB) was observed only when styrene was applied in concentrations that were ototoxic without noise (Mäkitie et al. 2003).

3.2.3 Mice

10 female and 10 male NMRI mice per dose group were exposed to 680, 864, 1420, 1528, 2983 or 3766 ppm styrene for 4 hours (BASF 1979a, see 3.1.2). Symptoms (not reported separately for both sexes) included hunched position at exposures exceeding 1420 ppm and rough fur at all concentrations. Styrene was irritating to the respiratory tract and to the eyes (intermittent breathing at all concentrations, rubbing of nose and mouth, secretion from nose and eyes, eyes closed at 2983 and 3766 ppm). Signs of CNS impairment (staggered or stalking gait) were noted from 1420 ppm upwards and were more severe at higher concentrations with apathy and narcosis occurring at 2983 and 3766 ppm.

In a subacute study, B6C3F1 and CD-1 mice (20 per sex and dose group) were exposed in 0.75 m^3 inhalation chambers to analytically confirmed concentrations of 0, 14, 58, 250 or 519 ppm styrene for 6 hours/day, 5 days/week for 14 days (Cruzan et al. 1997b). Mice exposed to all concentrations of styrene showed signs of irritation. At 500 ppm, animals adopted a prone position during exposure. Treatment related signs between exposures occurred in mice exposed to 250 or 519 ppm. These signs included lethargy, shallow breathing, and unsteady gait. 250 and 500 ppm caused liver lesions with

centrilobular hepatocyte necrosis and associated changes. B6C3F1 mice were more susceptible than CD-1 mice. Mortality also occurred at these two highest concentrations (see 3.1.2).

Sensory irritation

Male Swiss OF_1 mice (six at each concentration) were exposed "head only" to at least 4 different analytically (gas chromatography) controlled concentrations of styrene in dynamic 200 L inhalation test chambers for 5 minutes. For the determination of the reflex decrease in respiratory rate that served as an index of sensory irritation, the animals were secured in individual body plethysmographs. An RD_{50} of 586 ppm (no confidence limits given) was determined (de Ceaurriz et al. 1981).

In a similar study, Swiss Webster mice were exposed to styrene for 3 minutes (Alarie 1973). The test substance was solubilized in polyethylene glycol and aerosols were prepared. An RD_{50} of 666 µg/l (156 ppm) (95 % conf. limit 574 – 758 µg/l; 134 – 177 ppm) was determined. In a further study by the same author (cited in Bos et al. 1992), Swiss Webster mice were exposed for 10 minutes and an RD_{50} of 980 ppm (85 % conf. limit 826 – 1297 ppm) was determined.

Behavioral studies

Behavioral changes in a "despair swimming test" were studied in Swiss OF1 mice (de Ceaurriz et al. 1983). Groups of 10 male animals were exposed to analytically (gas chromatography) confirmed concentrations of 413, 610, 807, or 851 ppm styrene or air in 200-L chambers for 4 hours. Immediately afterwards, total duration of immobility during a 3-minute period in a "despair swimming test" was determined. Immobility was defined as cessation of struggling to get out of the water (the animal then remains floating passively in the water in a semihorizontal position only making movements necessary to keep its head above the water). Exposure to solvents including styrene caused a dose-dependent decrease in duration of immobility as compared to the corresponding controls. In case of styrene, the mean duration of immobility decreased significantly by 28, 60, 77, or 83 % of control at the concentrations noted above. An ID_{50} (50 % decrease in immobility) of 549 ppm (95 % confidence interval 522 – 573 ppm) was calculated.

Immunological effects

It is reported that female BALB/c mice (6 per group) exposed to 300 ppm but not to 200 or 100 ppm styrene showed an increase in IgM response of lung-associated lymph nodes in an anti-SRBC (sheep red blood cells) assay. Furthermore, the ex vivo release of γ-interferon from lung-associated lymph nodes decreased with increasing concentration of styrene but was higher than control values at all styrene concentrations. No effects were seen in the spleen (Ban et al. 2003). An evaluation of the results is not possible since the duration of exposure is not reported.

Studies with repeated inhalation exposure

Pulmonary toxicity was studied in CD-1 mice (Green et al. 2001b). Groups of 5 female and 5 male mice were exposed "whole body" in 3.4 m³ chambers to analytically (gas chromatography) controlled concentrations of 0, 40 or 160 ppm styrene for 6 hours/day for up to 5 days and, following a 2-day break, for further 5 days. At 40 ppm, in mice killed immediately after a single exposure, there was evidence of necrosis and loss of cells, believed to be Clara cells, from large bronchioles, while Clara cells in the terminal bronchioles were not overtly affected. At 160 ppm, no significant effect was seen at this time point. In animals killed 18 hours after exposure, minimal necrosis but treatment-related focal loss of cytoplasm from non-ciliated cells was observed, predominantly at the terminal bronchiolar area. Females seemed slightly more affected than males. The lesions observed at this time point were similar at both

styrene concentrations. In mice that had received 5-bromo-2-deoxyuridine 3 days prior to sacrifice, no evidence of an increase in cell replication in the alveoli, terminal or large bronchioles was observed after one day of exposure to styrene. In the lung of mice exposed to styrene for up to 10 times, focal crowding of non-ciliated cells was observed in the bronchiolar epithelium, particularly in the terminal bronchiolar regions. Again, the effects were similar at both styrene doses. Furthermore, there was an increase in the cell replication in the larger and the terminal bronchioles (but not in the alveoli) as indicated by an increased labelling index; the inhibition of styrene metabolism by 5-phenyl-1-pentyne abolished this effect.

In a further study of the authors, the toxicity of styrene to the nasal epithelium was studied in male CD-1 mice. 20 mice per dose group were exposed to analytically (gas chromatography) confirmed concentrations of 0, 40 or 160 ppm styrene for 6 hours/day for 3 days. Mice were killed 17 hours after the last exposure. At 160 ppm, degenerative, mostly focal changes in the olfactory tissue were observed in all mice. Most obvious was the presence of cellular and serious fluid exudate in the airways of the nasal passages in the olfactory epithelium of the dorsal meatus. Atrophy of the olfactory mucosa with loss of cellular organisation and focal decrease of Bowman´s glands were also observed. At 40 ppm, animals were largely unaffected, only one mouse showed minimal atrophy of the olfactory mucosa (Green et al. 2001a).

Effects on the nasal passages and the lung were also investigated by Cruzan et al. (2001). Groups of 55 male CD-1 mice were exposed to analytically (gas chromatography) confirmed concentrations of 0, 40 or 80 ppm styrene in 2.43 m³ inhalation chambers for 6 hours/day, 5 days/week for up to 13 weeks. A subgroup of 5 mice was terminated after one exposure (and further subgroups after repeated exposures). In the nasal olfactory epithelium, single cell necrosis was found after a single exposure to 80 ppm, but not to 40 ppm. No changes were observed in the lung at 40 or 80 ppm up to the end of the 13th week.

3.2.4 Guinea pigs

In the study of Spencer et al. (1942), guinea pigs generally showed the same reactions to styrene exposure than rats: irritation of mucous membranes, CNS-depression and pulmonary changes (see 3.2.2). The effects occurred at the same concentrations, however, it was reported that under comparable conditions of exposure the pulmonary changes were more severe in guinea pigs than in rats.

Studies with repeated inhalation exposure

In a study on ototoxicity of styrene, pigmented guinea pigs were exposed to 1000 ppm, 6 hours/day, for 5 days. Cochlear function as tested by DPOAE (distortion product otoacoustic emissions) was measured before exposure, immediately afterwards, and 2 and 4 weeks after exposure. In contrast to the observations made in rats similarily exposed in the same study (see 3.2.2), no disruption of auditory function was observed in guinea pigs (Lataye et al. 2003).

3.2.5 Rabbits

Studies with repeated inhalation exposure

In the study of Spencer et al. (1942), 2 rabbits (strain, sex, and further experimental details not reported) received up to 126 exposures to 2000 ppm for 7.5 – 8 hours/day, 5 days/week The rabbits were reported not to be affected by these exposures. In contrast, rats and guinea pigs showed marked eye and nose irritation during the exposures.

3.3 Developmental/Reproductive Toxicity

Data are available from studies with rats, mice, rabbits, and hamsters (for review, see Brown 1991; 2000; IARC 2002).

3.3.1 Rats

No studies were identified concerning the effects of a single inhalation exposure to styrene on developmental or reproductive toxicity.

Studies with non-inhalation exposure

Sprague-Dawley rats were treated on the 11th day of gestation with a single dose of 300 mg/kg styrene in corn oil by gavage (Daston et al. 1991). The dose led to maternal toxicity (decreased body weight, reduced food intake) but no effects on pre- and postimplantation losses, malformations and variations were observed on gestation day 20.

In a carcinogenicity study (Ponomarkov and Tomatis 1978), female BD IV rats were given 1350 mg/kg b.w. styrene in olive oil by gavage on day 17 of gestation (followed by weekly treatment of the offsprings with 500 mg/kg b.w. after weaning). Preweaning mortality in offsprings from styrene-treated dams was non-significantly higher (10 %) than in the control group (2.5 %). There was no effect on litter size at birth, postweaning survival, or body weight development.

Studies with repeated inhalation exposure

A "segment II" developmental toxicity study was conducted by Murray et al. (1978). Female Sprague-Dawley rats were exposed to 0, 300 or 600 ppm for 7 hours/day during day 6 – 15 of gestation. Both styrene concentrations were maternally toxic (decreased body weight gain and decreased food consumption). A greater incidence of skeletal variations but no other embryo or fetal developmental effects were observed in offsprings of styrene-treated dams compared to controls. The authors reported that the observed incidence was within the range (number not reported) of historical controls.

Postnatal neurochemical changes, growth, and physical landmarks of development were studied in offsprings of female Wistar rats that had been treated with 0, 50 or 300 ppm 6 hours/day during gestation day 6 – 20 (Katakura et al. 2001). To adjust for nutritional effects, pair-fed and ad-libitum controls were included. Food consumption of dams was decreased at 300 ppm, but maternal weight gain was not significantly different from that of both control groups. Litter size, birth weight and sex ratio were found to exhibit no effects within the variation range studied. At 300 ppm, an increased neonatal death rate was observed compared to the pair-fed control group. Postnatal development (incisor eruption, eye opening, air righting reflex) was also delayed at 300 ppm compared to both control groups. Furthermore, neurochemical alterations were observed as indicated by a significantly decreased 5-hydroxytryptamine concentration in the cerebrum at postnatal day 21 in offspring exposed in utero to 300 ppm styrene. These results suggest that the offspring were susceptible to the effects of styrene on a few developmental landmarks and the results support previous findings of alterations in postnatal development in offsprings of styrene treated dams (Kishi et al. 1992; 1995).

A two generation reproduction study was carried out with CD rats. Male and female animals were exposed daily to 0, 50, 150, and 500 ppm styrene for 6 hours/day for at least 70 consecutive days prior to mating for the F0 and F1 generations. Inhalation exposure for the F0 and F1 females continued throughout mating and gestation through gestation day 20. Inhalation exposure of the F0 and F1 females was suspended from gestation day 21 through lactation day 4. On lactation days 1 through 4, the F0 and

F1 females received styrene via oral gavage at dose levels of 66, 117, and 300 mg/kg · d, calculated to provide similar maternal blood peak concentrations as provided by the inhalation exposures. Inhalation exposure of the F0 and F1 females was re-initiated on lactation day 5. Rats in the 150- and 500-ppm groups in both parental generations gained weight more slowly than the controls. Degeneration of the olfactory epithelium was observed in the F0 and F1 generation at the highest dose. There were no adverse effects on fertility and reproduction at any dose. F2 birthweights were reduced at the highest dose and F2 offspring from both the 150- and 500-ppm exposure groups gained weight more slowly than the controls (Cruzan et al. 2005a).

In a companion developmental neurotoxicity to this two generation study, adverse functional and/or morphological effects on the neurological system in the F2 offspring following F0 and F1 generation exposure to styrene was assessed (for exposure conditions, see above). There were exposure-related reductions in mean body weights of the F1 and F2 offspring from the mid and high-exposure groups and an overall pattern of slightly delayed development in the F2 offspring from the highest dose group. This delay included reduced body weight (which continued through day 70) and a slightly delayed acquisition of some physical landmarks of development. Functional observational battery evaluations conducted for all F1 dams during the gestation and lactation periods and for the F2 offspring were unaffected by styrene exposure. 50 ppm was considered to be the NOAEL for growth of F2 offspring; and 500 ppm to be the NOAEL for F2 developmental neurotoxicity (Cruzan et al. 2005b).

Studies with non-inhalation exposure

In the study of Murray et al. (1978) (see above), pregnant rats were also treated with 90 or 150 mg/kg b.w. styrene by gavage twice daily from the 6^{th} to 15^{th} day of gestation. Compared to non-treated controls, maternal weight gain was reduced and the incidence of skeletal variation was higher in styrene-exposed groups. However, the authors reported that the observed incidences were within the range (numbers not reported) of historical controls.

In a further segment-II teratology study, albino rats were treated orally with 250 or 400 mg/kg b.w. styrene in peanut oil on gestation days 6 – 15 (Srivastava et al. 1990). At 400 mg/kg b.w., maternal toxicity (severe reduction in weight gain), increased pre- and postimplantation losses and reduction in fetal weight was observed but no gross or structural defects. No maternal toxicity and embryo/fetotoxic or developmental effects were seen at 250 mg/kg b.w.

Maternal toxicity (severe reduction in body weight, but no deaths) were also seen in a further study in which Sprague-Dawley rats were administered 1147 mg/kg b.w. styrene on gestation day 6 – 15 (Chernoff et al. 1990). No differences compared to control were observed with respect to fetal weight, embryo/fetal death, and skeletal or soft tissue malformations or variations.

Interactions of styrene exposure with protein malnutrition were studied by Khanna et al. (1991). Rats were given a diet of 20 % casein or 8 % casein throughout pregnancy and lactation, with or without 100 mg/kg b.w. of styrene given orally from day 6 of gestation onward. Low casein diet alone led to a reduction in postnatal weight gain and a delay in development (eye opening, behavioral responses). These effects were more pronounced in pups of dams that were treated with styrene and receiving the low casein diet. These pups also showed a decrease in brain enzyme activities. No such effects were seen in offsprings of dams that were given the normal casein diet.

In a three-generation reproductive toxicity study, female and male rats were continuously exposed to 125 or 250 ppm styrene in drinking water (7 – 10 mg/kg b.w. or 14 – 21 mg/kg b.w., respectively). Water consumption was reduced in both groups. In high dose females, body weight gain

was slightly reduced but no consistent treatment-related effects on pup survival, pup body weights, or developmental parameters could be observed (Beliles et al. 1985).

3.3.2 Mice

In a carcinogenicity study (Ponomarkov and Tomatis 1978), female O20 and C57Bl mice were given styrene in olive oil by gavage on day 17 of gestation (O20: 1350 mg/kg b.w.; C57Bl: 300 mg/kg b.w. each followed by weekly treatment of the offsprings with the same dose after weaning). In O20 mice, the maximum tolerated dose was exceeded. There was no effect on litter size at birth or on body weight gain but survival prior to weaning was decreased in the offspring of treated animals. In C57Bl mice, no effects on maternal mortality, litter size or preweaning mortality was observed.

Studies with repeated inhalation exposure

Pregnant female BMR/T6T6 mice were exposed to an analytically (infrared spectophotometry) controlled concentration of 250 ppm styrene or air (control) for 6 hours/day from the 6^{th} to the 16^{th} day of gestation and sacrificed the last day of exposure (Kankaanpää et al. 1980). The number of dead or resorbed fetuses was higher in styrene exposed mice (26.9 %) compared to controls (18.2 5) but did not reach statistical significance ($0.05 < p < 0.10$). Among 94 live fetuses in the styrene exposed group, 3 were malformed (rib fusion, extra rib), in the control group, among 76 live fetuses, one was malformed (exteriorization of the liver). No statistical evaluation of these results was presented in the report, but it seems unlikely that the effect would have been significant. In preliminary experiments with exposure to 500 and 750 ppm, high maternal mortality was observed (250 ppm: 2/6; 750 ppm: 3/5 died before gestation day 16). Surviving mice carried a high number of dead and resorbed fetuses (fetal death rate at 250 ppm: 47 %; at 750 ppm: 95 %).

