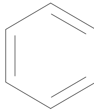
BENZENE
(CAS Reg. No. 71-43-2)



INTERIM ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

For
NAS/COT Subcommittee for AEGLS

2009

PREFACE

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

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

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

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

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

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

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EXECUTIVE SUMMARY

Benzene (or benzol, the old name for the commercial product benzene) is a clear colorless liquid with a characteristic sweet odor at low concentrations and disagreeable and irritating at high levels (Cavender, 1994). Benzene was the 17th highest volume chemical produced in the United States in 1994 and its production was about 14.7 billion pounds in 1994 (ATSDR 1997). Pure benzene can be isolated from coal tar oil and by refining crude oil. Benzene as a constituent of gasolines may account for levels up to 5% by volume until 2000 in Europe. Thereafter, a maximum of 1% is allowed. In the USA, benzene contributes about 1-2% of unleaded gasolines (ATSDR 1997). Benzene is also present in tobacco smoke and has been detected in expired air of smokers (Cavender, 1994). Benzene is industrially the most important of the so-called BTX aromatics (benzene, toluene, xylene). In industrial chemistry, benzene forms the basis for a great variety of aromatic intermediates and for the group of cycloaliphatic compounds. Benzene is used as the basis for the manufacture of plastics, synthetic rubber, dyestuffs, resins, raw materials for detergents, and plant protection agents (EU, 2002). In addition, the use of benzene as a solvent is widespread.

Benzene is responsible for various toxicity effects including CNS depression, eye and airway irritation, general developmental toxicity, genotoxicity, bone marrow toxicity and resulting carcinogenesis (leukemia).

The available animal data are not considered to be adequate for AEGL-1 development. For AEGL-1, both CNS effects and eye and airway irritation are relevant effects. It is expected that mild CNS effects will be the first noticable effects of benzene exposure and that irritation occurs only at higher exposures or are due to co-exposure to other substances. Therefore, the AEGL-1 values should be based on mild CNS effects. Also for toluene, mild CNS-effects were selected as the basis for the AEGL-1 values. Although CNS effects of benzene are already known for over 100 years, very little is known about the time-concentration-effect relationship of slight CNS effects due to benzene exposure in humans, and no quantitative point of departure is available for a solid PBPK modeling. The most controlled human study representing exposure within the AEGL time frames is Srbova et al. (1950) reporting no subjective symptoms during exposure to 110 ppm for 2h. However, no skin and eye exposure was involved in this study and clinical symptoms were not systematically investigated. Although the study by Srbova et al. (1950) has some weaknesses (no details on all individual exposures and time durations, the lack of symptoms was reported by a single remark, no active investigation of health effects), the 110 ppm level for 2h is taken as a NOEL for CNS effects. This is the starting point for AEGL-1 development. This NOEL appears to be supported by the fact that a substantial number of volunteers was exposed in a controlled or occupational setting to levels of 1 – 76 ppm for 7h TWA (Inoue et al., 1986; 64 men, 88 women), 32 ppm ± 25 ppm for 8h TWA (Inoue et al., 1988; 65 workers) or 19-125 ppm for 6-8h (Hunter and Blair, 1972; male laboratory staff) although none of these studies actually included statements on health effects. Because CNS effects are the consequence of systemic benzene exposure, time extrapolation should be applied. Based on the experiments described by Von Oettingen (1940) with cats, a n-value of 1 was identified for light and deep narcosis in cats (see section 6.2). It is not known whether this value is appropriate for humans but it does indicate that using n=3 for extrapolation short time periods is too conservative. Therefore, time extrapolation is performed using n=1 and n=2 for longer and shorter time periods respectively. Because human data are used, the interspecies uncertainty factor is 1. The intraspecies uncertainty factor is 3 because it has been found from experience with anesthetic gases that CNS

1 depression does no vary by more than a factor 2-3 between groups in the population. This results in the
2 AEGL-1 values displayed in the table below.
3
4 For AEGL-2 the following effects could be relevant: CNS-depression (reduced ability to escape),
5 general developmental toxicity, genotoxicty, and hematotoxicity / carcinogenicity. The developmental
6 toxicity effects of benzene are considered to be induced by repeated exposure and it is not likely that the
7 same extent of effects will be induced by a single exposure. Therefore, developmetnal toxicity will not be
8 used for AEGL-2. Genotoxicity of benzene is considered to be a marker of exposure but has limited
9 predictive value for leukemia or hematotoxicity. Therefore, genotoxicity will not be used for AEGL-2.
10
11 The AEGL-2 values will not be based on the risk of leukemia as explained in section 6.1. In
12 addition, hematotoxicity will not be used for setting the AEGL-2 values. With respect to hematotoxicity,
13 the first effects noted will be reduced numbers of ciculating cells and possibly unilineage progenitor cells.
14 However, these effects are in principle reversible although recovery may require some time. As such, these
15 effects are not the prefered AEGL-2 endpoints. Effects on the pluripotent stem cell, however, are serious
16 and irreversible. Unfortunately, no data are available showing effects on the pluripotent stem cell after a
17 single dose. The most important data are from Uyeki et al. (1974) showing reductions in pluripotent stem
18 cells after 3 x 8 hour exposures at 5020 ppm over three days. In general, it can be observed that major
19 hematotoxicity develops after a couple of days. In addition, Cronkite et al. (1989) showed that a two day
20 exposure to 3000 ppm showed less hematotoxicity than a 19 day exposure at 316 ppm, indicating that
21 some form a repeated exposure is necessary. It is expected that knocking out a substantial number of
22 pluripotent stem cells requires a couple of thousand ppm in a single dose (R Snyder, personal
23 communication). In this respect, CNS effects are expected to occur before major effects on the pluripotent
24 stem cell will be induced.
25
26 The most prominent effect of acute benzene, therefore, is exposure is CNS depression. Because
27 this is a continuum from very slight dizziness to narcosis, the level that impairs escape should be identified
28 for AEGL-2 derivation. There are no adequate dose-response studies available for humans, only
29 estimations and indications. Therefore, animal data will be used as the point of departure. Increased
30 activity is not considered an AEGL-2 endpoint but clear decreases in neurobehavioral function are an
31 AEGL-2 endpoint. The highest level showing no AEGL-2 effect, is 4000 ppm for 4h in rats (Molnar et al.,
32 1986). Based on the experiments described by Von Oettingen, an n-value of 1 was identified for light and
33 deep narcosis in cats. It is not known whether this value if appropriate for humans but it does indicate that
34 using n=3 for extrapolation short time periods is too conservative. Therefore, time extrapolation is
35 performed using n=1 and N=2 for longer and shorter time periods respectively. An interspecies factor of 3
36 is used because CNS depression due to benzene exposure does not appear to vary much between species.
37 In addition, the use of a larger interspecies factor would provide AEGL-2 values that do not match the
38 human experience (see below). Furthermore, with respect to CNS depression in animals, benzene is less or
39 equipotent to other alkylbenzenes and toluene in particular (Molnar et al., 1986; Tegeris and Balster, 1994,
40 Frantik et al., 1994) which means that the AEGL-2 values for benzene should end up within the same
41 order of magnitude than the CNS-based AEGL-2 values for toluene. The interim AEGL-2 values for
42 toluene are 990, 570, 510, 510, and 510 ppm for the 10 min, 30 min, 1 hour, 4 hour, and 8 hour period
43 respectively. Proposed AEGL-2 levels for xylenes are 990, 480, 430, 430, and 430 ppm for the 10 min, 30
44 min, 1 hour, 4 hour, and 8 hour period respectively. In contrast, to toluene and xylene which reach steady
45 status in the blood within 2 or 4 hours, benzene does not reach a steady state in blood and tissues before 4
46 hour. Therefore, for benzene time extrapolation should continue over the whole AEGL time frame.