3.3.3 Rabbits

Studies with repeated inhalation exposure

A “segment II” developmental toxicity study was conducted by Murray et al. (1978). Female New Zealand white rabbits were exposed to 0, 300 or 600 ppm for 7 hours/day during day 6 – 18 of gestation. No maternal toxicity, no embryo-/fetotoxicity and no teratogenic effects were evident in styrene exposed groups. Compared to the concurrent control, a higher incidence of a single skeletal variation was observed at 600 ppm. However, the authors state that the observed incidence was within the range of historical controls. It is reported (Brown et al. 2000) that styrene is not maternally toxic to rabbits at concentrations up to 1000 ppm so the validity of the study seems to be limited.

3.3.4 Hamsters

Studies with repeated inhalation exposure

Pregnant Chinese hamsters were exposed to analytically (infrared spectophotometry) controlled concentrations of 300, 500, 750, and 1000 ppm styrene or air (control) for 6 hours/day from the 6th to the 18th day of gestation and sacrificed the last day of exposure (Kankaanpää et al. 1980). No fetal/embryotoxic effects or malformations were seen at 300, 500, and 750 ppm. At 1000 ppm, the only effect seen was a significantly increased number of dead or resorbed fetuses (66 %) compared to 26.2 % in the control group.

3.4 Genotoxicity

A large number of studies have been published in which genotoxic effects of styrene and DNA-adducts were investigated *in vitro* and *in vivo*. These studies have been extensively evaluated and summarized in a number of reviews (ATSDR 1992; Cohen et al. 2002; IARC 1994; IARC 2002; Scott and Preston 1994; Vodicka et al. 2002; WHO 1983; WHO 2000). Since a detailed description of the findings from these studies is beyond the scope of this TSD, results described in these reviews are summarized.

Styrene itself does not react with DNA or other nucleophiles *in vitro* in the absence of metabolic activation. The genotoxic potential of styrene depends on the ability of the *in vitro* or *in vivo* system to metabolize styrene to reactive elecrophiles. The main primary metabolite of styrene in mammals is styrene oxide (SO), an electrophilic epoxide that is able to form covalent adducts with nucleophiles such as DNA. In accordance with this, SO binds to DNA and shows genotoxic activity *in vitro* and *in vivo.* The potency of styrene in metabolically active test systems is dependent on a number of additional factors, e.g., the ability to detoxify SO to non-reactive metabolites and to repair initial DNA-lesions.

In several studies with bacteria test systems (different strains of *Salmonella typhimurium)*, styrene was not mutagenic in the absence of exogenous metabolic activation system. In the presence of such activation system, mutagenic activity was observed in a few studies but most studies were negative . Styrene induced gene conversion and mitotic recombination in yeast cells *in vitro* and in a host-mediated assay using mice as hosts. In *Drosophila melanogaster,* somatic mutations were only observed in insecticide resistant strains that have a high bioactivation capacity.

In *in vitro* tests using rodent cells, styrene induced sister chromatid exchanges (SCE) in a study using whole-blood rat lymphocyte cultures. An increase in SCE was also observed in several studies with Chinese hamster ovary (CHO) cells, mostly in the presence of exogenous metabolic activation (S9 mix, human erythrocytes), and a further increase was observed by the addition of cyclohexane epoxide, an epoxide hydrolase inhibitor. Furthermore, styrene induced chromosomal aberrations (CA) in Chinese hamster lung (CHL) cells in the presence but not in the absence of exogenous metabolic activation.

In vivo assays with rodents were performed with rats, mice, and hamsters. Chromosomal aberrations (CA) and polyploidy, but not aneuploidy, in bone marrow were observed in one inhalation study in which Wistar rats were exposed to 300 ppm styrene (6 hours/day, 5 days/week, 9 weeks). No increase in CA was observed in other inhalation studies in bone marrow of Sprague-Dawley rats (600, 1000 ppm, 6 hours/day, 5 days/week, 12 months), in blood and spleen lymphocytes of B6C3F1 mice (124 – 491 ppm, 6 hours/day, 14 days), and in bone marrow of Chinese hamsters (300 ppm, 6 hours/day, 4 days or 5 days/week for 3 weeks). Also, no increase in CA in bone marrow was observed following oral administration of styrene in CD-1 mice (1000 mg/kg once; 500 mg/kg for 4 days, 200 mg/kg for 70 days) or i.p. administration in C57/BL6 mice (50 – 1000 mg/kg). Induction of micronuclei (MN) in bone marrow occurred following i.p. treatment of C57BL6 mice (250 – 1500 mg/kg), but not in blood

erythrocytes and spleen lymphocytes of B6C3F1 mice following inhalation exposure (124 – 491 ppm, 6 hours/day, 14 days) or in bone marrow of hamsters after i.p. administration (1000 mg/kg). Sister chromatid exchanges (SCE) were not observed in blood lymphocytes of F344 rats after inhalation of styrene (150 – 1000 ppm, 6 hours /day, 5 days/week, up to 4 weeks). In mice, however, an increase in SCE in liver, bone marrow, alveolar macrophages, lung, blood and spleen lymphocytes was observed after single (922 ppm, 6 hours) or repeated (387 ppm, 6 hours/day, 4 days) inhalation of styrene in BDF1 mice and in C57/Bl6 and B6C3F1 mice after i.p. administration of styrene. In a recently published study that is not included in the reviews mentioned above, no evidence of clastogenicity in bone marrow of NMRI mice was observed following styrene inhalation exposure at 750 mg/m³ (175 ppm) or 1500 mg/m³ (350 ppm), 6 hours/day, for 1, 3, 7, 14, or 21 consecutive days (Engelhardt et al. 2003).

DNA strand breaks were detected in a comet assay in liver, kidney, lymphocytes, and bone marrow of C57/Bl6 mice following single i.p. administration of 250 or 350 mg/kg b.w. styrene.

DNA-adducts of SO have been detected in several studies with rodents. In CD-1 mice and Sprague-Dawley rats exposed by inhalation to 160 ppm ^{14}C-styrene for 6 hours, an increase of *N*7-guanine DNA-adducts was found in lung in liver of both species 42 hours later. More recent studies use ^{32}P-postlabelling assays to detect and quantify different DNA-adducts. A dose respondent increase in *N*7- and O^6-guanine DNA-adducts of SO could be detected 3 hours after a single i.p. administration of styrene (up to 450 mg/kg b.w.) to NMRI mice. The adduct level in the lungs was higher than that in liver. Similar results were obtained in a further study with NMRI in which animals were exposed by inhalation to 175 or 350 ppm styrene, 6 hours/day, 7 days/week, for 1 – 21 days. The adduct levels increased linearily with time.

Differences in adduct levels between rats and mice with respect to differences in carcinogenicity between these two species were studied by Otteneder et al.(2002). In samples of liver tissue from CD rats treated with styrene via inhalation for 2 years, levels of O^6-SO-guanine adducts were above the limit of detection only in the highest dose group (1000 ppm). It was concluded that rat liver is able to tolerate a comparatively high level of styrene-derived DNA-adducts without a detectable increase of the tumor rate. Further, CD-1 mice were exposed 6 hours/day, 5 days/week, 2 weeks, to 0, 40, or 160 ppm styrene, CD rats were exposed to 0 or 500 ppm. No increase in O^6-SO-guanine adducts could be detected in any of the lung samples despite the observation from carcinogenicity studies that styrene increases the rate of lung tumors in mice but not in rats. The authors concluded that species- and site-specific tumor formation by styrene is not reflected by DNA-adducts in tissues.

3.5 Carcinogenicity

No carcinogenicity studies with single inhalation exposure of animals have been found in the literature.

Studies with non-inhalation exposure

No increase in tumor incidence compared to "vehicle only" controls was observed in 40 female and 40 male Sprague-dawley rats given a single subcutaneous dose of 50 mg styrene per animal in olive oil or four i.p. doses of 50 mg per animal in olive oil over a period of 4 months (Conti et al. 1988).

Studies with repeated inhalation exposure

Carcinogenicity studies with repeated inhalation or oral exposure were performed with different strains of rats (**TABLE 5**) and mice (**TABLE 6**). A detailed review with a critical comprehensive evaluation has recently been published (Cohen et al. 2002).

Rats

Sprague-Dawley rats (initially 96 females, 96 males) were exposed to 0, 600 or 1000 - 1200 ppm styrene in air for 6 hours/day, 5 days/week for 20.7 months (females) or 18.3 months (males). The higher concentration was reduced from 1200 ppm to 1000 ppm after 2 months because of excessive treatment-related effects (decreased weight gain) in male rats (Jersey et al. 1978). The authors observed an incidence (7/85 animals) of mammary adenocarcinoma at 600 ppm in females that was statistically higher compared to the corresponding control (1/85) but was within the range of historical controls (0 – 9 %). There was no significant association at 1000 ppm. The incidence of lymphosarcomas and leukemia in females was identical at both styrene exposures and was not statistically higher than in the corresponding control but exceeded that observed in historical controls.

In another inhalation study, Sprague-Dawley rats (30 females and 30 males) were exposed to 25, 50, 100, 200 or 300 ppm styrene for 4 hours/day, 5 days/week for 52 weeks. The study was terminated when the survival rate reached 50% in at least one experimental group (Conti et al. 1988). Inhalation exposure to styrene was associated with a higher incidence of overall mammary tumors (benign and malignant combined, control: 57 %, exposed: 70 – 83 %) and of malignant mammary tumors alone (control: 10 %, exposed: 13 – 40 %). However, in the colony of rats used the incidence of mammary tumors was quite high and fluctuating and there was no clear concentration-response.

In the most recently conducted inhalation study, groups of 60 female and 60 male CD (Sprague-Dawley derived) rats were exposed to 0, 50, 200, 500 or 1000 ppm styrene for 6 hours/day, 5 days/week for 104 weeks (Cruzan et al. 1998). In female rats, there was no increase of any tumor or in the number of tumor-bearing rats in the exposed groups compared to controls; there was a decrease in pituitary adenomas and mammary adenocarcinomas. In males, a significant trend for an increase in the incidence of interstitial cell testicular adenomas was observed (control: 3.3 %, exposed 3.3 – 11.5 %). However, all rates were within the range of historical controls (0 – 13.5 %), none of the incidences were significantly different from controls by pairwise comparison, and no treatment-related increase in histological alterations (cell hyperplasia, seminiferous tubular atrophy) typically associated with chemically induced interstitial cell tumors was observed. Therefore, the authors judged the observed effect to be incidental and not related to styrene exposure.

TABLE 5: SUMMARY OF RESULTS ON STUDIES OF CANCER IN RATS TREATED WITH STYRENE *

Strain	Exposure			Tumor incidence statistically elevated, type of tumor	Reference
	Route	Concentration or dose	Duration		
SD	Inhalation	600, 1000 - 1200 ppm	6 hours/day, 5 days/week f: 18.3 months, m: 20.7 months	Yes, for mammary adenocarcinoma, but no dose response and elevation within range of historical controls	Jersey et al. 1978
SD	Inhalation	25, 50, 100, 200, 300 ppm	4 hours/day, 5 days/week 52 weeks	Yes, for combined mammary tumors and for malignant mammary tumors only, but no clear concentration response	Conti et al. 1988
CD (SD-derived)	Inhalation	50, 200, 500, 1000 ppm	6 hours/day, 5 days/week 104 weeks	, Positive trend for benign testicular tumors, but elevation within range of historical controls, no pairwise statistical difference between control and any treat-ment group	Cruzan et al. 1998
SD	Gavage	50, 250 mg/kg b.w.	4 or 5 days/week	No	Conti et al. 1988
SD	Drinking water	125, 250 ppm [a]	2 years	No	Beliles et al. 1985
SD	Drinking water	15 – 19 mg/ animal x day	561 days	No	Oettel and Schulze 1962
F 344/N	Gavage	500 mg/kg b.w. 1000, 2000 mg/kg b.w.	5 days/week; 103 weeks 5 days/week, 78 weeks	No	NCI 1979b
F 344/N	Gavage	175, 350, 700 mg/kg b.w.	3 days/week, 79 weeks	No	NCI 1979a
BDIV	Gavage	500 mg/kg b.w.	once a week [b]	No	Ponomarkov and Tomatis 1978

* Table from Cohen et al. (2002), modified and supplemented;
a: doses at 125 ppm were 7.7 mg/kg b.w. in males and 12 mg/kg b.w. in females, at 250 ppm, 14 mg/kg b.w. in males and 21 mg/kg b.w. in females;
b: Dams were administered 1350 mg/kg b.w. styrene on day 17 of gestation, offspring received 500 mg/kg b.w. after weaning once weekly for lifetime.

Studies with non-inhalation exposure

Six studies were performed in which rats were exposed orally by gavage or via drinking water to styrene (Beliles et al. 1985; Conti et al. 1988; NCI 1979b; Oettel and Schulze 1962; Ponomarkov and Tomatis 1978) or to a mixture of 70 % styrene and 30 % ß-nitrostyrene (NCI 1979a). None of these studies did show an association between exposure to styrene and the development of tumors. However, it must be noted that none of these studies are fully acceptable under current standards (number of animals, maximum tolerated dose not reached, low survival, exposure to mixture, or only weekly dosing).

Mice

With mice, only one carcinogenicity study was performed in which the animals were exposed to styrene via inhalation. In this study, 50 female and 50 male CD-1 mice per group were exposed to 0, 20, 40, 80 or 160 ppm styrene for 6 hours/day, 5 days/week for 98 weeks (females) or 104 weeks (males). An increased incidence for lung tumors was observed in male and female mice. The incidence of bronchioalveolar adenomas was significantly increased in males at all except the lowest concentration of styrene, and in females at 20, 40, and 160 ppm. In males, the incidence of bronchio-alveolar carcinomas in the styrene-treated group was not significantly increased compared to the control. In females exposed to 160 ppm, the incidence of bronchio-alveolar carcinomas was higher than in controls. In interim sacrifices after 12 and months, respectively, did not reveal lung tumors in male or female mice. Since the lung tumors observed after 2 years in styrene-exposed and in control mice showed no difference in intensity of immunostaining, tumor location and type of tumor, the authors concluded that styrene increased the number of tumors seen spontaneously in this strain of mice (Cruzan et al. 2001a).

Studies with non-inhalation exposure

In three studies, mice were exposed orally by gavage to styrene (NCI 1979b; Ponomarkov and Tomatis 1978) or to a mixture of 70 % styrene and 30 % ß-nitrostyrene (NCI 1979a). It must be noted that none of these studies is fully acceptable under current standards (number of animals too low, exposure to mixture, or only weekly dosing).

In the NCI study with styrene (NCI 1979b), 50 female and 50 male B6C3F1 mice per styrene dose group (20 females, 20 males for control receiving vehicle only) received 150 or 300 mg/kg b.w. styrene in corn oil for 5 days/week for 78 weeks followed by a 27-week postexposure observation period. No treatment-induced effect on the incidence of tumors was seen in female mice. In males, an increase in the combined incidence of lung adenomas and carcinomas was observed. The incidence in the low-dose group (13.6 %) was clearly higher than in the corresponding control (0 %) but about as high as in historical controls (12 %, range 0 – 20 %). In the high-dose group, the incidence (20.9 %) exceeded that observed in historical controls. In the NCI study using a mixture of styrene and ß-nitrostyrene (NCI 1979a), no increase in the incidence of any tumor was observed.

In the study of Ponomarkov and Tomatis (1978), 29 pregnant O20 mice were treated with 1350 mg/kg b.w. in olive oil on day 17 of gestation. Offsprings (39 females, 45 males) received 1350 mg/kg b.w. in olive oil once a week for 16 weeks when exposure was ended due to high mortality with hepatic necrosis, lung congestion, and spleen hypoplasia (20 % of females and 50 % of males had died). Controls (22 females, 20 males) received vehicle only. Animals were sacrificed after 120 weeks. The combined incidence of lung adenomas and carcinomas was significantly higher in the group of styrene-treated animals. No treatment-related effects were described with respect to other tumors. It must be noted that the maximum tolerated dose had been exceeded.