An uncertainty factor of 3 is used for intraspecies variation because it has been found from experience with anesthetic gases that CNS depression does no vary by more than a factor 2-3 between groups in the population. The total uncertainty factor is therefore 10. This provides the AEGL-2 values displayed in the table below.

There are no reliable human data available for setting an AEGL-3. Human data, however, can be used as supporting evidence, especialli since the 'old data' are based on many years of experience with benzene exposure with large number of workers. Only few adequate LC50 studies are available in animals and the available studies do not allow the derivation of a substance specific value for the factor of n.

The observed NOEL for mortality of 5940 ppm for a 4h exposure rats (Molnar et al., 1986) is taken as starting point AEGL-3 development. Because the mortality of benzene is caused by severe CNS depression (paralysis of the respiratory center), this effect is correlated to the benzene level in the brain lipid fraction (De Jongh et al., 1998). This concentration will be related directly to a build-up of benzene in the tissue, which is directly related to the inhalation rate (see e.g. the data from Sabourin et al., 1987). Therefore, it is expected that humans require higher external concentrations compared to rodents, to obtain a similar level of benzene in the blood or brain as is observed also for other VOC's (trichloroethylene, toluene). In the technical support document for toluene, kinetic information that supports this view is available for various species (including humans). For this reason, an interspecies uncertainty factor of 1 is used. The use of a higher uncertainty factor for interspecies variation, would actually result in conservative AEGL-3 values which are too low compared to the – although limited – human experiences. In addition, values would not match with those of toluene since benzene is less or about equipotent with regard with CNS depression and mortality (Tegeris and Balster, 1994; Bonnet et al., 1982). it has been found from experience with anesthetic gases that CNS depression does no vary by more than a factor 2-3 between groups in the population. It should be noted that especially for toluene, a range of human studies is avialable. An uncertainty factor of 3 is used for intraspecies variation because it has been found from experience with anesthetic gases that CNS depression does no vary by more than a factor 2-3 between groups in the population. This is supported by the small range in concentrations found between 0 and 100% mortality (Svirbely et al., 1943; Bonnet et al., 1982). Time extrapolation is performed using n=1 and n=2 for longer and shorter time periods respectively (see above). This approach results in the AEGL-3 values displayed in the table below. The values are extrapolated from a 4h endpoint to 10 min because the resulting values are supported by other animal data within a exposure time of 15 min to 2h (Von Oettingen, 1940; Furnas and Hine, 1958, Nielsen and Alarie, 1982; Magos et al, 1990). The resulting AEGL-3 values are in good agreement with the limited quantitative human experiences from occupational and accidental exposures.

In addition, these values are considered to be protective also for sudden cardiac arrest due to cardiac sensitization.

1

SUMMARY TABLE OF PROPOSED AEGL VALUES FOR BENZENE in ppm (mg/m^3)						
Classification	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour	Endpoint (Reference)
AEGL-1 (Nondisabling)	130 (420)	73 (240)	52 (170)	18 (58)	9.0 (29)	Highest level available without AEGL-1 effect in humans. 110 ppm for 2h no subjective symptoms (Srbova et al., 1950)
AEGL-2 (Disabling)	2000* (6500)	1100 (3600)	800 (2600)	400 (1300)	200 (650)	Highest level without AEGL-2 effect (CNS depression, i.e. reduced activity in animals). 4000 ppm for 4h. Molnar et al., 1986.
AEGL-3 (Lethal)	See below¶	5600* (18,000)	4000* (13,000)	2000* (6500)	990 (3300)	Highest reliable NOAEL for mortality in rats. 5940 ppm for 4h. Molnar et al., 1986.

2 * The AEGL-2 or AEGL-3 value is higher than 10% of the lower explosive limit of propane in air (LEL = 1.4 %
3 (14,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.
4 ¶ The 10-min AEGL-3 value is higher than 50% of the lower explosive limit of propane in air (LEL = 1.4 % (14,000
5 ppm)). Therefore, extreme safety considerations against hazard of explosion must be taken into account.
6 The calculated 10-min AEGL-3 value is 9700 ppm (31,000 mg/m^3).
7
8 **Key References**

9 Srbova, J., Teisinger, J., Skramovsky, S. (1950) Absorption and elimination of inhaled benzene in man.
10 Arch. Ind. Hyg. Occup. Med. 2; 1-8.
11
12 Molnar, J., Paksy, K., Naray, M. (1986) Changes in the rat's motor behaviour during 4-hr inhalation
13 exposure to prenarcotic concentrations of benzene and its derivates. Acta Physiol. Hung. 67; 349-354.
14

15

1 INTRODUCTION

Benzene (or benzol, the old name for the commercial product benzene) is a clear colorless liquid with a characteristic sweet odor at low concentrations and disagreeable and irritating at high levels (Cavender, 1994). Chemical and physical data are summarized in table 1. The natural sources of benzene are petroleum and, to a lesser extent, condensate from natural gas production. In industry, benzene is produced by different petroleum conversion processes in petroleum refinery and chemical plant processes, primarily by catalytic reforming, steam cracking and dealkylation. Pure benzene can be isolated from coal tar oil and by refining crude oil. Over half of the benzene demand is covered by means of extractive distillation of pyrolysis gasoline in the EU (EU 2002) but in the USA over 90% of the benzene is derived from petroleum sources (EHC 1993). Benzene was the 17th highest volume chemical produced in the United States in 1994 and its production was about 14.7 billion pounds in 1994 (ATSDR 1997).

Benzene as a constituent of gasolines may account for levels up to 5% by volume until 2000 in Europe. From 2000, a maximum benzene content of 1% is allowed in Europe. In the USA, benzene contributes about 1-2% of unleaded gasolines (ATSDR 1997). Benzene is also present in tabacco smoke and has been detected in expired air of smokers (Cavender, 1994).

In the chemical industry, benzene is industrially the most important of the so-called BTX aromatics (benzene, toluene, xylene). In industrial chemistry, benzene forms the basis for a great variety of aromatic intermediates and for the group of cycloaliphatic compounds. Benzene is used as the basis for the manufacture of plastics, synthetic rubber, dyestuffs, resins, raw materials for detergents, and plant protection agents (EU, 2002). In addition, the use of benzene as a solvent is widespread.

Table 1 Physical and chemical properties of benzene.

Parameter	Value	Reference
Molecular formula	C_6H_6	BUA 1988
Molecular weight	78.114 g/mole	BUA 1988
CAS Registry Number	71-43-2	BUA 19988, ATSDR 1997
Physical state	Strongly reflectant liquid	
Color	Colorless	
Synonyms	Benzol, coal naphta	ATSDR, 1997
Vapor pressure	99.6 hPa (at 20°C)	BUA 1988, EU 2002
Density	0.8787 g/cm^3 (at 15°C) 0.8786 g/cm^3 (at 20°C)	ATSDR, 1997 BUA 1988
Melting point	5.5 °C	ATSDR 1997, EU 2002
Boiling point	80.1 °C	BUA 1998, ATSDR 1997, EU 2002
Solubility	In water: 1.77 g/l (at 25°C)	BUA 1988
Explosive limits in air	1.4% (lower limit), 8% (upper limit)	ATSDR 1997
Flammability	Highly flammable Auto flammability at 555 °C.	EU 2002

Conversion factors	1 ppm = 3.24 mg/m^3 1 mg/m^3 = 0.308 ppm	BUA 1988

2 HUMAN TOXICITY DATA

2.1 Acute Lethality

Numerous cases of fatal or nearly fatal benzene exposures have been reported in the public literature, especially in publications from the first part of the 20th century (e.g. Greenburg, 1926a; Flury, 1928; Hamilton, 1931). Most cases deal with accidental poisoning with benzene in industrial settings such as coal tar and benzene tanks or in and around distillation facilities. A substantial number of fatal exposures specifically occured in enclosed spaces filled with (residual) benzene vapor. Such spaces can easily contain high concentrations of benzene vapor due to the high vapor pressure of this substance. In various publications, necropsy findings are reported and sometimes also tissue levels of benzene have been measured. However, concentrations and exposure durations were never determined or reported, except for a single case in which concentrations were measured in a simulation experiment afterwards.

Lethality data in humans are summarized in Table 2 and Table 3.

2.1.1 Case Studies

Greenburg (1926a) describes numerous cases of fatal acute benzene exposures. In many cases it was argued by the author that lethality was the consequence of both the acute toxic actions of benzene itself and possibly asphyxiation that follows the presence of benzene vapors. No cases were described which included any indication for exposure concentrations or durations, except for a single case with oral exposures (see section on non-inhalation toxicity). Greenburg (1926a) also referred to findings by Lehmann indicating that levels of 4700 ppm will produce confusion within 30 minutes while levels of 6160 to 9190 ppm will produce ‘definite symptoms of poisoning within a few hours’ (no reference provided; basis of these statements cannot be assessed).

Flury (1928) does not describe actual cases of humans poisoning but provides raw estimates of acute lethality levels for humans. It was stated that a level of 20.000 ppm for 5 to 10 minutes would be lethal, a level of 7500 ppm for 30 to 60 minutes would be dangerous (toxic) while a level of 3000 ppm for 30 to 60 minutes would be tolerable. However, these values should be viewed with care since they result probably from extrapolations from experiments with mammals.

Hamilton (1931) and Cronin (1924) provide reviews of clincal and pathological findings in fatal and non-fatal acute benzene poisonings. However, their findings were not coupled to exposure estimates. Benzene exposure results in depression of the CNS, irritation, muscle twitchings, deepening of respiration, increased pulse rate, decreased body temperature and, in fatal cases, narcosis, convulsions and death from paralysis of the respiratory center. Typical necropsy findings after acute benzene poisoning are: dark red blood, cyanotic organs (both resulting from asphyxiation), hemorrhages (often punctate in origin) in brain (especially the surface), lungs, heart, abdominal organs and interstitial membranes, and sometimes the skin, (interstitial) edema of the lungs, and congestion of brain, lungs, and abdominal organs (especially liver and kidneys).

Winek et al. (1967) describe the case of a 16-yr Caucasian boy who was found in the cellar of his home probably due to “glue sniffing” from a can of rubber cement. Necropsy revealed cerebral and pulmonary edema,

1 and congestion of spleen, liver, and mucosal surfaces of the stomach and duodenum (see Table 2 for tissue
2 levels). Exposure concentration and duration are unknown.
3
4 A 45-yr old healthy white male died from acute exposure to “light oil” (67.7% benzene, 5.7% xylene,
5 14.5% toluol, 2.0% forerunnings from a benzene destillate, 4.8% crude solvents, 5.3% residues) when he was
6 trying to correct the overflow from a storage tank. Upon noticing the overflow from a control room he ran towards
7 the tanks and was found lying on the floor about 2 minutes later some distance from the spill. No distinct aromatic
8 odor was noticed by the colleague that found him. Based on the conditions of the tanks, the victim had managed
9 to stop the spill. He received mouth-to-mouth resuscitation and later a supply of oxygen. Exposure concentrations
10 were not known but exposure duration was within minutes. Autopsy revealed no structural damage. Benzene
11 levels in various tissues are reported in Table 2 (Tauber, 1970).
12
13 An 18-yr old white male was found on the floor with a plastic bag over his head containing a folded
14 handkerchief. A partly emptied bottle of reagent grade benzene was found nearby. The boy was taken to hospital
15 and was pronounced death (no emergency treatment had been given). Autopsy showed acute granular tracheitis,
16 laryngitis and bronchitis, massive hemorrhages of the lungs, congestive gastritis, spleen infarct, acute congestion
17 of kidneys, and cerebral edema (Winek and Collom, 1971). See for benzene tissue levels Table 2.
18
19 A16-yr old white male was found death on the bedroom floor with a bullet wound through his throat. A
20 plastic bag together with empty tubes of model airplane glue were found in the same room. The boy had not
21 shown any signs of depression or unusual behavior in the days before his death. Autopsy was not performed but
22 heart blood was analyzed for benzene (see table below). It was concluded that the boy was sniffing glue and shot
23 himself, accidentally, while being in euphoric state (Winek and Collom, 1971).
24

25 **Table 2 Benzene tissue levels in case reports of fatal benzene exposure of humans.**