Ponomarkov and Tomatis (1978) also exposed 15 pregnant C57 mice and their offspring (27 females, 27 males) to styrene similarly as described above but to a lower dose of only 300 mg/kg b.w. There were no effects of styrene on survival, growth or the incidence of any tumor in this experiment.

In a further study with i.p. administration, groups of 25 female A/J mice received a total amount of 200 µmol styrene (20.8 mg) in olive oil 3 times a week for a total of 20 injections (Brunnemann et al. 1992), 25 control animals received vehicle only. 20 weeks after the last dose, three treated animals and one control animal had lung adenoma, the difference was not statistically significant. No lung adenocarcinomas were found in any animal. It must be noted that the total dose applied was very low.

Taking together, these studies provide evidence of an increase of lung tumors in styrene-treated mice.

TABLE 6: SUMMARY OF RESULTS ON STUDIES OF CANCER IN MICE TREATED WITH STYRENE *

Strain	Exposure			Tumor incidence statistically elevated, type of tumor	Reference
	Route	Concentration or dose	Duration		
CD-1	Inhalation	20, 40, 80, 160 ppm	6 hours/day, 5 days/week f: 98 week, m: 104 weeks	Yes, for lung tumors	Cruzan et al. 2001a
O20	Gavage	1350 mg/kg b.w.	once a week [a]	Yes, for lung tumors	Ponomarkov and Tomatis 1978
C57	Gavage	300 mg/kg b.w.	once a week [b]	No	Ponomarkov and Tomatis 1978
B6C3F1	Gavage	150, 300 mg/kg b.w.	5 days/week, 78 weeks	Yes, for lung tumors	NCI 1979b
B6C3F1	Gavage	200, 400 mg/kg b.w.	3 days/week, 78 weeks [c]	No	NCI 1979a
A/J	I.p.	total: 200 µmol (20.8 mg)	3 days/week, 20 injections	No	Brunnemann et al. 1992

* Table from Cohen et al. (2002), modified.
a: Dams were administered 1350 mg/kg b.w. styrene on day 17 of gestation, offspring received 1350 mg/kg b.w. after weaning once a week for lifetime.
b: Dams were administered 300 mg/kg b.w. styrene on day 17 of gestation, offspring received 300 mg/kg b.w. after weaning once a week for lifetime.
c: Mice were given a mixture of 70 % styrene wih 30 % ß-nitrostyrene.

3.6 Summary

Lethality data were available for rats, mice, and guinea pigs. Mice were much more sensitive than the other species as death in this but not in the other species was observed in a number of studies with single or short-term repeated 6-hour exposures to 250 and 500 ppm (Cruzan et al. 1997b; Mahler et al. 1999; Morgan et al. 1993c; Morgan et al. 1993a; Sumner et al. 1997). In contrast, no death occurred in rats upon subchronic daily 6-hour exposures to 1500 ppm (Cruzan et al. 1997b). Guinea pigs could be more sensitive than rats as indicated by the lethality data provided by Stewart et al. (1942), but the data base is

too limited to allow firm conclusions. Limited data for monkeys (4 animals, species not reported; Stewart et al. 1942) that were exposed in a subchronic study at 1300 ppm of styrene for 7-8 hours/day do not provide any evidence that monkeys may be more sensitive to styrene than rats.

In studies with rats, the reported lethality data (LC_0; LC_{50}; LC_{100}) show considerable differences between individual studies:

10,000 ppm	1 hour	LC_0	(Spencer et al. 1942)
5000 ppm	1 hour	LC_0	(Niklasson et al. 1993)
5000 ppm	2 hours	LC_0	(Spencer et al. 1942)
10,000 ppm	3 hours	LC_{100}	(Spencer et al. 1942)
2761 ppm	4 hours	LC_{50}	(Shugaev 1969)
2700 ppm	4 hours	LC_{50}	(Jaeger et al. 1974; abstract only)
6410 ppm	4 hours	LC_{50}	(BASF 1979b)
7769 ppm	4 hours	LC_0	(Lundberg et al. 1986)
1500 ppm	6 hours	LC_0	(Cruzan et al. 1997; repeated exposure)
4618 ppm	6 hours	LC_{50}	(Bonnet et al. 1982)
2500 ppm	8 hours	LC_0	(Spencer et al. 1942)
7769 ppm	8 hours	"LC_{40}"	(Lundberg et al. 1986)
5000 ppm	8 hours	LC_{100}	(Spencer et al. 1942)

Most notable, the 4-hour LC_{50} reported in two studies (Jaeger et al. 1974; Shugaev, 1969) was lower than the LC_{50} in a third study (BASF 1979b) and only 1/3 of the 4-hour LC_0 in another study (Lundberg et al. 1986). Also, it must be noted that these two "low" 4-hour LC_{50} were even lower than the 6-hour LC_{50} in another study (Bonnet et al. 1982b) and similar to an 8-hour LC_0 in a further study (Spencer et al. 1942). Furthermore, the 4-hour LC_{50} of BASF (1979b) and the 6-hour LC_{50} are lower than the 8-hour concentration which caused death in 4/10 animals ("LC_{40}") (Lundberg et al. 1986).

Experimental differences between the studies are likely to have affected the outcomes of the studies. In the study of BASF (1979b), animals were observed for 14 days after the exposure, and delayed deaths that were observed up to 3 days after exposure were taken into account. In contrast, Lundberg et al. (1986) counted the number of deaths only 24 hours after start of the inhalation exposure but not at later time points. Therefore, delayed deaths will have been missed in this study. Niklasson et al. (1993) obviously observed no death during the neurological studies they performed in rats, but it cannot be deduced from their data whether the rats exposed to the highest concentration were observed for one week after exposure or not.

The data of Jaeger et al. (1974) only were published in an abstract lacking any experimental details. Shugaev (1969) also did not report important data, especially, the number of animals and of dose groups used were not given. In addition to data for rats, Shugaev (1969) also reported a 2-hour LC_{50} of 4914 ppm for an unspecified strain of mice. Interestingly, this value is much higher than the LC_{50} for rats although it is clear from a vast number of studies that mice are more susceptible to styrene than rats. Furthermore, whereas the LC_{50} reported by Shugaev (1969) for rats is much lower than the LC_{50} determined in other studies, the opposite is true fot the LC_{50} for mice in Shugaev (1969); this value is much higher than others reported in other studies, even when the different exposure times are taken into account (BASF 1979a; Bonnet et al. 1979b; Izmerov et al. 1982). Although it cannot be ruled out that differences in the susceptibility of different strains of mice to styrene could play some role, it is tempting to speculate that the LC_{50} for rats and mice could have been erroneously mixed up with each other in the publication of Shugaev (1969).

In the study of Spencer et al. (1942), the concentration of 10,000 ppm may be doubted in view of the observation of Lundberg et al. (1986) that 7769 ppm was the highest attainable (analytically confirmed) vapor concentration of styrene. Therefore, it seems possible that in the study of Spencer et al. (1942) some condensation of styrene vapor at the highest concentration used had occurred (leading to a lower vapor concentration but possibly to additional dermal exposure).

The studies of BASF (1979b) and Bonnet et al. (1982a) both reported the experimental conditions in detail (especially, number of animals and dose groups, analytically determined vapor concentrations, and consideration of delayed deaths during post-exposure period). These studies thus provide the most reliable and relevant acute lethality data for rats.

Following i.p. treatment of rats with styrene, no differences in LD_{50} and the corresponding confidence limits were observed when deaths were counted 24 days and 14 days after the injection (Lundberg et al. 1986). This indicates that the delayed deaths observed after inhalation exposure are not due to a systemic effect but probably are related to the local toxic effects that are observed in the lung of animals dying some days after styrene exposure.

Very limited data from one study are available for styrene toxicity in monkeys (species not reported) (Spencer et al. 1942). In this study, none of 4 animals died during subchronic exposure to 1300 ppm styrene, 7 – 8 hours/day, 5 days/week. It was further reported that there were no signs of irritation or intoxication and no pathological findings in inner organs or in hematology compared to 3 control animals.

At non-lethal concentrations, rats showed CNS-depression. Animals were in state of deep narcosis after 1 hour of exposure to the 4-hour LC_{50} (reported to be 2761 ppm, but see discussion above) (Shugaev 1969) and lost consciousness at 2000 ppm after 5 hours (Withey and Collins 1979). Reduced attention was described to occur at 6-hours of exposure to 1500 ppm (Jarry et al. 2002), and an inability to supress nystagmus in an optokinetic test were seen at 1730 ppm after about 30 minutes of exposure (Niklasson et al. 1993). Animals were mostly recumbent at 12 hours of exposure to 600 ppm (Mäkitie et al. 2003), this may also indicate CNS-depression. In mice, signs of CNS-depression that occurred during a 4-hour exposure included staigered gait at 1420 ppm and apathy and finally narcosis at higher concentrations (2983 and 3766 ppm) (BASF 1979a).

In rats, immediate sensory irritation occurred at 1300 ppm (Spencer et al. 1942). Cruzan et al. (1997b, 1998) observed a concentration-dependent increase of irritation reaching from closed eyes at 200 ppm to salivation and rubbing of paws and chin at higher concentrations (500, 100, 1500 ppm). Signs of sensory irritation were also observed at 500 ppm during initial exposure in one study (Jarry et al. 2002), but in another study, no clear signs of eye, skin or mucous membrane irritation could be observed at 600 ppm (Mäkitie et al. 2003). In mice, RD_{50} for sensory irritation of 156 ppm (3 minutes), 586 ppm (5 minutes) and 980 ppm (10 minutes) were reported (Alarie 1973; de Ceaurriz et al. 1981; Bos et al. 1992).

In rats, pulmonary lesions at acute exposure only were observed at concentrations that also caused severe and mostly lethal CNS effects. Mice were more sensitive to styrene than rats. At 250 ppm and 500 ppm, upper respiratory tract and lung toxicity, liver lesions and sometimes death were observed following one or few exposures, and differences in sensitivity between strains were observed; B6C3F1 were most sensitive (Morgan et al. 1993a, c; Mahler et al. 1999; Cruzan et al. 1997; Sumner et al. 1997).

Ototoxicity of styrene was observed in rats after repeated exposure. Exposure for 6 hours/day, 5 days/week, for 4 weeks to 850 ppm and higher concentrations caused a permanent increase in auditory threshold, at 500 ppm, no effect was observed (Loquet et al. 1999). In another study, no effect was seen immediately after exposure to 1000 ppm, 6 hours/day, for 5 days, but tests indicated a disruption of

cochlear auditory function 2 and 4 weeks after the end of exposure. In the same study, no effects were detected in similarily exposed guinea pigs (Lataye et al. 2003). Histological lesions of the cochlea and worsening of the electrophysiological results after the end of exposure were also described in another publication of the same group after exposure of rats to 1000 ppm, 6 hours/day, 5 days/week, for up to 4 weeks (Campo et al. 2001). No hearing impairment was detected in rats exposed to 100 or 300 ppm styrene for 12 hours/day, 5 days/week, for 4 weeks; at 600 ppm, a hearing loss of ~ 3 dB was observed only at the highest test frequeny of 8 kHz, and cytocochleograms showed a substantial loss of the outer hair cells of the cochlea from these animals (Mäkitie et al. 2003).

No studies were available concerning effects of a single inhalation exposure to styrene on reproductive or developmental toxicity. A single oral administration of 300 mg/kg b.w. of styrene on day 11 of gestation caused maternal toxicity in rats, but had no developmental or fetotoxic effects (Daston et al. 1991). In another study with rats and mice that were treated orally with styrene on gestation day 17, 1350 mg/kg b.w. styrene had no significant effect on preweaning mortality, litter size at birth, or body weight development in BD IV rats. In O20 mice, survival prior to weaning was reduced after 1350 mg/kg b.w., no effect was seen in C57Bl mice given 300 mg/kg b.w. (Ponomarkov and Tomatis 1978).

Following repeated 6-hour exposure of rats to 300 ppm during gestation day 6 – 20, an increased neonatal death rate was observed compared to pair-fed controls (that were included to control for a styrene-induced reduction of food intake; maternal weight gain was not affected). Postnatal development (incisor eruption, eye opening, air righting reflex) also was delayed. No effects were seen at 50 ppm (Katakura et al. 2001). These findings supported those from earlier studies in which similar effects were described but no pair-fed controls were used (Kishi et al. 1992; 1995). In a study with hamsters, the number of dead or resorbed fetuses was increased in the group exposed to 1000 ppm 6 hours/day from gestation day 6 -18, but not at 750 ppm or lower concentrations (Kankaanpää et al. 1980). However, in a more recent two generation study with rats, F2 birthweights were reduced at the highest dose and F2 offspring from both the 150- and 500-ppm exposure groups gained weight more slowly than the controls, but there was no increased mortality (Cruzan et al., 2005a). In a companion developmental neurotoxicity study, no effects on the developing neurological system could be observed in offspring of the F1-generation exposed to styrene (Cruzan et al., 2005b). In other studies with repeated oral or inhalation exposure of rats, mice, and rabbits, no significant developmental effects were seen (Murray et al. 1978; Srivastava et al. 1990; Chernoff et al. 1990; Beliles et al. 1985; Kankaanpää et al. 1980).

Styrene is genotoxic *in vitro,* provided there is sufficient activation to styrene oxide (SO), and *in vivo.* Data from laboratory animals indicate that styrene exposure may lead to the formation of DNA-adducts, sister chromatid exchange, and chromosomal aberrations.

With respect to carcinogenicity, no clear effect was observed in rats. In mice, the studies provide evidence for an increase of lung tumors. IARC recently has re-evaluated the data on carcinogenicity of styrene and concluded that there is “limited evidence” in experimental animals for the carcinogenicity of styrene. In the overall evaluation, it was concluded that styrene is “possibly carcinogenic to humans (Group 2B)” (IARC 2002). Styrene is being reassessed under the IRIS Program of the US-EPA, no quantitative carcinogenicity assessment for lifetime exposure is currently proposed (US EPA 1998).

4 SPECIAL CONSIDERATIONS

4.1 Toxicokinetics

The toxicokinetics of styrene both in humans and laboratory animals has been reviewed (e.g. ATSDR 1992; Bond 1989; Cohen et al. 2002; Engelhardt et al. 2003; Government Canada 1993; Linhart 2001; Sherrington and Routledge 2001; Sumner et al. 2001).

Uptake and distribution

In studies with human volunteers and occupationally exposed workers, retention of styrene was about 70 % of the inhaled dose. E.g., in two studies on male healthy volunteers who were exposed to 300 mg/m³ (70 ppm) of styrene during light exercise, the average uptake of styrene was 68 % of the amount supplied; the percentage of uptake was nearly constant during the whole exposure period (0 – 30 minutes: 71.0 %; 90 – 120 minutes: 66.7 % (Wigaeus et al. 1983; Wigaeus et al. 1984).

Experimental and simulation studies suggest a washin-washout effect for styrene in the upper respiratory airways of humans (Jonsson and Johanson 2002). In rats and mice, styrene has been shown to be taken up and metabolized in surgically isolated upper respiratory tract preparations; uptake amounted to about 10 % of styrene at 200 ppm (Morris 2000).

For styrene, a high *in vitro* blood:air partition coefficient at equilibrium of 32 was reported by Astrand (1975). Even higher coefficients of 40 for rats and mice and 52 for humans were reported by Ramsey and Andersen (1984). In controlled human studies *in vivo,* the blood:air coefficient was found to depend on the work load during exercise: At 50 and 150 ppm styrene, the coefficient of the concentration of styrene in alveloar air:blood was 15 at rest and increased to 50, 85, and 105 at work loads of 50 W, 100 W, and 150 W during subsequent 30-minute exposure periods, respectively (Astrand 1975).

Concentrations of styrene determined in blood of humans and rats and in rat brain are summarized in **TABLE 7**. In humans exposed to 69 ppm for two hours during light physical exercise (50 W), the concentration of styrene in arterial blood rose steeply during the first 30 minutes and reached a plateau at about 60 minutes (**FIGURE 3**) (Wigaeus et al. 1984). Controlled studies further show that the concentration of styrene in blood depends on the intensity of physical work load. When volunteers were exposed at 154 ppm styrene for consecutive 30-min periods with increasing intensity of physical exercise (0, light exercise: 50 W, 100 W, heavy exercise: 150 W), the arterial blood concentration increased with increasing exercise activity and was approximately 3-fold higher at 50 W, 5-fold higher at 100 W, and 10-fold higher at 150 W than at rest (Astrand 1975). However, care must be taken when interpreting these observations since a 30-minute exposure period (at 154 ppm) is not sufficient to reach a plateau level of styrene in blood. Thus, the experimental condition in the study of Astrand (1975) leads to an overestimation of the effect of work load.