	Winek and Collum, 1971 18 yr boy	**Winek and Collum, 1971 16 yr boy**	**Winek et al., 1967 16 yr boy**	**Tauber, 1970 45 yr old male**	**Avis and Hutton, 1992 25 yr male case 1**	**Avis and Hutton, 1992 30 yr male case 2**	**Avis and Hutton, 1992 39 yr male case 3**	**Barbera et al., 1998 41 yr male**
	Blood values in mg/l – all other tissue levels in mg/kg tissue							
Blood	20	65	0.94	3.8	120	30	54	31.7
Urine	0.6							2.3
Kidney	19		5.5					75
Liver	16			2.6	15	38	25	379
Bile	11				trace	trace	45	
Abdominal / body fat	22.3				> 120	68	88	
Brain	39		44	13.8	58	62	63	179
Stomach contents	10							

26
27 Avis and Hutton (1993) described an accident on a cargo ship resulting in three fatalities. Three workers
28 opened a flange valve in the cofferdam of a ship and were acutely exposed to benzene fumes, which remained
29 from the previous cargo. Another worker put on an oxygen facemask, rescued one of the three workers but was

1 then overwhelmed by the benzene fumes himself. When his body was found the facemask was partially removed.
2 Exposure concentrations were unknown, exposure duration was probably only a few minutes. The three victims
3 showed second degree skin burns on the face, trunk and limbs. Autopsy revealed hemorrhagic airless lungs with
4 alveolar hemorrhage and pulmonary edema. Brains appeared normal but showed vascular congestion
5 microscopically. Benzene levels in body tissues are presented in Table 2.
6
7 A 41-yr old white male went inside an empty storage tank for inspection after the tank was discharged of
8 benzene and was washed and ventilated. After several rescue attempts the victim was removed from the tank
9 unconscious and he died before arriving at the hospital. Exposure concentrations were unknown but exposure
10 duration was certainly less than 30 min (since estimated time to death was 30-45 minutes). Autopsy showed
11 marked congestion of meningeal vasa, edema and congestion of the parenchymal vessels. The lungs were swollen,
12 large blood clots were found within the heart, and multi-organ congestion was evidenced (Barbera et al., 1998).
13 Tissues were analyzed for benzene content (see Table 2).
14
15 In a number of fatal cases associated with benzene exposure (often some form of "sniffing"), it has been
16 reported that sudden death occurs directly following a short period of euphoria with strenuous activity (running
17 for about 30-100 m). It has been argued that these cases of sudden death are related to benzene-induced cardiac
18 sensitization (Bass, 1970; Reinhardt et al., 1971; Litovitz, 1988). However, no quantitative human data are
19 available with respect to this toxicity endpoint. A number of animal studies of varying quality have addressed this
20 issue but most of them involve probably very high levels of exposure (see section 3.2).
21
22 ***Studies with non-inhalation exposure***
23 A workman who swallowed 80 grams of commercial benzene developed acute symptoms (no details
24 provided, (the present reviewer calculated that this dose is equivalent to 1.14 mg/kg bw, assuming a body weight
25 of 70 kg). Five cm^3 of 10% lecithin emulsion given i.v. after 2h resulted in definitive improvement (Greenburg,
26 1926a).
27
28 A 3 yr old child accidentally had been drinking from a bottle of commercial benzene (no estimation of the
29 actual dose provided). The child died after 2 hours (Heyndrickx et al., 1943). Analysis of tissues showed that
30 benzene was primarily located in the brain and the liver (determined colorimetrically).
31
32 Bonnichsen et al. (1966) described three fatal cases in which benzene levels were found in tissues of the
33 individuals. All three cases involved individuals who had been drinking or were chronic alcoholics; co-exposure
34 to ethanol was likely. Oral intoxication by benzene was most likely accidental. Benzene levels were detected in
35 stomach contents but measurements on other tissues showed no clear pattern or were not available. The dose to
36 which the individuals were exposed was unknown.
37
38 **Table 3 Summary of data on lethality in humans**

SUMMARY OF DATA ON LETHAL EFFECTS IN HUMANS					
Subject information	**Exposure route**	**Exposure information**	**Estimated dose**	**Effect**	**Reference**

Male 16 yr	inhalation	Glue sniffing	Unknown	Death	Winek et al. 1967
Male 45 yr	inhalation	A few minutes with strenuous activity	High levels for a few minutes	Death	Tauber, 1970
Male 18 yr	inhalation	Sniffing from plastic bag	Unknown	Death	Winek and Collum 1971
Male 16 yr	inhalation	Sniffing from glue	Unknown	Death (see summary)	Winek and Collum 1971
Three males 25-39 yr	Inhalation	High levels in confined space	Within minutes, concentration unknown	Death with severe skin and airway irritation	Avis and Hutton, 1993
Male 41 yr	Inhalation	Benzene vapor in storage tank	Duration less than 30-45 min, concentration unknown	Death	Barbera et al., 1998

2.2 Nonlethal Toxicity

In his monograph of 1960, Gerarde provides a table summarizing the toxicological effects of exposure to benzene vapors for humans. The table re-appeared in Cavender (1994) with some additions based on the publication by Van Oettingen from 1940. Although this table is probably based on estimations from both animal experiments and early human experience with benzene exposure, it is not clear from any of these papers on which particular primary data this table is constructed. The table below is taken from Cavender (1994) but with corrections based on the original publication of Gerarde (1960).

In his review of 1984, Fishbein stated that the primary acute toxic effect of benzene is on the central nervous system, which would be initially seen at exposures above 250 ppm. Exposure to massive concentrations (about 25,000 ppm) would be fatal within minutes (Fishbein, 1984). However, no primary data were referenced on which these statements were based.

1 **Table 4 Estimated toxicity levels for humans (according to Gerarde 1960).**

Concentration in ppm	Concentration in mg/m^3	Exposure duration	Effects
1.5	4.86	-	Olfactory threshold
25		480 min (8h)	No effects; benzene detectable in blood
50-150	162-486	300 min (5h)	Headache, lassitude, weariness
500	1620	60 min	Symptoms of illness
1500	4860	60	Serious symptoms
3000	9720	30 min	May be tolerated for 0.5-1 hr
3100-5000	10,044-48,600	30 min	Subtle signs of intoxication, absorbed 79.8-84.8 %
7500	24,300	60 min	Signs of toxicity in 0.5-1 hr, dangerous to life
19,000-20,000	61,560-64,800	5-10 min	May be fatal in 5 to 10 min.