At higher inhalation concentrations, no constant styrene concentration in blood is reached (data for humans see **FIGURE 4**, Löf and Johanson 1993). In rats exposed to 520, 1274, and 2850 ppm styrene by inhalation for up to 5 hours, the level of styrene in jugular venous blood rose continuously. After about 90 minutes, a nearly linear increase was observed at the three higher concentrations and no equilibrium was reached during exposure. At 45 ppm, no marked increase with continuing exposure was observed (**FIGURE 5**, Withey and Collins 1979).

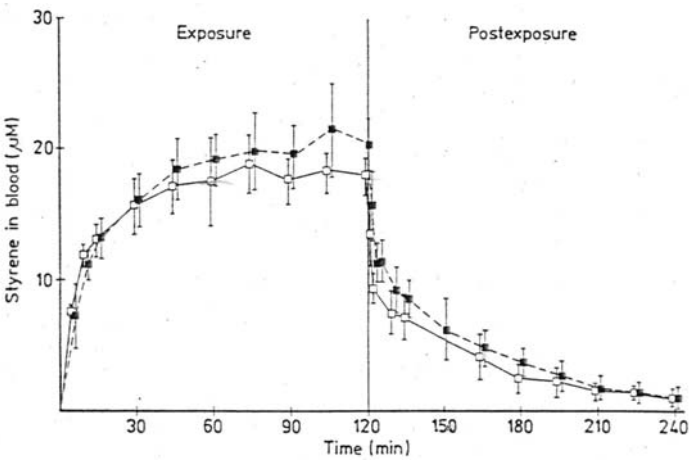

FIGURE 3: STYRENE CONCENTRATION IN ARTERIAL BLOOD OF HUMANS DURING AND AFTER A 2-HOUR EXPOSURE TO 69 PPM STYRENE IN AIR

(Human volunteers (n = 5) were exposed to 69 ppm styrene (open symbols) or a mixture of 70 ppm styrene and 520 ppm acetone (closed symbols) during a work load of 50 W (light exercise) (Graph from Wigaeus et al. 1984). 1 µM = 104 µg/l.).

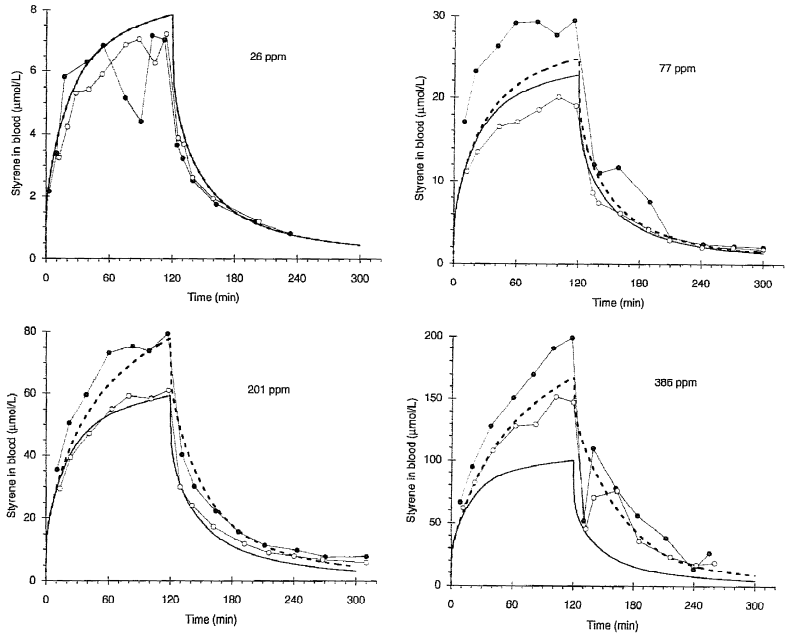

FIGURE 4: OBSERVED (CIRCLES) AND SIMULATED CONCENTRATIONS OF STYRENE IN ARTERIALIZED CAPILLARY BLOOD FROM TWO HUMAN VOLUNTEERS

(2 hours of exposure during light (50 W) exercise. Continuous line: PBPK model simulation with a linear model (nonsaturable metabolism in liver); broken lines: same model with saturable metabolism. The study did not indicate which of the values were determined in the female and the male volunteer. Graph from Löf and Johanson (1993)).

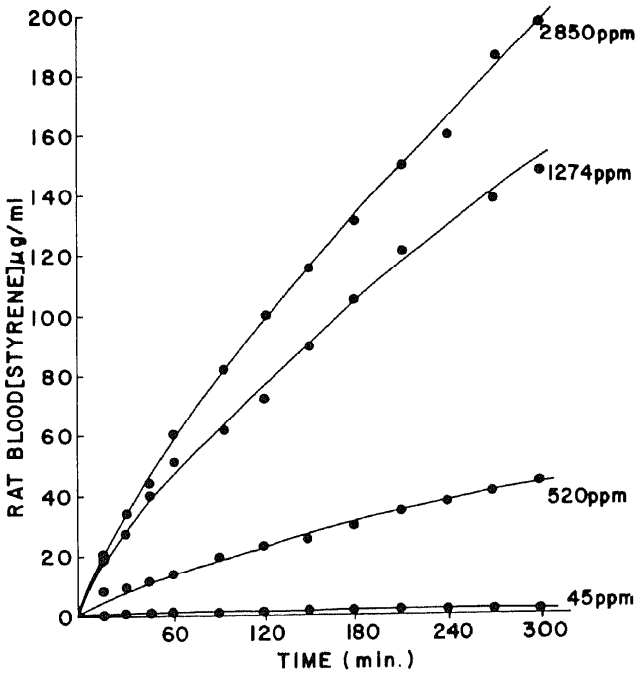

FIGURE 5: STYRENE CONCENTRATION IN BLOOD OF RATS DURING A 5-HOUR EXPOSURE TO DIFFERENT CONCENTRATIONS OF STYRENE IN AIR

(Animals were exposed to the styrene vapor concentrations indicated and styrene was determined in blood from jugular vein at different time points by means of an indwelling cannula fixed prior to exposure. Graph from Withey and Collins 1979.)

Styrene is widely distributed throughout the body. From its high lipophilicity, the highest concentrations may be expected in lipid-rich tissues. However, it must be taken into account that the distribution of styrene is affected by its rapid metabolic clearance (see below). Thus, at low exposure concentrations (54 ppm), the concentration of styrene in rat brain was lower than in blood. At higher concentrations (≥ 470 ppm) where metabolic clearance approaches saturation (see below), the concentration in rat brain was 1.17 – 1.89-fold higher than in blood. Similar effects were observed in heart, lung, liver, and spleen, but not in kidney where the styrene concentration was higher than in blood at all concentrations. Concentrations at least 10-fold higher than in every other tissue were observed in perirenal fat (Withey and Collins 1979). In humans, the ratio between the styrene concentration in subcutaneous adipose tissue (from the gluteal region) and arterial blood reached 3 at the end of a 2-hour exposure to 70 ppm styrene. This value is far below the value in equilibrium that can be calculated from the oil:air (about 5.5) and blood:air partition (52) coefficient of styrene indicating that styrene in adipose tissue does not reach equilibrium under the condition of the study. Taking into account the long half-life of styrene in subcutaneous adipose tissue (2.2 – 5.2 days), the authors further estimated that several days of continuous exposure would be necessary to reach 90 % of steady state (Wigaeus et al. 1983).

TABLE 7: EXPOSURE CONCENTRATIONS AND BLOOD LEVEL OF STYRENE IN HUMANS AND RATS				
Exposure time	**Conc. in air (ppm)**	**Concentration in blood (mg/L) or brain(mg/kg)**	**Remarks**	**Reference**
Humans				
50 min	87-139	2.7	Exposure at light physical exercise (50 W)	Ödkvist et al. 1982
55 min 1 h 55 min 3 h 30 min	51.4 116.7 116.7 99	0.2 – 0.7 (vb) 1.7 (vb) 2.7 (vb) 0.9 – 1.4 (vb)	Exposure at rest	Stewart et al. 1968
30 min 1 h 2 h	69	1.8 (ab) 2.1 2.2	Exposure with light physical exercise (50 W)	Wigaeus et al. 1983; 1984
30 min	154	~ 2 (ab) ~ 6 ~ 9 ~ 16	Exposure at rest 50 W exercise 100 W exercise 150 W exercise; all values estimated from figure	Astrand 1975
2 h	69	1.6 (ab)	Exposure with light physical exercise (50 W); study with occupationally exposed workers	Löf et al. 1984
6 h	80	0.92 (vb)	Exposure at rest	Ramsey et al. 1980
2 h	26 77 201 386	~ 0.7/ 0.7 (acb) ~ 2/ 3.1 ~ 6.2/ 8.3 ~ 15/ 21	Values for 2 volunteers exposed with light physical exercise (50 W); values estimated from figure	Löf and Johanson 1993
Rats				
2 h	520 1274 2850	~ 24 ~ 73 ~ 100	Values estimated from graph	Withey and Collins 1979
5 h	45 520 1274 2800	< 2 (vb) ~ 43 ~ 149 ~ 198	Values estimated from graph	Withey and Collins 1979
5 h	54 470 1018 1522 2144 2240	0.65 (vb)/ 0.2 (brain)[2] 31.8 / 43 65.3 / 76 72.8 / 105 173.7 / 302 135.5 / 256		Withey and Collins 1979
		> 75 (ab)	i.v. exposure; effects on vestibular system as indicated by changes in nystagmus	Tham et al. 1982
6 h	80 1200	1.0 (wb) 63		Ramsey and Andersen

TABLE 7: EXPOSURE CONCENTRATIONS AND BLOOD LEVEL OF STYRENE IN HUMANS AND RATS				
Exposure time	**Conc. in air (ppm)**	**Concentration in blood (mg/L) or brain(mg/kg)**	**Remarks**	**Reference**
				1984
6 h	50 200 500 1000	0.43/ 0.29 (m/f) 2.8/ 1.95 12.5/ 9.5 33.2/ 29.7	Values determined in week 95 of chronic study	Cruzan et al. 1998
4 h 1 h	2760 * 2760 *	250 (brain) 218 (brain) 222 (brain) 177 (brain) 86 (brain) 0 - max. 44 (brain)	Exposure to LC_{50} End of exposure, animal in deep narcosis 15 min after end of exposure 30 min after end of exposure 60 min after end of exposure 90 min after end of exposure	Shugaev 1969
6 h + 4 h[1]	1750	37.5 (ab) 68 (brain)	At end of exposure	Campo et al. 1999

ab: arterial blood; acb: arterialized capillary blood; vb: venous blood; wb: whole blood;
m/f: values for males/females;
1: 6 hours on 1st and 4 hours on 2nd day; 2: approximate concentration, calculated from values presented as brain concentration relative to blood in original reference.
* see section 3.6 for discussion of validity of data from this study.

Metabolism

The metabolism of styrene was compared in male Sprague-Dawley rats and B6C3F1 mice. In both species, the rate of metabolism of inhaled styrene was found to increase linerarily with concentration up to about 300 ppm. In this concentration range, delivery of styrene to the site of metabolism but not metabolic capacity was the rate-limiting step for metabolism. Above 300 ppm, the rate of metabolism at steady state became more and more limited by metabolic parameters. Metabolism approached nearly saturation at about 700 ppm in rats and 800 ppm in mice; exposure concentrations at half maximum rates of metabolism were 190 ppm in rats and 270 ppm in mice, respectively. Repeated exposure for 6 hours/day on 5 consecutive days to 150 or 500 ppm caused no detectable changes in the rates of styrene metabolism (Filser et al. 1993). However, evidence for an induction of styrene metabolism in male rats following pre-exposure to styrene was observed in another study in which prior exposure to styrene (1000 ppm, 6 hours/day, 4 days) increased V_{max} about two-fold. Significant induction of styrene metabolism was observed in 24-hr continuous exposure to 400, 600, or 1200 ppm. Calculations using a physiological model of styrene inhalation kinetics to estimate the dynamics of the induction indicated that induction at 1200 ppm began 4.6 hr after the start of exposure and reached 4.4 times the V_{max} of naive rats. No induction occurred in 48-hr exposure to 200 ppm (Andersen et al. 1984).

In humans, evidence from controlled studies indicates that saturation of metabolic capacity to clear styrene becomes noticeable at concentrations around 200 ppm. In one study, one female and one male volunteer were exposed to analytically confirmed concentrations of 26, 77, 201 and 386 ppm styrene vapor for 2 hours with light exercise (50 W). During the 2-h exposure, the concentration of styrene in blood reached a plateau at the lower concentrations in both individuals. At 386 ppm, the arterial styrene concentration only approached a plateau in one individual but continued to increase steadily in the other

(**FIGURE 4**); it was not indicated in the study which of the values were determined in the male and in the female volunteer). This non-linear relationship between the level of exposure to styrene and the concentration of styrene in arterial blood (and also the 0 – 5 hours cumulative excretion of mandelic acid, see below) indicated metabolic saturation. A physiologically based pharmacokinetic model was used to estimate metabolic parameters. According to the model, transition to metabolic saturation is seen at about 100 ppm (at 50 W physical activity) and at about 200 ppm at rest (Löf and Johanson 1993). Previous studies also provided evidence that styrene metabolism in rats, mice and humans becomes saturated at concentrations exceeding 200 ppm. At lower concentrations, the ratio of styrene concentration in blood to inhaled air is controlled by perfusion limited metabolism, while at higher concentrations, the ratio is controlled by the blood:air coefficient (Ramsey and Andersen 1984).

An overview of the pathways of styrene metabolism is presented in **FIGURE 6**. The major metabolic pathway starts with the formation of styrene-7,8-oxide (SO) by cytochrome P450-dependent monoxygenases. SO is either conjugated with glutathione (GSH) to finally produce mercapturic acids or it is hydrolyzed by epoxide hydrolase to styrene glycol that is subsequently oxidized to mandelic and phenylglyoxylic acid. Phenylacetic acid and benzoic acid or rather hippuric acid, its glycine conjugation product, are also found in urine.

Different CYP isozymes are involved in the oxidation of styrene to SO. Based on *in vitro* studies, CYP2B6 and CYP2E1 seem most important in liver and CYP2F2 in lung, but other isozyms also seem to play a role. In mice devoid of CYP2E1 activity (Cyp2e1-null mice), the amount of metabolites derived from SO was higher and that derived from phenylacetaldehyde was lower as compared to control mice. The excretion of total urinary metabolites was higher in "null mice" than in wild-type controls. These data indicate that CYP2E1 may not be a major isozyme involved in the metabolism of styrene to SO in mice (Sumner et al. 2001). Further research has indicated that CYP2F2 is critically involved in the metabolic process of styrene to cause cytotoxicity in mouse lung and nasal epithelium (Cruzan et al. 2002). In humans with individual differences in xenobiotic metabolism capacity determined with enzyme-specific substrates for CYP2E1, CYP1A2, and CYP2D6, no correlation was found between the blood clearance of styrene and the metabolic capacity as measured by urinary excretion of mandelic and phenylglyoxylic acid. Under the experimental conditions (24 and 84 ppm styrene, 1 hour exposure, light exercise), the apparent blood clearance of styrene (1.4 l/min) was similar to the hepatic blood flow (IARC 2002). These data further support the assumption that styrene metabolism at low concentrations is limited by perfusion and not by the capacity of the metabolism.

Qualitatively, styrene metabolism is similar in humans and rodents (**FIGURE 6**), and in both, humans and animals, more than 90 % of styrene taken up is metabolized. However, there are quantitative differences between rats and humans, and, more pronounced, humans and mice. With respect to the metabolism of styrene in the liver, the experimental data indicate that the order of the oxidation rate of styrene to SO is mice > rats > humans, while the order for microsomal epoxide hydrolase activity of the liver is humans > rats > mice (Mendrala et al. 1993). However, enzymatic activities in tissues other than in liver may be different (Vodicka et al. 2002). In humans, conversion of SO preceeds predominantly via oxidation to styrene glycol, whereas conjugation with GSH and formation of mercapturic acid is important in mice and, to a much lesser extent, in rats. Furthermore, in mice up to 30 % of styrene metabolism leads to the formation of phenylaceturic acid. This pathway likely involves the formation of phenylacetaldehyde as a reactive intermediate that is able to react with proteins. In rats and humans, this pathway is of minor impotance (rats: 5 %; humans: less than 5 %) (Sumner et al. 2001).

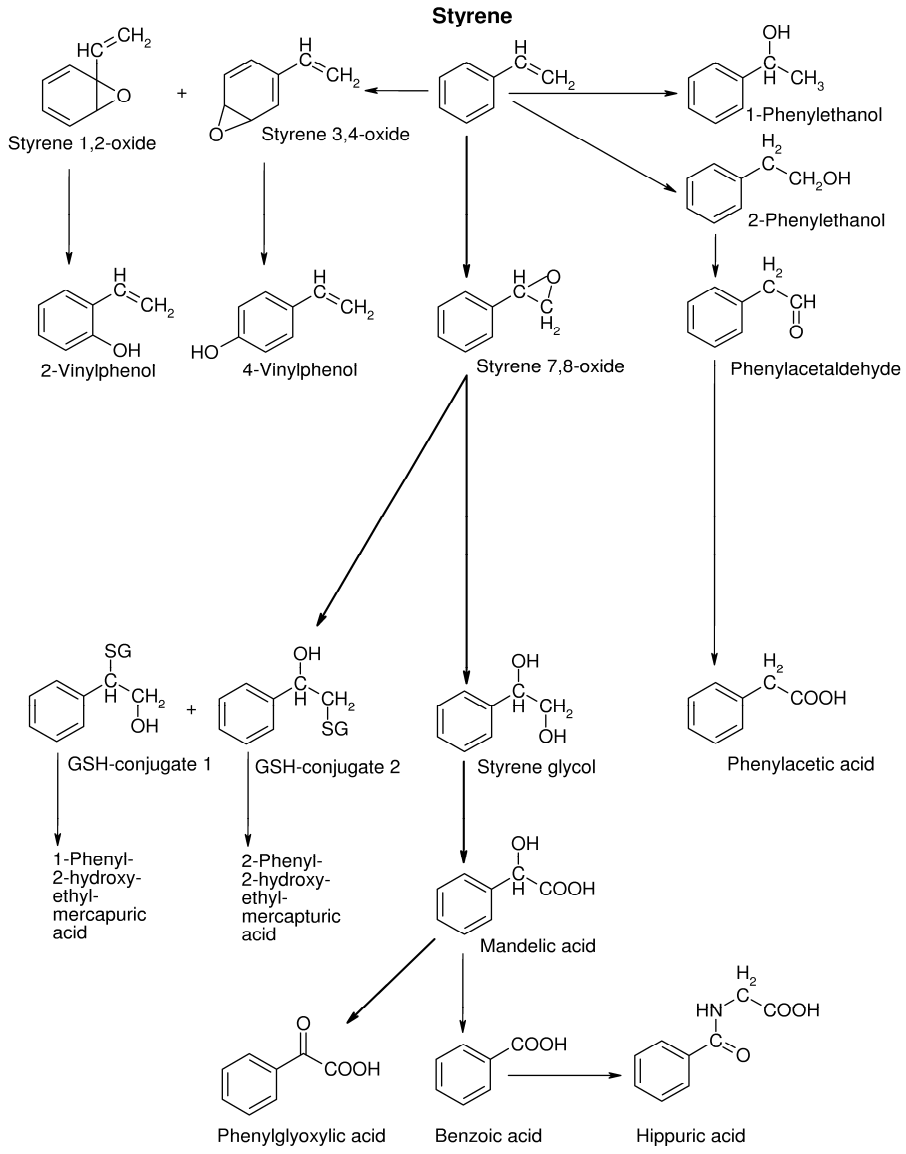

FIGURE 6: PATHWAYS FOR THE METABOLISM OF STYRENE IN HUMANS AND RODENTS

(modified from IARC 2002; main pathways are illustrated by arrows in bold)

Nasal metabolism of styrene is thought to be related to olfactory epithelium toxicity that is pronounced in mice and less or marginal in rats (Cruzan et al. 2002). The metabolism of styrene in nasal epithelia of rats, mice, and humans was studied by Green et al. (2001a). Pretreatment of mice with 5-phenyl-1-pentyne, an inhibitor of CYP2F2 and CYP2E1, prevented the development of nasal lesions upon styrene exposure. Determination of enzyme activities *in vitro* in samples from respiratory and olfactory mucosa of rats and mice revealed that the rates of metabolic formation of SO from styrene in olfactory tissue of both species were similar and higher than in liver, whereas in preparations from respiratory tissue the rates were about half those in the olfactory region and comparable to samples from liver. However, SO is much more efficiently metabolized by both epoxide hydrolase and glutathione transferase in rat

olfactory tissue compared to respiratory tissue or to either region in the mouse. In a limited number of nasal tissue fractions derived from 9 fresh human biopsies in which both respiratory and olfactory epithelium was present in variable amounts, neither metabolism of styrene to styrene oxide nor GSH-transferase activity could be detected. In contrast, epoxide hydrolase activity was detectable and comparable to that in mice. Overall, with respect to the relation between SO-formation and SO-metabolism in nasal epithelia, humans seem similar to rats as the rate of deactivation greatly exceeds activation rates.

Excretion

In a study with human volunteers exposed to 80 ppm styrene, styrene was cleared from the blood in a bi-phasic manner with calculated half-lives of 0.58 and 13 hours for the rapid and the slow clearance phase, respectively (Ramsey et al. 1980). In humans and laboratory animals, very little styrene (less than 5 %) is exhaled unchanged via the lungs. The majority of styrene (> 90 %) is oxidized to water soluble metabolites that are excreted in urine. In humans, mandelic acid (MA) followed by phenylglyoxylic acid (PGA) are the two predominant styrene metabolites that are excreted in urine. The excretion of these metabolites is used as a biological exposure index to monitor exposure in occupationally exposed workers (Schaller and Triebig 2000). Elimination of MA and PGA in urine also follows bi-phasic kinetics with half-times of 4 – 9 and 17 – 26 hours determined for MA and of 10 and 26 hours for PGA (ACGIH 1997). At occupational exposure to average concentrations exceeding 150 ppm, urinary excretion of MA and PGA determined at the end of the worshift appeared to reach a plateau indicating saturation kinetics (Götell et al. 1972). In a controlled human study, cumulative excretion of MA in urine 5 hours after onset of a 2-hour exposure leveled off when the exposure concentration was increased from 201 to 386 ppm. However, cumulative 24-hour excretion of MA was proportional to styrene exposure up to the highest concentration indicating that the metabolism to MA was delayed (Löf and Johanson 1993).

4.2 Mechanism of Toxicity

Styrene has an acute CNS depressant action that is also observed with other alkyl and alkenyl benzenes. This effect is likely related to the physico-chemical properties of the substance and the amount of parent substance in the brain and is not dependent on styrene metabolism. In accordance with this, the acute toxicity (as indicated by the LC_{50}) of styrene for rats falls within the range of LC_{50} for xylenes and toluene (Bonnet et al. 1982a). However, styrene is more irritant than the alkyl benzenes.

Other toxic effects of styrene have been attributed to the formation of reactive metabolites. The main primary metabolite of styrene in mammals is styrene oxide (SO), an electrophilic epoxide that is able to form covalent adducts with nucleophiles such as DNA, but also with proteins and glutathione. However, the underlying mechanisms of toxicity following styrene exposure, most important the development of respiratory lesions and tumors in the mouse, are not yet fully understood.

4.3 Other relevant information

4.3.1 PBPK-Modelling

Several physiology-based pharmacokinetic (PBPK) models have been developed to describe toxicokinetics of styrene in animals and humans (Filser et al. 1993; Jonsson and Johanson 2002; Pierce et al. 1998; Ramsey and Andersen 1984). In recent models, efforts have focussed on the description and prediction of species-specific differences of styrene and styrene oxide metabolism in target organs, especially the lung of mice (Csanady et al. 1994; Csanady et al. 2003; Filser et al. 1999; Filser et al. 2002; Sarangapani et al. 2002; for review see Cohen et al. 2002).

4.3.2 Species variability

Mice are more sensitive to styrene than rats. Lethality was observed in mice, but not in rats, following single or few exposures to 250 and 500 ppm. At these concentrations, lethality was related to respiratory tract toxicity and hepatic lesions which are seen in mice, but not in rats.

In humans, no life threatening effects or long-lasting adverse health effects or death were observed in controlled studies at single exposures up to 800 ppm for 4 hours. Also, no data describing such effects following acute or short-term exposure were identified in occupational studies. Therefore, it is considered that the higher susceptibility of mice is not relevant for the derivation of AEGL values.

The species difference between rats and mice with respect to respiratory tract and hepatic toxicity and carcinogenicity is not only observed in case of styrene, but also with other epoxide-forming chemicals and their epoxides, e.g. butadiene, isoprene, chloroprene, vinyl chloride, and ethylene oxide (IARC 2002).

4.3.3 Susceptible populations

Individuals with a high level of physical activity during exposure may be considered more susceptible than individuals at rest because the concentration of styrene in blood is strongly affected by physical activity. Subjects with high physical activity during exposure will take up more styrene and thus have a higher concentration of styrene in blood than subjects at rest. The influence of physical activity can be explained by physiological models taking into account the blood:air partition efficient and the effect of physical activity on basic physiological parameters (alveolar ventilation, cardiac output, hepatic perfusion) and (Csanady and Filser 2001). In controlled studies, the observed increase of styrene in arterial blood at exposure to about 150 ppm styrene was approximately 3-fold when the physical activity was increased from rest to light exercise (50 W), 5-fold at moderate exercise (100 W), and 10-fold at heavy exercise (150 W). These values are conservative estimates since – as outlined above (see section 4.1) – the experimental conditions lead to an overestimation of the effect.

Individual cases of respiratory or skin sensitization to styrene have been described (Hayes et al. 1991; Moscato et al. 1987; Sjöborg et al. 1982). Taking into account the wide use of styrene both in industry and in consumer products, sensitization seems to be a rare event. However, sensitized individuals may not be able to tolerate styrene concentrations that are without effect in non-sensitized individuals and may not be protected by the AEGL-levels developed for styrene in this TSD.

4.3.4 Concurrent exposure issues

In rats, acetone potentiated the lung toxicity of styrene (Elovaara et al. 1990). No data were available for humans with respect to acute toxic interactions of styrene and other chemicals.

In humans, the toxicokinetics of styrene was not affected by co-exposure with acetone, methanol, or toluene. However, co-administration of ethanol was found to decrease the excretion of mandelic acid and phenylglyoxylic acid in urine (IARC 2002).

5 DATA ANALYSIS AND PROPOSED AEGL-1

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

5.1 Summary of Human Data Relevant to AEGL-1

In humans, the effects associated with acute inhalation exposure are irritation of eyes and mucous membranes and central nervous system depression.

In a study on psychological reactions related to chemosensory irritation, ratings for odor and annoyance increased similarily with increasing styrene concentrations ranging from 0.5 – 40 ppm, while there was only a marginal increase for irritation. Effects sizes comparing the ratings between exposure to 20 ppm and pre-exposure were higher for odor, irritation, and annoyance. Effects sizes were also higher compared to "clean air only"-exposure. However, the ratings for irritation indicated only marginal effects in this respect (Seeber et al. 2002). No increase in irritation or headaches compared to control was noted at 20 ppm in a further study (Hake et al. 1983). Subjective signs and symptoms of irritation and CNS effects were not negatively influenced during a 6-hour exposure at 25 or 50 ppm or at 50 ppm with 4 peak exposures of 15 minutes at 100 ppm (Ska et al. 2003; Vyskocil et al. 2002a,b). At 50 ppm, a further study indicated a slight increase in subjective symptoms ratings for eye and nose irritation, headache, and fatigue (Oltramare et al. 1974). At 100 ppm, Oltramare et al. (1974) reported that signs of irritation and of mild subjective CNS effects (headaches, fatigue, poor concentration, sleepiness) were felt more often than at 50 ppm. Complaints of mild eye and throat irritation at 99 ppm in one test but not in another at 116 ppm were reported by Stewart et al. (1968). Complaints of eye and nose irritation were frequent at about 200 ppm (Oltramare et al. 1974; Stewart et al. 1968) and the severity increased with a further increase in concentration to 376 ppm. 300 – 400 ppm caused immediate lacrymation, and previously non-exposed subjects reported that they could not withstand 500 – 800 ppm for more than 1 – 2 minutes (Götell et al. 1972).

5.2 Summary of Animal Data Relevant to AEGL-1

The RD_{50} as a measure of sensory irritation in mice varied – depending on the exposure duration – between 156 ppm (3 minutes), 586 ppm (5 minutes), and 980 ppm (10 minutes) (Alarie 1973; de Ceaurriz et al. 1981, Bos et al. 1992). In rats, closed eyes at exposure to 200 ppm possibly indicate eye irritation (Cruzan et al. 1997b). Other signs of irritation were salivation (500 ppm), lacrymation (1300 ppm), and rubbing of paws and chin (1500 ppm) (Cruzan et al. 1997b; Stewart et al. 1942; Jarry et al. 2002).

5.3 Derivation of AEGL-1

In an evaluation of reactions related to chemosensory irritation in humans, the ratings for irritation at 20 ppm indicated only marginal effects in this respect. No increase in irritation or headaches compared to control was noted at 20 ppm in a further study. At 50 ppm, a marginal increase in subjective symptoms ratings for eye and nose irritation, headache, and fatigue was described in one, but not in a second study. At 100 ppm, signs of irritation and of mild subjective CNS effects were reported in some studies, but no such effects were seen in others. Complaints of eye and nose irritation were more frequent at about 200 ppm and the severity increased with a further increase in concentration.

Therefore, 20 ppm were selected to derive AEGL-1. Because this concentration represents a NOAEL for local as well as CNS effects and in other studies effects at 50 and 100 ppm were only weak or absent, an intraspecies factor of 1 is applied. The value of 20 ppm was used for all timepoints since slight irritation and subjective discomfort that were reported at higher concentrations did not increase within several hours of exposure.

The derived values are listed below.

TABLE 8: AEGL-1 VALUES FOR STYRENE					
AEGL Level	**10-Minute**	**30-Minute**	**1-Hour**	**4-Hour**	**8-Hour**
AEGL-1	20 ppm	20 ppm	20 ppm	20 ppm	20 ppm

The level of distinct odor awareness (LOA) for styrene is 0.54 ppm. The LOA derivation follows the guidance as described (Van Doorn et al. 2002). The LOA represents the concentration above which it is predicted that more than half of the exposed population will experience at least a distinct odor intensity, about 10 % of the population will experience a strong odor intensity. The LOA should help chemical emergency responders in assessing the public awareness of the exposure due to odor perception. The derived LOA is considered to have warning properties, but it must be noted that accomodation to odor usually occurs within minutes.

6 DATA ANALYSIS AND PROPOSED AEGL-2

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

6.1 Summary of Human Data Relevant to AEGL-2

As outlined above, the effects in humans associated with acute inhalation exposure are irritation and central nervous system depression.

Nasal and mild eye irritation were reported by volunteers exposed to 376 ppm (Stewart et al. 1968). Local irritation was nearly absent when the odor was masked (Gamberale and Hultengren 1974). In their study of styrene exposed workers, Götell et al. (1972) reported that they themselves suffered from lacrymation and irritation of the nasopharynx immediately when exposed to 300 – 400 ppm. Concentrations of 500 – 800 ppm caused irritation that was intolerable to the investigators within 1 or 2 minutes. Strong eye and nasal irritation was also reported by volunteers exposed to concentrations ≥ 600 ppm (Carpenter et al. 1944; Wolf et al. 1956).