2
3
4 **2.2.1 Experimental Studies**
5 In a historical study by Lehmann (1910), 3 humans were exposed to 11-16 mg/l (± 3400 – 4900 ppm) for
6 5 to 15 minutes. It was stated that this level induced airway irritation as well as some excitement and dizziness
7 after 10 min.
8
9 Srbova et al. (1950) studied the uptake and elimination of inhaled benzene in human volunteers (23
10 students and laboratory assistants). Volunteers inhaled benzene at levels between 47 and 110 ppm (measured
11 concentrations, polarographic method used not specified) for periods up to 2h (sometimes up to 3h). The benzene
12 was delivered by breathing equipment containing a valve separating the inhaled and exhaled air streams. This
13 method probably excludes contact of the skin and eyes with benzene vapor. It was reported that the volunteers had
14 no subjective troubles (volunteers probably did not complain, no active questionnaire involved). From this
15 statement it can be concluded that exposure up to 110 ppm for 2h does not induce subjective complaints in
16 healthy humans.
17
18 In a metabolism study, Inoue et al. (1986) investigated the relation between benzene exposure and urinary
19 phenol levels in 64 men and 88 women. Geometric mean benzene exposure in several subgroups ranged from 1.0
20 ppm to 76.4 ppm (7h TWA, badge type passive samplers). In a second metabolism study, Inoue et al. (1988)
21 investigated urinary metabolites of 65 benzene exposed workers and 55 benzene + toluene exposed workers.
22 Exposure to benzene was 31.9 ± 24.8 ppm (arithmetic mean) or 17.9 ± 29.3 ppm benzene + 20.5 ± 25.8 ppm
23 toluene (8h TWA, personal diffusive samplers). No information on health effects was provided in either of these
24 two reports.
25
26 Hunter and Blair (1972) exposed male volunteers (laboratory staff) to levels of 19 – 125 ppm for periods
27 up to 6-8h and sometimes to 25 – 30 ppm for 2 periods of 3-4h (separated by lunch break) up to 5 consecutive
28 days. No information on health effects was provided. In addition, Sato et al. (1974) exposed three male volunteers
29 to 25 ppm for 2h by breathing mask in a metabolism study. No information on health effects was provided in
30 either paper.
31

1 **2.2.2 Case Studies**
2 Four employees were involved in the renovation of a confined room (19 m^3) for 2 days in which the floor
3 tile was taken up and remaining adhesive was removed by using benzene (Drozd and Bockowski, 1967).
4 Dissolved adhesive in benzene was wiped up using rags which were then thrown in an open container. This work
5 was primarily performed on the first day which consisted of 4h work divided in a morning shift of 2h (door
6 closed) and an afternoon shift of 2h (door open). When the benzene vapor became annoying, the men left the
7 room for a few minutes. On the second day a morning shift of 2h was followed by an afternoon shift of 3h during
8 which new tile was laid using a water-based adhesive. Benzene was then used primarily for cleaning hands and
9 removal of residual adhesive. One employee (19-yr old white male) returned in the evening for another shift of
10 3,5h. After this period he felt sick, complaining of malaise, vertigo, nausea and dizziness. He was treated for his
11 symptoms and was advised to sleep in a well-ventilated room. The next morning, he was hospitalized. Body
12 temperature was 38°C, pulse rate 88 beats/min and blood pressure 90/40 mm Hg. Other examinations did not
13 shown abnormalities except for paravertebral tenderness. The ratio of organic and inorganic sulfate in his urine
14 was changed (see section 4.2) indicating possible benzene intoxication. The patient was discharged after 13 days
15 of hospitalization with no residua. In a simulation experiment in the workspace with two containers filled with
16 benzene wetted rags, benzene concentrations were measured using Draeger tubes (5 times during 1h). With the
17 door closed, benzene levels in the room ranged from 750 ppm (after 30 min from the start of the experiment) to
18 1500 ppm (after 50 minutes). When the door was opened, levels decreased to 600 ppm within10 minutes (Drozd
19 and Bockowski, 1967). These simulation values should be related primarily to the first 2h of work of the first day
20 in which benzene was used with the doors closed. Because the simulation experiments only involved passive
21 evaporation from containers filled with rags, whereas the actual work involved wiping with benzene soaked rags,
22 the values might be an underestimation of the personal exposure of the workers, at least in the first 2 hours*.
23
24 Fifteen male workers (age 29-62 yr, 14 black, one Caucasian) performed degassing operations in fuel
25 tanks of a ship (Midzenksi et al., 1992). The operations involve cleaning of the tanks from residual fuel by using
26 pressurized hot water. Workers were partially protected by boots and rain suits. After several days, inspection
27 revealed the presence of benzene in the tanks by Draeger equipment. Multiple samples showed benzene levels >
28 60 ppm. Gaschromatographic analysis of a gas sample from an uncleaned tank of the same ship (with about 7500
29 L fuel left) showed a concentration of 653 ppm. Due to analytical aspects, the authors propose that benzene levels
30 could have been even higher and calculated that levels could have been maximally 987 ppm based on a 0.01%
31 level of benzene in the fuel (0.01% is detection limit, benzene was not found above this level in fuel analysis).
32 The tanks are confined spaces with limited openings, limited ventilation, and were not designed for continuous
33 worker occupancy. The time that workers spent in the contaminated tanks was between 1 day to 3 weeks (mean 5
34 days, median 3 days) with a range of 2.5 to 8 hours/day (mean 5.5 h, median 5h). The time of employment before
35 the incident ranged from 3 months to 10 years (mean 50 months). Hematological profiles of the workers were
36 made within 2 days after the acute exposure and regularly thereafter up to 1 year. A month after the incident
37 questionnaires research was done in order to obtain medical and occupational history, smoking habits, alcohol
38 consumption, and symptoms experienced during the incident. Symptoms encountered during working in the tanks
39 included mucous membrane irritation (eye, nose, throat, mouth) (80% of the individuals), peculiar or strong odor
40 compared to other ships (73%), dyspnea (67%), dizziness/lightheadedness (60%), nausea (47%), chemical taste

* Using all the parameters described in the original poublication, the personal exposure of a worker was calculated by the present reveiwer, using the exposure model CONSEXPO 3.0 developed by RIVM (Van Veen, 2001). Exposures ranged from 850 ppm (with 10cm^3 evaporation surface) to 2000 ppm (with 25cm^3 evaporation surface) using low ventilation rates (1 volume/h). Equilibrium concentrations were reached within a few minutes. These exposure estimations are in good agreement with the Draeger-tube measurements.