With respect to CNS-depression, at 99 ppm intermittent difficulties in performing a modified Romberg test were observed in 3/6 subjects exposed for 7 hours with a 30-minute break in between. Other tests on coordination and on manual dexterity were normal, and no effects were noted at the end of exposure. No CNS effects were seen in another experiment with 116 ppm exposure for 2 hours or 216 ppm for 1 hour in the same study (Stewart et al. 1968). In another study, 6 hour exposure at 50 ppm with 4 repeated 15-minute peaks at 100 ppm had no negative influence on performance to neuropsychological tests (Vyskocil et al. 2002a,b). Headaches, but no effects on equilibrium and cognitive function tests were noted in male and female volunteers at repeated exposures to 100 and 125 ppm for at least one hour (Hake et al. 1983). Oltramare et al. (1974) noted that slight difficulties in balance performance at 50 – 200 ppm (1.5 hours), but there was no concentration-response, and slight difficulties in balance performance at 200 ppm (1 hour), but the variation of data was large. No effects on simple and choice reaction time was seen following exposure to 250 ppm for 30 minutes. However, when the concentration was raised to 350 ppm for 30 minutes directly afterwards, both simple and choice reaction time were increased (Gamberale and Hultengren 1974). More pronounced effects were observed during exposure to 376 ppm for one hour: One subject complained of nausea that persisted one hour after the end of exposure, 2 subjects had a feeling of being inebriated, 3 of 5 subjects exposed were unable to normally

perform the modified Romberg test, and also 3 subjects (unclear, if the same 3 subjects) had significant decrements in other tests of coordination and manual dexterity (Stewart et al. 1968).

In a toxicokinetic study, 2 subjects were exposed to 386 ppm styrene for 2 hours while performing light physical exercise of 50 W (Löf and Johanson 1993). In that study, no information was presented as to the presence or absence of subjective or objective signs of intoxication or irritation. However, it may reasonably be assumed that no severe effects will have occurred in such a study.

At higher concentrations, the irritation becomes very strong (see above), and only one controlled study was located that was conducted at this level (Carpenter et al. 1944). In this study, 2 subjects exposed to 800 ppm for 4 hours suffered from listlessness, drowsiness, impairment of balance, and, after cessation of exposure, muscular weakness and unsteadiness with inertia and depression. A "steadiness test" (measuring manual dexterity) indicated a marked decreased of performance compared to pre-exposure level. Besides CNS-depression, the subjects complained of eye and throat irritation.

Limited data from studies in workers do not provide evidence that styrene exposure leads to lesions of the upper respiratory tract or will impair the sense of smell. In a cross-sectional study, no differences in histological characteristics of the mucosa from the nasal inferior turbinates could be observed in biopsies from styrene exposed workers and matched controls (Ödkvist et al. 1985). In a controlled study, the olfactory threshold for styrne was 32-fold higher in styene-exposed workers than in age-matched controls. However, the odor threshold for the olfactory standard phenyl ethylalcohol was not altered nor was the ability to identify 20 different aroma compounds in an odor identification test (Dalton et al. 2003).

6.2 Summary of Animal Data Relevant to AEGL-2

Limited data from one study are available for styrene toxicity in monkeys (species not reported) (Spencer et al. 1942). In this study, no death occurred in animals during subchronic exposure to 1300 ppm styrene, 7 – 8 hours/day, 5 days/week. It was further reported that there were no signs of irritation or intoxication and no pathological findings in inner organs or in hematology.

Rats exposed to 2760 ppm for one hour were in a state of deep narcosis (Shugaev 1969). Also, "many" rats lost consciousness during exposure to 2000 ppm for 5 hours (Withey and Collins 1979). In rats exposed to concentrations ≥ 2983 ppm, signs of CNS impairment (staggered or staling gait, tremors, lying on the side, and narcosis) were observed. In mice, signs of CNS-depression that occurred during a 4-hour exposure also included staigered gait at 1420 ppm and apathy and finally narcosis at higher concentrations (2983 and 3766 ppm) (BASF 1979a).

At 1500 ppm, reduced attention was described to occur during 6-hours of exposure in rats (Jarry et al. 2002), and an inability to supress nystagmus in an optokinetic test were seen at 1730 ppm after about 30 minutes of exposure (Niklasson et al. 1993). Rats were mostly recumbent at 12 hours of exposure to 600 ppm (Mäkitie et al. 2003), this may also indicate weak CNS-depression.

Behavioral changes were observed in a "despair swimming test" in mice (de Ceaurriz et al. 1983). The mean duration of immobility decreased by 28 – 83 % of control after a 4-hour exposure to 413 – 851 ppm styrene. An ID_{50} (50 % decrease in immobility) of 549 ppm was calculated.

Ototoxicity of styrene with an increase of the auditory threshold in functional tests and a substantial loss of the outer hair cells of the cochlea was observed in rats after repeated exposure at 600 ppm and higher concentrations, but not at 500 ppm (6 hours/day, 5 days/week, 4 weeks) (Mäkitie et al. 2003; Loquet et al. 1999; Lataye et al. 2003 Campo et al. 2001); no effects were detected in similarily

exposed guinea pigs (Lataye et al. 2003). No studies were available in which ototoxicity was investigated after a single exposure, so the relevance of these effects with respect to a single exposure of humans is not known. Therefore, these results will not be used for the derivation of AEGL-2.

In rats, pulmonary lesions following acute inhalation exposure only were observed at concentrations that also caused severe and mostly lethal CNS effects. Mice were more sensitive to styrene than rats. At 250 ppm and 500 ppm, upper respiratory tract and lung toxicity, liver lesions and sometimes death were observed following one or few exposures, and differences in sensitivity between strains were observed; B6C3F1 were most sensitive (Morgan et al. 1993a, c; Mahler et al. 1999; Cruzan et al. 1997; Sumner et al. 1997). At similar concentrations, no such effects have been observed in humans in controlled studies and in numerous studies on occupationally exposed workers with long-term exposure to styrene. Obviously, the high susceptibility of mice with respect to liver and respiratory tract lesions is species- (and strain-) specific and these data are not relevant for the derivation of AEGL.

No developmental toxicity was observed in rats following single oral administration of a maternall toxic dose on day 11 of gestation in one study (Daston et al. 1991) and on gestation day 17 in another (Ponomarkov and Tomatis 1978). In O_{20} mice, survival prior to weaning was reduced after a maternally toxic dose given orally on gestation day 17, no effect was seen in C57Bl mice given a lower dose (Ponomarkov and Tomatis 1978). Following repeated 6-hour exposure to 300 ppm, but not to 50 ppm, during gestation day 6 – 20, an increased neonatal death rate was observed and delayed postnatal development was observed (Katakura et al. 2001). In hamsters, the number of dead or resorbed fetuses was increased at exposure to 1000 ppm 6 hours/day from gestation day 6 -18, but not at 750 ppm (Kankaanpää et al. 1980). In other studies with repeated oral or inhalation exposure of rats, mice, and rabbits, no significant developmental effects were seen (Murray et al. 1978; Srivastava et al. 1990; Chernoff et al. 1990; Beliles et al. 1985; Kankaanpää et al. 1980). The relevance of an exposure duration of about half the gestation period in rodents to a less than one day exposure in humans is questionable. Therefore, these results will not be used for the derivation of AEGL-2.

6.3 Derivation of AEGL-2

The AEGL-2 is based on the CNS effects observed in humans following exposure to 376 ppm for one hour: nausea in one subject; feeling of being inebriated in two, and unability to normally perform the modified Romberg test and significant decrements in other tests of coordination and manual dexterity in three of five subjects (Stewart et al. 1968). The effects described address a level of CNS depression that seems still below a level for an impairment of the ability to escape and therefore a concentration of 376 ppm is considered a NOAEL. However, this concentration is close to the range where irritation in humans becomes intolerable: Exposed subjects reported immediate lacrymation at 300 – 400 ppm and described the irritation above 500 or 600 ppm as very strong or even intolerable after 1 or minutes. Clearly, such irritating levels may limit the ability to escape and thus are above AEGL-2.

Generally, for volatile substances with CNS-depressant effects an intraspecies factor of 3 is applied to account for sensitive individuals because the effective concentration range does not differ more than 2-3-fold between individuals. In case of styrene, it must be taken into account that physical activity has a marked effect on the uptake of styrene and its level in blood. In the studies used to derive AEGL-2, the subjects were at rest. In controlled studies, the observed increase of styrene in arterial blood at exposure to about 150 ppm styrene was approximately 3-fold when the physical activity was increased from rest to light exercise (50 W), 5-fold at moderate exercise (100 W), and 10-fold at heavy exercise (150 W) (Astrand 1975). These values are conservative estimates since – as outlined above (see section 4.1) – the experimental conditions lead to an overestimation of the effect.

Therefore, it could be argued that an intraspecies uncertainty factor of 10 to account for sensitive subgroups would be necessary to protect individuals at heavy physical exercise. Application of a factor of 10 would lead to a 1-hour AEGL-2 of 38 ppm and similar values at longer time periods. On the other hand, the following two points which indicate that a factor of 3 is justified, are believed to outweigh the above rationale.

Firstly, due to physiological limitations, heavy physical exercise (150 W) cannot be performed continuously for longer periods of time. Therefore, it is unrealistic to consider an exposure scenario with heavy exercise for one or several hours. In contrast, light exercise (50 W) may be performed over a longer period of time. In this case, the increase of the styrene concentration in blood will be about 3-fold which is within the range of an uncertainty factor of three.

Secondly, an AEGL-2 value in the range of 38 ppm as mentioned above would be in conflict with styrene exposure data at occupational workplaces. At workplaces, such concentrations are or were frequently observed (IARC 2002) without workers showing signs of CNS depression that would have limited their ability to escape.

Therefore, an intraspecies uncertainty factor of 3 is considered adequate to protect sensitive subgroups including groups exposed to styrene during longer periods of light exercise. This leads to a value of 130 ppm as AEGL-2 for 1 hour.

This experimentally derived exposure value was scaled to shorter periods of time using the equation $c^n \times t = k$ (Ten Berge et al. 1986). As outlined in NRC (2001), a default of n = 3 for shorter periods of time (30 minutes and 10 minutes) was applied, due to the lack of suitable experimental data for deriving the concentration exponent. The “n” value of 1.2 used for calculations of AEGL-3 (see below) was not used for AEGL-2 for following reasons: Firstly, the exponent was derived from lethality studies in which delayed mortality was observed that was not related to narcotic effects on the CNS (which are relevant for AEGL-2) but probably to pulmonary lesions observed at these very high concentrations (in addition to CNS effects which are the major cause of death). Secondly, toxicokinetics at high exposure concentrations over several hours of exposures (as in the lethality studies) is different from that at lower concentrations for shorter time periods.

Toxicokinetic studies with humans exposed to styrene concentrations at 70 – 200 ppm show that most of the increase of the styrene concentration in blood is seen during the first 30 minutes of exposure and that there is no or very little increase at 1 – 3 hours at these concentrations (Löf and Johanson 1993; Ramsey et al. 1980; Wigaeus et al. 1983). Therefore, no additional extrapolation is necessary and the AEGL-2 of 130 ppm derived for 1 hour is applied to longer periods of time.

The derived values are listed below.

TABLE 9: AEGL-2 VALUES FOR STYRENE

AEGL Level	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour
AEGL-2	230 ppm	160 ppm	130 ppm	130 ppm	130 ppm

As outlined above, ototoxicity was observed in short-term studies with rats. At present, the relevance of these findings for acute exposure in humans cannot be assessed. However, taking into account that the NOEL of 500 ppm is derived from a subacute study (6 hours/day, 5 days/week, 4 weeks), the derived AEGLs are considered to be protective against ototoxic effects.

Individual cases of respiratory sensitization to styrene were described (Hayes et al. 1991; Moscato et al. 1987). Taking into account the wide use of styrene both in industry and in do-it-yourself products, sensitization seems to be an exceptionally rare event. Although the risk of sensitization following a single exposure at AEGL-2 is considered negligible, individuals already sensitized may not be able to tolerate styrene concentrations that are without effect in non-sensitized individuals and may not be protected by the AEGL developed for styrene in this TSD.

7 DATA ANALYSIS AND PROPOSED AEGL-3

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

In lethality studies with rats (see below), most animals died of CNS-depression but delayed death was observed in some rats. These animals showed pulmonary edema and hemorrhages. Since no reports of death in humans following exposure to styrene were located in the literature, it is not known if pulmonary lesions may occur in humans at high, acute life-threatening exposure, but this cannot be excluded.

7.1 Summary of Human Data Relevant to AEGL-3

No studies were located in which it was reported that styrene exposure has caused death in humans.

7.2 Summary of Animal Data Relevant to AEGL-3

At high concentrations, CNS-depression progresses to loss of consciousness and finally death. Furthermore, delayed deaths were observed that seem to be related to pulmonary lesions. As outlined in section 3.6, the most reliable data for lethal toxicity in rats are those of BASF (1979b) and Bonnet et al. (1982). In these studies, delayed deaths which were seen in some animals were included in the determination of the LC_{50}. Other lethality studies did not include such observations or lack important experimental details.

Limited data are available for nonhuman primates. In one study with monkeys (species not reported), no death was observed at repeated exposures to 1300 ppm, 7 – 8 hours/day, 5 days/week for at least 7 months (Spencer et al. 1942).

Mice were more sensitive to styrene than rats. At 250 ppm and 500 ppm, upper respiratory tract and lung toxicity, liver lesions and sometimes death were observed following one or few exposures, and differences in sensitivity between strains were observed; B6C3F1 were most sensitive (Morgan et al. 1993a, c; Mahler et al. 1999; Cruzan et al. 1997; Sumner et al. 1997). At similar concentrations, no such effects were observed in humans in controlled studies and in numerous studies on occupationally exposed workers with long-term exposure to styrene. Obviously, the high susceptibility of mice with respect to liver and respiratory tract lesions is species- (and strain-) specific and these data are not relevant for the derivation of AEGL.

Developmental toxicity including embryolethality data are summarized above (see 6.2). For the same reason as outlined there, these results will not be used for the derivation of AEGL-2.

7.3 Derivation of AEGL-3

In rats, exposure to high concentrations of styrene leads to progressive CNS depression with narcosis and, finally, death. Pulmonary lesions were also described in these studies but only at concentrations leading to severe or lethal CNS effects.

In humans, the acute effects on the CNS are also well known. However, no reports were identified describing lethal intoxication of humans following styrene exposure. Therefore, it is not known if the pulmonary lesions observed in rats may also occur in humans exposed to life-threatening or potentially lethal concentrations of styrene.

For a conservative approach, data from studies with rats taking into account delayed deaths with pulmonary lesions were taken to derive AEGL-3. From the data of the 4-hour exposure study of BASF (1979b), a benchmark calculation was performed with the lethality data using different models. Calculations were performed for males/females combined and for both sexes separately. According to these calculations, female rats could be slightly more susceptible than male rats as indicated by the higher mortality at lower concentrations. Therefore, a BMDL05 for female rats of 3409 ppm (rounded to 3400 ppm) was used as a starting point to derive AEGL-3.

A total uncertainty factor of 10 was applied. This total factor may formally be split up into an interspecies factor of 3 and an intraspecies factor also of 3.

For volatile solvents like styrene with a CNS-depressant effect, an interspecies uncertainty factor of 3 has been applied in the derivation of AEGL for several substances. This is based on the similarity of effects manifested in rodents compared to humans.

In case of styrene, limited data indicate no gross differences in the concentration of styrene in blood between rats and humans. According to a toxicokinetic model, at concentrations exceeding 200 ppm styrene in air, the non-steady-state concentration of styrene in blood of humans (calculated for 6 hours of exposure) will always be lower than that in blood of rats since (Ramsey and Andersen 1984). Styrene levels in human blood were in accordance with this model up to 376 ppm in air, however, no experimental human data are available at higher concentrations.

An intraspecies uncertainty factor of 3 was applied to account for sensitive individuals since the threshold for CNS impairment is not expected to vary much among individuals. As in case of the derivation of AEGL-2 (see 6.3), an intraspecies uncertainty factor of 3 is considered adequate to protect sensitive subgroups including groups exposed to styrene during longer periods of light exercise.

Extrapolation was made to the relevant AEGL time points of 30 minutes and 1 hour using the relationship $C^n \times t = k$ (Ten Berge et al. 1986) with a value of $n = 1.2$ which was derived from extrapolation of the LC_{50} in rats for 4- and 6 hours (BASF 1979b (1979b) and Bonnet et al. 1982a (see Appendix B). The 10-minutes AEGL-3 was assigned the same value as that for the 30-minutes AEGL-3 as it was considered inappropriate to extrapolate from an experimental period of 4 or 6 hours to 10 minutes. The 8-hour AEGL-3 was assigned the same value as that for the 4-hour AEGL-3 as toxicokinetic data indicate that there is at most little increase of internal dose after 4 hours of exposure; moreover, lower values which would be derived by default calculations are not supported by toxicological data for humans.#

The derived values are listed below.

TABLE 10: AEGL-3 VALUES FOR STYRENE					
AEGL Level	**10-Minute**	**30-Minute**	**1-Hour**	**4-Hour**	**8-Hour**
AEGL-3	1900 ppm *	1900 ppm *	1100* ppm	340 ppm	340 ppm

*: The lower explosive limit (LEL) of styrene in air is 1.1 % (11,000 ppm). The AEGL-3 value of 1900 ppm (8090 mg/m³) for 10 minutes and 30 minutes is higher than 1/10 of the LEL. Therefore, safety considerations against hazard of explosion must be taken into account.

The derived values can be interpreted to the effect that the average population might be exposed to a concentration 3-fold higher than susceptible subgroups without experiencing AEGL-3 effects. Taking this into account, limited data from controlled human studies support the derived AEGL-3 values. Especially, Carpenter et al. (1944) observed marked CNS depression (and severe irritation) but no life-threatening effects in 2 resting volunteers exposed to 800 ppm for 4 hours.

8 SUMMARY OF PROPOSED AEGLS

8.1 AEGL Values and Toxicity Endpoints

TABLE 11: SUMMARY/RELATIONSHIP OF PROPOSED AEGL VALUES					
Classification	**10-Minute**	**30-Minute**	**1-Hour**	**4-Hour**	**8-Hour**
AEGL-1 (Nondisabling)	20 ppm	20 ppm	20 ppm	20 ppm	20 ppm
AEGL-2 (Disabling)	230 ppm	160 ppm	130 ppm	130 ppm	130 ppm
AEGL-3 (Lethal)	1900 ppm *	1900 ppm *	1100* ppm	340 ppm	340 ppm

*: The lower explosive limit (LEL) of styrene in air is 1.1 % (11,000 ppm). The AEGL-3 value of 1900 ppm (8090 mg/m³) for 10 minutes and 30 minutes is higher than 1/10 of the LEL. Therefore, safety considerations against hazard of explosion must be taken into account.

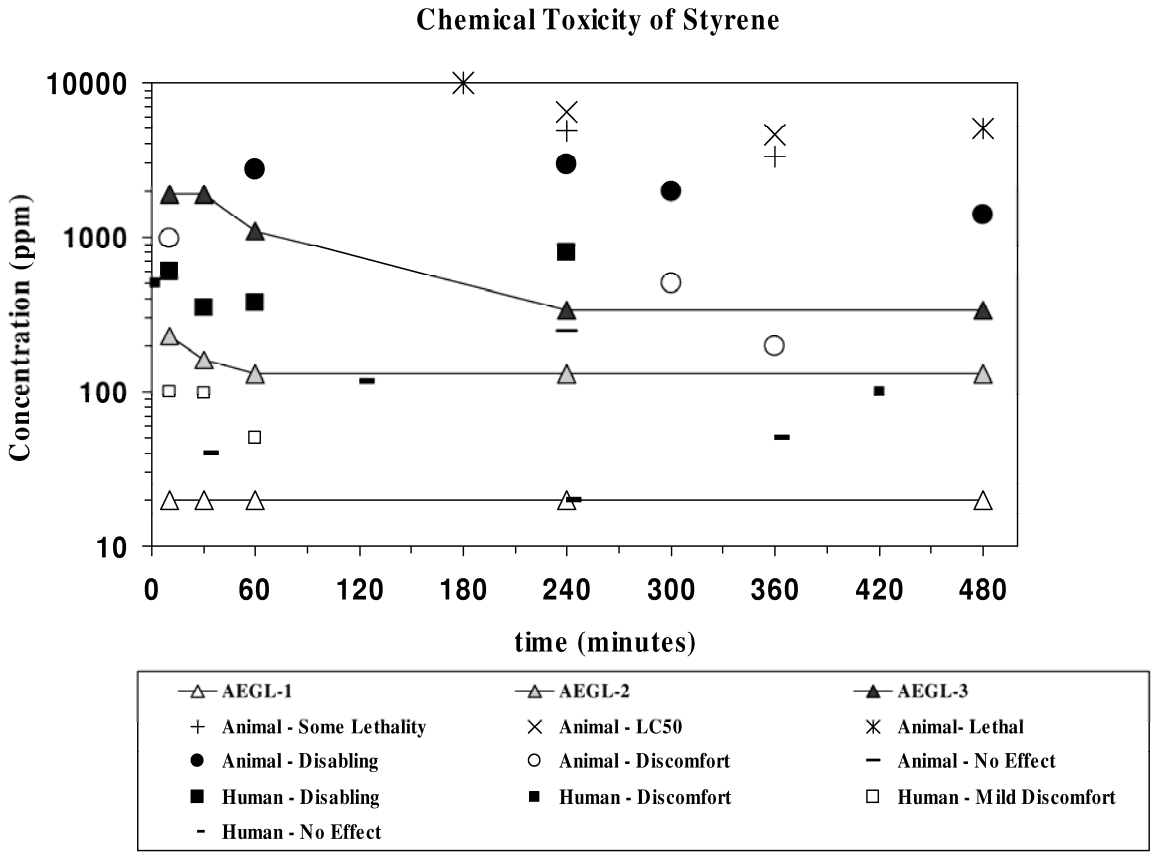

FIGURE 7: CATEGORICAL REPRESENTATION OF STYRENE INHALATION DATA

(Lethality data for mice were excluded from the plot since the data were considered not to be relevant for the assessment of risk for humans. See 4.3.2)

8.2 Comparison with Other Standards and Guidelines

Other standard and guidance levels for workplace and community are listed in **TABLE 12**.

TABLE 12: EXTANT STANDARDS AND GUIDELINES FOR STYRENE

Guideline	Exposure duration					
	10 min	30 min	1 h	4 h	8 h	24 h
AEGL-1	20 ppm	20 ppm	20 ppm	20 ppm	20 ppm	
AEGL-2	230 ppm	160 ppm	130 ppm	130 ppm	130 ppm	
AEGL-3	1900 ppm *	1900 ppm *	1100* ppm	340 ppm	340 ppm	
ERPG-1 (AIHA 1995)[a]			50 ppm			
ERPG-2 (AIHA 1995)[a]			250 ppm			
ERPG-3 (AIHA 1995)[a]			1000 ppm			
IDLH (NIOSH 1996)[b]		700 ppm				
PEL-TWA (OSHA, 1989)[e]					100 ppm	
Acceptable peak (OSHA)[f]	600 ppm [5 min]					
REL-TWA (NIOSH)[g]					50 ppm	
STEL-NIOSH	100 ppm [15 min]					
TLV-TWA (ACGIH 1997)[h]					20 ppm	
TRGS 900 (Germany)[i]						
TRGS 900 (Germany) Spitzenbegrenzung[j]						
MAK (DFG 1987, Germany)[k]					20 ppm	
MAK (DFG, Germany) Kurzzeitkategorie[l]					II,1	
Einsatztoleranzwert[m]				40 ppm		

*: The lower explosive limit (LEL) of styrene in air is 1.1 % (11,000 ppm). The AEGL-3 value of 1900 ppm (8090 mg/m³) for 10 minutes and 30 minutes is higher than 1/10 of the LEL. Therefore, safety considerations against hazard of explosion must be taken into account.

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

The ERPG-1 is the maximum airborne concentration below which nearly all individuals could be exposed for up to one hour without experiencing or developing effects more serious than mild irritation, other mild transient health effects, or perception of a clearly objectionable odor. The ERPG-1 for styrene is based on the observation that controlled exposure to 50 ppm for 1 hour or more does not produce any adverse health effects except mild to moderate odor perception.

The ERPG-2 is the maximum airborne concentration below nearly all individuals could be exposed for up to one hour without experiencing or developing irreversible or other serious adverse health effects or symptoms that could impair an individual‘s ability to take protective action. The ERPG-2 for styrene is derived on the basis that simple reaction time was increased after 30 minutes exposure at 350 ppm, but not at 250 ppm and exposure to 200 ppm was well-tolerated.[2]

The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing life-threatening health effects. The ERPG-3 for styrene of 1000 ppm is derived on the basis that this value is well below lethality levels observed in acute animal studies, exposure to 2 human volunteers at 800 ppm caused only irritation and CNS depression, and that repeated exposures to 1300 ppm are well-tolerated in animal species.

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

Basis for revised IDLH: Acute inhalation toxicity data in humans (drowsiness, nausea, headache, fatigue, and dizziness reported to occur in workers exposed to 200 – 700 ppm; AIHA 1959) (NIOSH 1996).

e: **OSHA PEL-TWA** (Occupational Health and Safety Administration, Permissible Exposure Limits - Time Weighted Average) for 8 hours (OSHA). Additionally, in 1996, the styrene industry agreed to establish a voluntary compliance program, the objective of which is to encourage all facilities to comply with PEL established for styrene during the 1989 rulemaking; that is, inhalation exposures that do not exceed 50 ppm on an 8-hour TWA, and a 100 ppm 15-minute ceiling (OSHA 2003).

f: **Acceptable Peak OSHA** (Occupational Health and Safety Administration, Permissible Exposure Limits; OSHA).

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

h: **ACGIH TLV-TWA** (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average) (ACGIH, 1999):

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.

k: **MAK** (Maximale Arbeitsplatzkonzentration [Maximum Workplace Concentration], Deutsche Forschungs-gemeinschaft [German Research Association], Germany.

is defined analogous to the ACGIH-TLV-TWA.

l: **MAK Spitzenbegrenzung** (Kategorie II, 1) (Peak Limit Category II, 1) (DFG 2000): 5 times MAK

constitutes the maximum average concentration for substances with a half-life of less than two hours to which workers may be exposed for a period up to 30 minutes (mean value) no more than 4 times per workshift.

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

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

[2] The rationale, however, also stated that there was a loss of balance in volunteers exposed for 1 – 3 hours to 200 ppm or more.

8.3 Data Adequacy and Research Needs

The data base on humans includes controlled clinical studies and studies at the workplace. These studies showed that styrene is irritating to eyes and mucous membranes of the upper respiratory tract. Effects on the central nervous system were observed in controlled human studies, in studies on workers occupationally exposed to styrene, and in accidents following exposure to higher but unknown concentrations. Toxicokinetic studies are also available. The derived AEGL-1 and -2 values are based on well-described controlled human studies.

Studies with acute to subacute exposure of animals – mostly rats and mice, and very limited data for other species (monkeys, guinea pigs, hamsters, rabbits) – addressed irritation, effects on the central nervous system including behavior, ototoxicity, and lethality. Developmental toxicity, genotoxicity and carcinogenicity studies are also available. Species differences observed between rats and mice were observed with respect to respiratory toxicity and carcinogenicity. Toxicokinetic studies indicate that the higher susceptibility of mice compared to rats with respect to pulomary toxicity and carcinogenicity is related to differences in the metabolic activation and detoxification of styrene. The derived AEGL-3 values are based on a well-conducted and -described lethality study with rats.

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APPENDIX A: DERIVATION OF AEGL VALUES

Derivation of AEGL-1

Key study:	Seeber et al. 2002
Toxicity endpoint:	NOAEL for slight irritation: 20 ppm
Scaling:	None
Uncertainty/ modifying factors	None
Calculations	The 20 ppm concentration is used for all exposure durations.
10-minute AEGL-1	20 ppm (85 mg/m³)
30-minute AEGL-1	20 ppm (85 mg/m³)
1-hour AEGL-1	20 ppm (85 mg/m³)
4-hour AEGL-1	20 ppm (85 mg/m³)
8-hour AEGL-1	20 ppm (85 mg/m³)

Derivation of AEGL-2

Key study:	Gamberale and Hultengren 1974; Stewart et al. 1968
Toxicity endpoint:	CNS effects (impairment of reaction time, difficulties in balance performance test, decrement in coordination test, nausea, feeling of inebriation) in humans exposed to 350 ppm for 30 minutes or 376 ppm for 1 hour.
Uncertainty/ modifying factors	3 for intraspecies variability
Scaling:	$C^3 \times t = k$ for extrapolation to shorter time points $k = 376^3$ ppm³ x 1 h = 5.31574×10^7 ppm³ h Applying the uncertainty factor of 3 results in a concentration at which toxicokinetic data indicate that the blood level of styrene does not or only very slightly increases after 1 hour. Therefore, 1-hour AEGL-2 = 4-hour AEGL-2 = 8-hour AEGL-2.
Calculations	
10-minute AEGL-2	$C^3 \times 0.167$ h = 5.31574×10^7 ppm³ h C = 683 ppm 10-min AEGL-2 = 683 ppm/3 = 230 ppm (980 mg/m³)
30-minute AEGL-2	$C^3 \times 0.5$ h = 5.31574×10^7 ppm³ h C = 473 ppm 30-min AEGL-2 = 473 ppm/3 = 160 ppm (680 mg/m³)
1-hour AEGL-2	C = 376 ppm 1-hour AEGL-2 = 376 ppm/3 = 130 ppm (550 mg/m³)
4-hour AEGL-2	4-hour AEGL-2 = 1-hour AEGL-2 4-hour AEGL-2 = 130 ppm (550 mg/m³)
8-hour AEGL-2	8-hour AEGL-2 = 1-hour AEGL-2 8-hour AEGL-2 = 130 ppm (550 mg/m³)

Derivation of AEGL-3

Key study:	BASF 1979b
Toxicity endpoint:	BMDL05 = 3400 ppm for female rats, 4-hour exposure, acute toxicity study
Scaling:	$C^{1.2} \times t = k$ for extrapolation to 30 minutes, 1 hour $k = 3400^{1.2}$ ppm$^{1.2} \times 4$ h $= 6.91568 \times 10^4$ ppm$^{1.2}$ h The 10-minutes AEGL-3 was set at the same concentration as the 30-minutes AEGL-3. The 8-h AEGL-3 was set at the same concentration as the 4-h AEGL-3.
Uncertainty/ modifying factors	Combined uncertainty factor of 10 3 for interspecies variability 3 for intraspecies variability
Calculations	
10-minute AEGL-3	10-minutes AEGL-3 = 30-minutes AEGL-3 = 1900 ppm (8090 mg/m³)
30-minute AEGL-3	$C^{1.2} \times 0.5$ h $= 6.91568 \times 10^4$ ppm$^{1.2}$ h C = 19233 ppm 30-min AEGL-3 = 19233 ppm/10 = 1900 ppm (8090 mg/m³)
1-hour AEGL-3	$C^{1.2} \times 1$ h $= 6.91568 \times 10^4$ ppm$^{1.2}$ h C = 10794 ppm 1-hour AEGL-3 = 10794 ppm/10 = 1100 ppm (4690 mg/m³)
4-hour AEGL-3	C = 3400 ppm 4-hour AEGL-3 = 3400 ppm/10 = 340 ppm (1450 mg/m³)
8-hour AEGL-3	8-hour AEGL-3 = 4-hour AEGL-3 = 340 ppm (1450 mg/m³)