1 (47%), headache (33%), cough (27%), drowsiness (20%), fatigue (20%), and skin irritation (13%). An analysis of
2 the relation between individual exposure duration and reported symptoms showed that those worked for more
3 than 2 days reported nausea and dizziness more frequently compared to those exposed < 2 days. Hematological
4 profiles showed no consistent trends following exposure, although 9 of the 15 workers showed at least one
5 hematological abnormality (6 workers showed numerous large granular lymphocytes (LGL)). Neither the duration
6 of the acute exposure, the total time of employment as a degasser, nor individual alcohol consumption was related
7 to the occurrence of LGL's. Except for a correlation between low WBC counts and low MCV counts, no relations
8 were observed between any of the hematological parameters. It should be remarked that the presence of other fuel
9 constituents (n-hexane, xylenes, toluene) was not analyzed but may contribute to the observed findings.
10
11 Ten men present in a dockside were briefly exposed to a spillage of some 1200 UK-gallons of benzene
12 (about 5500 liter) (Clare et al., 1984). Urinary concentrations of phenol (as a marker for benzene exposure, see
13 section 4.2) in these workers were clearly increased on the day of the spillage (the highest concentrations
14 measured in each individual were 127, 20, 213, 331, 184, 16, 154, 226, 256, and 97 mg/L respectively) Urinary
15 phenol levels measured in 5 of these men a few days before the spillage ranged from 2 to 21 mg/L. Exposure
16 duration or concentrations in air were not provided or estimated. Blood samples of these exposed workers were
17 investigated for chromosomal abnormalities 4 months after the incident and compared to a group of 11 control
18 workers from the same company which were not present during the spillage. Chromosome aberrations were not
19 increased in the exposed group. The number of SCE's per chromosome was slightly increased (0.14 ± 0.02 vs.
20 0.12 ± 0.01 in controls). When the number of SCE's per chromosome was plotted against the highest phenol level
21 measured in urine, a positive but non-significant relationship was obtained ($r=0.55$, $0.05<p<0.10$). The number of
22 cells (standardized at 30 cells p.p.) showing more then 10 SCE's, however, was clearly increased (2.9 ± 2.2 vs.
23 1.6 ± 0.89 in controls).
24
25 Very recently, a spill of "benzene heartcut" (40-60% benzene, 40-60% naphta) was reported in the UK.
26 About 250 tonnes of this mixture were released from a storagetank along the Tees river. The incident was
27 identified by a ship crew reporting a benzene-like odor. First measurements indicate concentrations above the
28 Maximal Exposure Limit (> 3ppm). Seventeen men were sent to hospital for investigation. Further information
29 indicates that workman on a jetty had a burning sensation in their nose and some skin irriation. Measurements
30 indicated 50 ppm in the open air and 100 ppm in a control cabin at the jetty. No details about the measurements
31 are known (A. Keddie, UK-HSE, personal communication).
32
33 ***Studies with non-inhalation exposure***
34 No studies summarized.
35
36 **2.2.3 Occupational Exposure**
37 Benzene has been used for more than 100 years in various types of industries (Brief et al., 1980; Verma
38 and Des Tombes, 1999). Some types of activities in particular have been associated with substantial exposure of
39 workers to benzene vapor: artificial leather- and shoe manufacture, the rubber industry and the rotogravure
40 industry. There is a massive amount of literature, that deals with occupational exposure. Direct associations
41 between personal exposure and health effects are mostly absent since the majority of these papers deal with
42 chronic exposure estimates in relation to hematotoxicity and carcinogenicity. Over the last century, exposure to
43 benzene has progressively decreased along with the development of the benzene TLV (ACGIH 1997, Verma and
44 Des Tombe, 1999). The highest benzene exposure levels were thus reported in the 20's and 30's of the 20th
45 century. Other information comes from various sources in the 1960's and 1970's. Because the technical and
46 scientific standards in the past with respect to exposure measurements (including transparant description of

1 methods) were quite different from current accepted guidelines, the rather ‘historic’ exposure data do not always
2 match our high standards of today. However, this does not necessarily implicate that the data that were generated
3 are not useful. Just because of the fact that measurements have been reported from various factories and
4 workplaces involving many thousands of people, the general picture that emerges from the total amount of
5 information should be considered as highly valuable. In addition, the rather ‘historical’ data generated in the US
6 and Europe is supported by rather recent exposure data from industries in China.
7
8 In this section a general overview will be given of occupational exposure levels that have been reported
9 with special attention to the highest values reported and peak levels involved.
10
11 As reported eslwhere in this document, Greenburg (1926b; 1939; see below for detailed descriptions)
12 reports workplace measurements in several factories within the range of 70 to 1800 ppm *on average* depending on
13 the season. Maximum levels in 18 different places were 100, 110, 120, 130, 190, 340, 340, 350, 360, 390, 450,
14 460, 480, 860, 890, 1020, 2640, and 4140 ppm. No details about the analytical procedure were provided and the
15 numbers represent area samples. Greenburg (1926b) also refers to other studies from 1920 indicating workplace
16 levels (most likely area samples) of 210 – 1050 ppm, and one observation of 2800 ppm in a ventilated room with
17 low ceiling.
18
19 The studies performed by Aksoy and collegues in Turkey in 1960-70’s involved workplace levels up to
20 650 ppm on a regular basis (Aksoy et al., 1971, 1972; Haley, 1977). Haley (1977) indicates that the majorty of
21 *average* workplace levels in the 1960-70’s were within the range of about 10 – 400 ppm.
22
23 Fishbein (1984) provides an overview of occupational exposure data primarily used in evaluations by
24 NIOSH and EPA. Workplace concentrations ranged from virtually zero up to 650 ppm in a range of industrial
25 settings with one source with levels up to 1060 ppm (probably all average values). Peak levels were reported up to
26 1400 ppm (Fishbein, 1984).
27
28 In a recent review paper Wong (2002) summerised various actual exposure data of Chinese factories. It
29 should be emphasized that these figures represent regular occuring exposure levels involving up to 500.000
30 exposed individuals. In addition, Wong (2002) pointed out that in Chinse factories people work(ed) for about 12-
31 14 hours per day instead of the normally assumed 8 hours/day. Although no details were provided in this paper, it
32 may be assumed that the values summarised by Wong (2002) were determined using relatively modern sampling
33 and analysing techniques (compared to the e.g. Greenburg 1926b, 1939) because these exposure data were
34 determined to a large extent in the last decade. Wong (2002) indicated that one case of leukemia identified in the
35 studies of Yin et al (see carcinogenicity section) was associated with an average exposure of 149 ppm with a
36 range of 0-1265 ppm. In a railroad company, levels were reported up to ~ 460 ppm (most likely time weighted
37 average levels instead of short term peak levels). Exposure levels in several Chinese shoe factories ranged from
38 about 50 ppm up to ~ 840 ppm. Maximum levels were about 600 – 1235 ppm. The latter value was the highest
39 level measured within a series of 30 ‘highest’ measurements. In addition, levels in a furniture factory were
40 reported to be as high as 1130 ppm with an average of 1040 ppm while employees were working 10-12h per day.
41 (Wong, 2002).
42
43 In conclusion, regular occuring concentrations in workplaces in the past were mostly within the range of
44 10 to about 500 ppm. However, also area concentrations of 1000 – 1500 ppm were observed. To illustrate to
45 exent of these high exposures, Yin et al. (1987) reported that about 1.3% of the factories included in their analysis
46 had *mean* exposure levels above 308 ppm (1.3% of 50255 workplaces involves > 600 workplaces). In most cases