APPENDIX B:
DERIVATION OF EXPONENTIAL FUNCTION FOR TEMPORAL SCALING

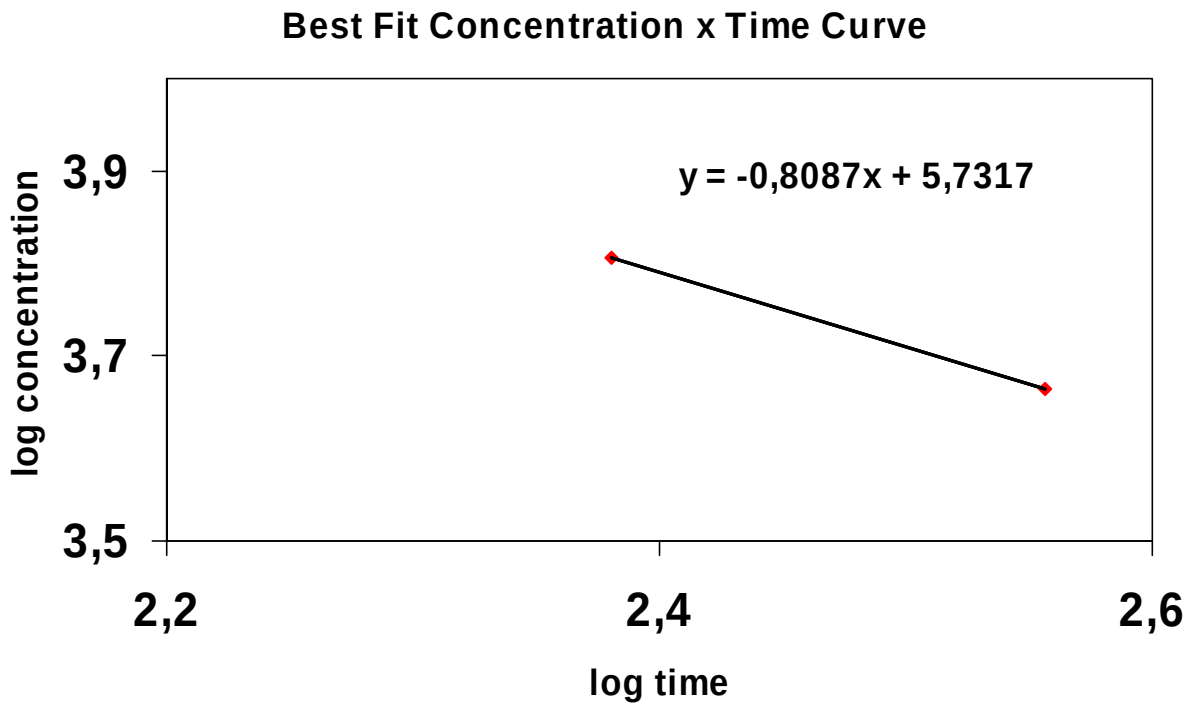
Concentration-Time Mortality Response Relationship for Rats

Data source: BASF 1979b; Bonnet et al. 1982a

Time (min)	Conc. (ppm)	lg Time	lg Conc.
240	6410	2.3802	3.8069
360	4618	2.5563	3.66445

n = 1.2

k = 1.224×10^7



APPENDIX C:
BENCHMARK CALCULATIONS FOR STYRENE

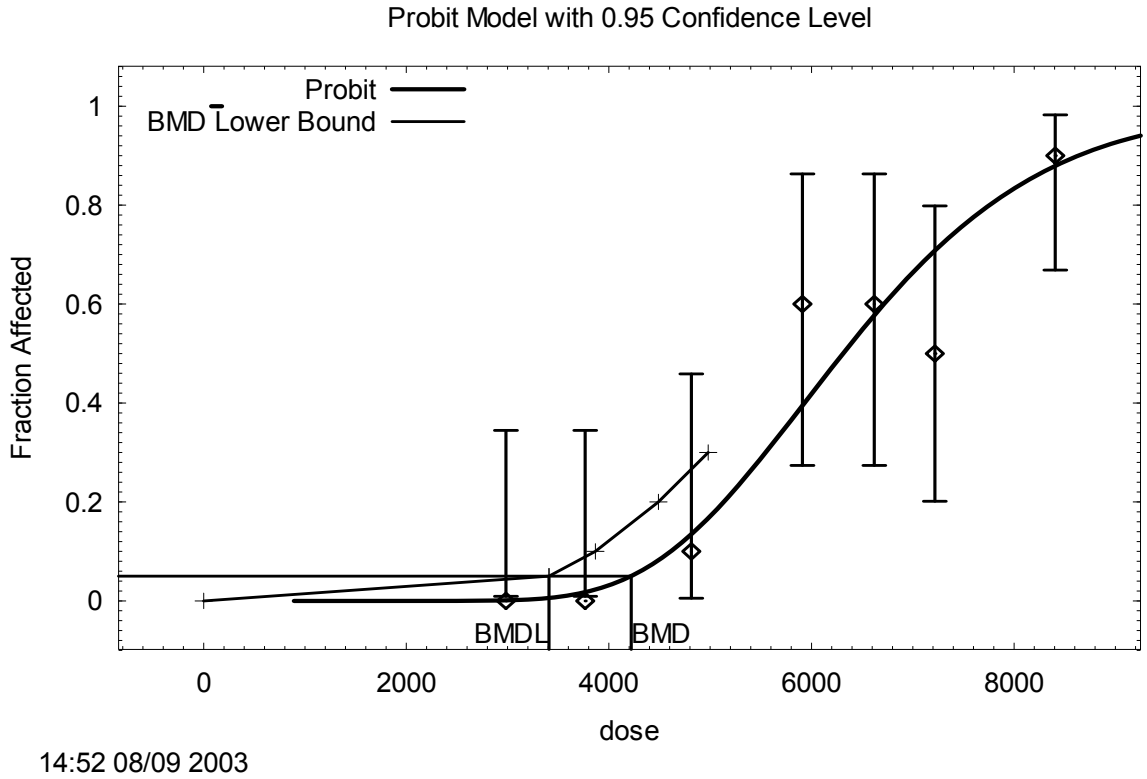
Benchmark calculations

The benchmark calculations were carried using the US-EPA benchmark software packet BMDS version 1.3.2. Calculations are based on lethality data for rats (BASF 1979b, see **TABLE 3**) and were performed with data for male and female rats separately and with the combined data set. Background was set to Zero (at dose 0, no lethality assumed).

According to default assumptions (SOP, NRC 2001), the Log Probit model was used for calculations. For the AEGL-3 derivation a value of 3409 ppm (BMDL05) was used.

$BMDL_{05}$ (female rats) = 3409 ppm

BMC_{01} (female rats) = 3571 ppm



Calculation of $BMDL_{05}$ for female rats

Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $

BMDS MODEL RUN

The form of the probability function is:

P[response] = Background + (1-Background) * CumNorm (Intercept+Slope*Log(Dose)),

where CumNorm (.) is the cumulative normal distribution function.

Dependent variable = Let_femal
Independent variable = ppm
Background parameter is set to zero
Slope parameter is restricted as slope >= 1
Total number of observations = 7
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model.

Default Initial (and Specified) Parameter Values
background = 0 Specified
intercept = -26.5274
slope = 3.04574

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -background have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-1
slope	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.
intercept	-35.6988	7.66807
slope	4.07942	0.872206

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-30.1442			
Fitted model	-32.2497	4.21094	5	0.5195
Reduced model	-55.0511	49.8138	6	<.0001

AIC: 68.4993

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Residual
2983.0000	0.0011	0.011	0	10	-0.1051
3766.0000	0.0174	0.174	0	10	-0.4212
4814.0000	0.1339	1.339	1	10	-0.3145
5911.0000	0.3933	3.933	6	10	1.338
6621.0000	0.5761	5.761	6	10	0.1531
7218.0000	0.7068	7.068	5	10	-1.437
8407.0000	0.8782	17.564	18	20	0.2979

Chi-square = 4.25 DF = 5 P-value = 0.5134

Benchmark Dose Computation

Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95

BMC = 4220.71
BMCL = 3408.98

Benchmark Dose Computation

Specified effect = 0.01
Risk Type = Extra risk
Confidence level = 0.95

BMC = 3571.36
BMCL = 2683.76

Table: Comparison of calculated benchmark doses for male, female, or male + female rats. All values presented in ppm.

Sex	**Model**	**BMD_{05}**	**$BMDL_{05}$**	**BMD_{01}**	**LC_{50}**	**Remark**
Male	Logprobit	4884	4213	4344	6477	LC_{50} (BASF): 6480
Female	Logprobit	4221	**3409**	3571	6317	LC_{50} (BASF): 6310
Male + female	Logprobit	4551	4036	3950	6405	LC_{50} (BASF): 6410

APPENDIX D:
DERIVATION SUMMARY FOR STYRENE AEGLS

ACUTE EXPOSURE GUIDELINE LEVELS
FOR STYRENE

DERIVATION SUMMARY

AEGL-1 VALUES				
10 minutes	**30 minutes**	**1 hour**	**4 hours**	**8 hours**
20 ppm	**20 ppm**	**20 ppm**	**20 ppm**	**20 ppm**

Key References: Seeber, A., C. van Thriel, K. Haumann, E. Kiesswetter, M. Blaszkewicz, and K. Golka. 2002. Psychological reactions related to chemosensory irritation. Int. Arch Occup. Environ Health 75: 314-325.

Test Species/Strain/Number: 16 or 24 human subjects; 4 subjects/group

Exposure Route/Concentrations/Durations: : Inhalation
0.5 ppm (4 hours), 20 ppm (3 hours), 40 ppm (30 minutes, interim peak exposures during exposure to 0.5 ppm)

Effects: Increase of ratings for odor and annoyance, but only marginally for irritation. Effects sizes comparing ratings during exposure to 20 ppm and during pre-exposure higher for odor, annoyance, and irritation, and also higher compared to “clean air only”, however, verbally rated as “hardly at all”.

Endpoint/Concentration/Rationale: NOAEL for slight irritation/subjective discomfort at 20 ppm

Uncertainty Factors/Rationale:
Interspecies: 1, test subjects were humans
Intraspecies: 1, intensity of irritation is not expected to vary greatly among the general population.

Modifying factor: NA

Animal to Human Dosimetric Adjustment: NA

Time Scaling: Not applied, complaints about discomfort were reported not to increase during several hours of exposure.

Confidence and Support for AEGL values: Values are based on data from well-described controlled human study and are supported by data from other controlled human studies.

AEGL-2 VALUES				
10 minutes	**30 minutes**	**1 hour**	**4 hours**	**8 hours**
230 ppm	160 ppm	130 ppm	130 ppm	130 ppm

Key References: Stewart, R.D., H.C. Dodd, E.D. Baretta, and A.W. Schaffer. 1968. Human exposure to styrene vapor. Arch Environ Health 16: 656-662.
Gamberale, F. and M. Hultengren. 1974. Exposure to styrene. II. Psychological functions. Work Environ Health 11: 86-93.

Test Species/Strain/Number: 1 - 5 human subjects/dose (Stewart et al. 1968)
12 human subjects/dose (Gamberale and Hultengren 1974)

Exposure Route/Concentrations/Durations: Inhalation
51 ppm (1 h), 99 ppm (2 x 3.5 h), 117 ppm (2 h), 216 ppm (1 h), 376 ppm (1 h) (Stewart et al. 1968)
50 ppm, 150 ppm, 250 ppm, 350 ppm (30 minutes) (Gamberale and Hultengren 1974)

Effects: 376 ppm: difficulties in balance performance tests, decrement in manual dexterity tests, nausea, inebriation, headaches (Stewart et al. 1968);
250 ppm: no effect; 350 ppm: increase in simple and choice reaction time (Gamberale and Hultengren 1974).

Endpoint/Concentration/Rationale: NOAEL for CNS depression impairing ability to escape: 376 ppm (1 hour)

Uncertainty Factors/Rationale:
Interspecies: 1, test subjects were humans
Intraspecies: 3, CNS effects not expected to vary greatly within the general population.

Modifying factor: NA

Animal to Human Dosimetric Adjustment: NA

Time Scaling: Extrapolation was made to the relevant AEGL time points using the relationship $C^n \times t = k$ with a default value of n = 3 for extrapolation to shorter time periods. Toxicokinetic studies with humans exposed to styrene concentrations at 70 – 200 ppm show that most of the increase of the styrene concentration in blood is seen during the first 30 minutes of exposure and that there is no or very little increase at 1 – 3 hours at these concentrations. Therefore, no additional extrapolation is necessary and the AEGL-2 of 130 ppm derived for 1 hour is applied to longer periods of time.

Confidence and Support for AEGL values: Values are derived from controlled human studies reported in sufficient detail, supporting studies available addressing irritation, CNS-effects, and toxikokinetics.

AEGL-3 VALUES				
10 minutes	**30 minutes**	**1 hour**	**4 hours**	**8 hours**
1900 ppm	1900 ppm	1100 ppm	340 ppm	340 ppm

Key References: BASF. 10-12-1979b. Bericht über die Bestimmung der akuten Inhalationstoxizität LC50 von Styrol als Dampf bei 4stündiger Exposition an Sprague-Dawley-Ratten. Unveröfffentlichte Untersuchung. [Report on the determination of the acute inhalation toxicity LC_{50} of styrene as vapor at a 4-hour exposure on Sprague-Dawley rats. Unpublished study.] BASF AG, Ludwigshafen, Germany. [In German].

Test Species/Strain/Number: Rats/ Sprague-Dawley/ Groups of 10 (20) males (m) and 10 (20) females (f)

Exposure Route/Concentrations/Durations:
Inhalation 2983, 3766, 4814, 5911, 6621, 7218, 8407 ppm, 4 hours

Effects: 18/20 f, 20/20 m, 38/40 m + f died
5/10 f, 8/10 m, 13/20 m + f died
6/10 f, 3/10 m, 9/20 m + f died
6/10 f, 1/10 m, 7/20 m + f died
1/10 f, 2/10 m, 3/20 m + f died
0/10 f, 0/10 m, 0/20 m + f died
0/10 f, 0/10 m, 0/20 m + f died

Endpoint/Concentration/Rationale:
Death (due to CNS depression and additionally to pulmonary lesions at concentrations causing severe to lethal CNS-effects). Benchmark calculation of $BMDL_{05}$ for female rats: 3400 ppm

Uncertainty Factors/Rationale:
Interspecies: 3; no gross interspecies differences expected for substances with acute CNS-depression
Intraspecies: 3; CNS effects not expected to vary greatly within the general population.

Modifying factor: NA

Animal to Human Dosimetric Adjustment: NA

Time Scaling: Extrapolation was made to the relevant AEGL time points of 30 minutes and 1 hour using the relationship $C^n \times t = k$ with a value of n = 1.2 which was derived from extrapolation of the LC_{50} in rats for 4- and 6 hours (BASF 1979b; Bonnet et al. 1982a). The 10-minutes AEGL-3 was assigned the same value as that for the 30-minutes AEGL-3 as it was considered inappropriate to extrapolate from an experimental period of 4 or 6 hours to 10 minutes. The 8-hour AEGL-3 was assigned the same value as that for the 4-hour AEGL-3 as toxicokinetic data indicate that there is at most little increase of internal dose after 4 hours of exposure; moreover, lower values which would be derived by default calculations are not supported by toxicological data for humans.

Confidence and Support for AEGL values: Well conducted study, described in sufficient detail, data supported from other toxicity studies with rats.

APPENDIX E:
DERIVATION OF THE LEVEL OF DISTINCT ODOR AWARENESS

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

Van Doorn et al. (2002) present results of odor threshold determinations for styrene that were a) measured by olfactometry methods considered compatible with a precursor of the NVN2820 and EN13725 method or b) were measured by TNO in the Netherlands using a precursor of the NVN2820 and EN 13725 methods, with a mean n-butanol threshold of 25 ppb. Results of both were converted to the reference agreed in EN13725 of 400 ppb n-butanol by using a factor of 40:25 = 1.6. Thereby, odor thresholds of 0.049 ppm and 0.025 ppm, respectively, were obtained. Taking into account the threshold value of 0.033 ppm obtained by the Japanese method (Hoshika et al. 1993), Van Doorn et al. (2002) calculated an n-butanol corrected mean odor threshold of 0.0345 ppm for styrene.

Corrected odor detection threshold (OT_{50}) for styrene (Van Doorn et al. 2002): 0.0345 ppm

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

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

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

$$3 = 2.33 * \log (C / 0.0345) + 0.5 \quad \text{and}$$

$$C = 0.41 \text{ ppm}$$

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

$$LOA = C * 1.33 = 0.41 \text{ ppm} * 1.33 = 0.54 \text{ ppm}$$

The **LOA** for styrene is set to **0.54 ppm**.