no details about sampling strategy, duration of sampling and analytical methods were provided. The massive amount of data that fit the general picture (including the recent data summarised by Wong (2002)) provides sufficient confidence in the exposure ranges. Unfortunately, acute occuring health effects were almost never reported and at least not coupled to exposure data on the individual level. Most studies focussed on hematological changes and carcniogenicity when studying health effects. As described by Wong (2002), benzene intoxication is substantial after prolonged exposure in these conditions (intoxication rates of 25-38% of the workers have been reported). However, one must assume from the large extent of people involved in these occupational exposures, and the fact that these exposure levels occurred on a regular basis that those condition were not severely adverse on an acute scale and will certainly not have resulted in an impaired ability to escape, although the occurrence of some health effects such as headache, dizziness, and irritation cannot be excluded at these levels (see also below).

In the following sections, only studies are summarised in which (high) exposure data were to some extent coupled with reports on health effects.

Study descriptions

Greenburg (1926b) investigated a number of plants and workrooms where benzene was used (in various processes) for benzene levels. In a part of these plants a medical survey of workers was performed (mainly focussed on hematological changes). Unfortunately, exposure levels were not coupled to health outcomes on an individual level but only at group level. In addition, levels of benzene in the workroom air in summer were mostly based on 2 or 3 measurements, other samples and all winter values were based on 10 to 20 measurements. Measurements were performed non-specifically using charcoal tubes (using 20L air samples, short term area sampling). In summer, mean 'benzene' levels ranged from 70 to 1800 ppm (minimum and maximum individual measurements were respectively 20 and 4140 ppm). In winter, mean 'benzene' levels ranged from 90 to 580 ppm (minimum and maximum individual measurements were respectively 0 and 1020 ppm). In the health surveys, 81 individuals were investigated for symptoms and signs (questionnaire) and for hematological changes (Hb, RBC,WBC and differential WBC counts). Based on a ≥ 25% decrease in WBC (associated with increased lymphocyte and decreased PMN counts) an individual was marked as "positive". In total 26 out of 81 individuals were classified as positive. When examining the mean benzene concentration and the number of positive individuals for the various occupational settings no clear dose-response like pattern is present. From this paper it can be concluded that at mean benzene levels of about 100 ppm in occupational settings, "positive" individuals can still be found. Reporting on symptoms and signs was done only for 9 positive individuals. Findings include pallor (6/9), dizziness (4/9), headache, loss of appetite and spongy gums (all 2/9). Exposure durations or total employment time were not provided. Although only short-term area samples were used (personal breathing zone sampling was not performed in those days) which can over- or underestimate the actual exposure, it can be concluded that mean 'benzene' exposure levels up to about 1000 ppm occured on a routine basis and will not form an inability to escape.

In an additional study, Greenburg et al (1939) showed concentrations of 11-1060 ppm in rotogravure and dry-cleaning workplaces (plants A, B, and C; total 332 workers). Exposure was 4-7 days/week for 3 to 54 years. In plant A, 11 samples showed levels in the range of 50-1060 ppm with 4 samples > 400 ppm (440, 480, 740, 1060 ppm). In plant B, 24 samples showed levels in the range of 24-675 ppm with 6 samples > 300 ppm (320, 328, 395, 473, 675 ppm). In plant C, 13 samples showed levels in the range of 11 – 298 ppm. In plant C (the lowest concentration) workers reported symptoms as fatigue and dizziness, in plant B (medium concentration) fatigue, dryness of mucous membranes, hemorrhaging, nausea, vomiting, and lethargy, while in plant A (highest concentration) weakness, fatigue, epistaxis, dryness of mucous membranes, loss of appetite, nausea, vomiting, dizziness, insomnia, and lethargy were reported. Of the total group workers examined, 130 showed various

1 degrees of benzene poisoning (decreased RBC, mean corpular volume (MCV), platelets, and WBC). Because
2 workers were present in different places of the plant during a day, the given values cannot be directly translated
3 into personal exposure levels. However, these concentrations will have been present on a routine basis and are not
4 expected to have impaired the ability to escape.
5
6 In a survey of 70 workers of a coke oven by-product recovery facility, Hancock et al. (1984) investigated
7 the relation between cumulative benzene exposure and hematological findings. The workers (all males) were
8 presently or formerly employed at the facility at various locations. Mean exposure duration was 207.4 months
9 (range 30-249). Exposure levels were estimated from a) time-weighted average personal monitoring, b) general air
10 sampling values, or c) estimates from plant environmental health engineers. Measurements were only available for
11 the last 2 years and for a limited number of facility locations (associated with specific jobs). For each individual a
12 cumulative exposure was estimated using the exposure level estimates and time on the job. Mean exposure levels
13 were 10.5 ppm (range 0.1-67 ppm) with a mean cumulative exposure of 2026 ppm-months (range 12-26900 ppm-
14 months). The hematological records of these workers (sampled annually for the last 20 years) were used for
15 analysis. A control group of 21 male supervisory employees, assumed to have no benzene exposure, was used
16 (average 202 months on the facility). Three groups were created: low (< 240 ppm-months, N=17), intermediate
17 (240-2400 ppm-months, N=37), and high (> 2400 ppm-months, N=16). These groups correspond with mean
18 exposure levels of <1, 1-10, and > 10 ppm benzene. Analysis of WBC counts, RBC counts, and Hb levels showed
19 no changes compared to controls in any of exposure groups.
20
21 Kellerova (1985) investigated changes in EEG-pattern in benzene exposed workers. The benzene-exposed
22 workers came from two different locations. On location A, 28 male workers were studied (exposure for 0.5 to 4
23 years; mean 2 years; mean age 32.4 years). On location B, 12 workers (9 men, 3 women) were studied (exposure
24 for 2 to 20 years, mean 11.45 years, mean age 41.5 years). On location A, short-term sampling (stationary
25 sampling in breathing zone for 1-2 h) revealed benzene concentrations of 45-154 ppm with isolated cases up to
26 308 ppm. Benzene concentrations in location B were reported to be ‘in the range of the highest permissible
27 concentration of phenol’ without further specification. The results showed that the percentage of people having
28 threshold-type or abnormal EEG findings was significantly increased in benzene exposed workers (67.5% vs.
29 16.7% in controls). The observed abnormalities were episodic or diffuse, or a combination of both. In addition, a
30 higher percentage of benzene exposed workers showed sleep phenomena in their EEG’s (60.0% vs. 25.0% in
31 controls). In particular the occurrence of deeper sleep stages was increased (15% in benzene-exposed vs. 0% in
32 controls). Because of the limited exposure measurements, benzene exposure could not be linked to the occurrence
33 of effects in this study.
34
35 Yin et al. (1987) investigated over 300 solvent workers in factories in China. Three groups were studied:
36 primary exposure to benzene (n=171, 55% females), primary exposure to toluene (n=101, 37% females), and a
37 group exposed to an equal mixture of these two substances (n=43, 56% females). A control group was recruited
38 from the same factories but included individuals without direct exposure to benzene (n=127, 68% females).
39 Benzene concentrations were determined using diffuse personal dosimeters. The mean 7h time-weighted average
40 exposure to benzene amounted to 47.9 ± 41.6 ppm (maximum 7h TWA value was 210 ppm) for men and women
41 combined (mean for men 33.3, mean for women 59.2 ppm). Peak levels were not determined. The groups
42 consisted of adults with a mean age of 31.5 years (controls) or 28.8 years (benzene). Total time exposed
43 (employment duration) was about 5 years in the benzene group. Hematological investigation was performed once
44 on cubital venous blood and finger tip blood. Clinical biochemistry (GOT, GPT, LDH, γ-GT, ALP, LAP,
45 bilirubin, BUN, creatinine, inorg. P) was performed on venous blood and urinalysis involved protein, sugar and

occult bleeding. A self-completion questionnaire for subjective symptoms and was employed and was directed to both symptoms at work and symptoms in the last 6 months.

Hematology in venous blood showed no effects on total WBC, RBC, Hb and Hct for the benzene exposed group. A slight but significant decrease of WBC was observed in finger tip blood (mainly in men) associated with an increased percentage of persons with abnormal cell counts. A significant negative relationship (regression analysis) between WBC counts and exposure duration was observed for finger tip blood but a relation between benzene exposure en WBC counts was absent in venous blood. Differential cell counts in venous blood showed a decrease in lymphocytes and an increase in eosinophils (both only in women).

Urinalysis and serum biochemistry revealed no effects in the benzene exposed group except for a slight increase in inorganic phosphorus in benzene exposed women. Experience of subjective symptoms in the benzene group was significantly increased both during work and during the last 6 months. The main symptoms reported were dizziness, sore throat and headache, followed by nose/eye irritation and difficulty in sleep out of working time. When separated into a low and high benzene exposed group, a dose-related pattern emerges (see table 5). Pancytopenia related symptoms (increased bleeding) was clearly increased in the benzene exposed group but no consistent differences are observed between the low and high benzene group.

Table 5 Symptoms in benzene exposed workers in China (Yin et al., 1987)

Symptom	Control (% affirmative respondents)	Low benzene 1- 40 ppm (% affirmative respondents)	High benzene ≥ 41 ppm (% affirmative respondents)
Number of respondents	87	40	47
Sore throat	1.2	5.0	12.8
Dizziness	6.9	55.0	63.8
Headache	0.0	17.5	21.3
Pancytopenia related symptoms			
Gingival bleeding	21.8	50.0	48.9
Hypermenorrhea	1.2	10.0	8.5
Epistaxis	3.5	2.5	12.8

Kraut et al. (1988) investigated the neurotoxic effects of solvent exposure in 19 male sewage treatment workers (age 24-62 yr (median 43 yr)). Time of employment as sewage worker varied between 10 mo and 37 yr with an average of employment of 5.0 year. In the sewage plant, unusual odors were intermittently reported and 4 measurements with Draeger tubes revealed benzene levels of 30, 50, 85, and 300 ppm during the presence of such odors. However, benzene levels when the odor was absent were not reported and, in addition, it is likely that exposure to other organic solvents (especially toluene) occurred as well. The workers were medically investigated for signs and symptoms, complete blood counts, serum biochemistry, urinalysis, chest x-ray, and pulmonary function tests. In addition, the workers were subjected to 5 neurobehavioral tests aimed at perceptual abilities, attention, concentration, constructional abilities, eye-hand coordination, and higher cognitive processes.

Workers complained of a range of CNS symptoms and irritation. These included increased fatigue (n=8), lightheadedness (n=9), increased sleep requirements (n=5), headache (n=4), eye irritation (n=3), nose and throat irritation (n=2), shortness of breath (n=2), and cough (n=1). The irritation responses occurred usually in the presence of an unusual odor. CNS-related symptoms resolved when the workers were transferred away from the plant. Three workers had elevated urinary phenol levels (22, 31, 52 mg/L compared to an upper normal level of

20 mg/L for unexposed persons). Of the workers employed at plant A for up to 6 years about 30% had abnormal scores in the neurobehavioral tests (deviating more than 2 x SD from the mean score matched for age, sex, race, and education). Of the 4 workers in plant A for 9 to 12 years, all had abnormal neurobehavioral test scores. No effects were observed for blood counts, serum biochemistry, chest x-rays, urinalysis, or pulmonary function tests. Because of the limited exposure measurements and the limited number of workers studied, a relation between exposure and effects at the individual level cannot be defined.

In a recent study by Qu et al. (2002), hematological changes were investigated in Chinese workers exposed within the range of 0.06 – 122 ppm benzene (workday averages using personal sampling). Significant negative correlations were observed between benzene exposure and RBC, WBC and neutrophils. No information was provided on other (acute) health effects in these workers.

2.3 Developmental/Reproductive Toxicity

Information on developmental and reproductive toxicity of benzene exposure in humans is scant. A number of monographs have addressed the issue and summarized the studies available (ECETOC, 1984; BUA, 1988; EHC, 1993; most comprehensive review by ATSDR, 1997). Some data suggest that benzene might be responsible for menstrual disorders in female workers and decreased fertility of women (ATSDR, 1997). However, these studies are all limited in one or more ways. Benzene exposure levels and exposure duration were not well characterized (one study > 1 ppm, another study lower than 0.25 ppm), no adequate controls were included, simultaneous exposure to other chemicals occurred, little follow-up was done on the investigated subjects, or no attempts were made to exclude other medical causes for observed effects (ATSDR, 1997). A much more recent case control study (Stücker et al., 1994) investigated the association between paternal benzene exposure and spontaneous abortions: no association was found. According to ATSDR (1997) studies on developmental effects in humans are limited because of concomitant exposure to other chemicals, inadequate sample size, and lack of quantification of exposure levels. One study was cited in which 14 children from exposed women (not specified) had increased chromosome aberrations and SCE's.

In addition to this information on reproductive and developmental effects in humans, parental exposure to benzene has been suggested as a risk factor for childhood leukemia (OEHHA, 2001). It should be noted that the major leukemia type associated with benzene exposure in adults is acute myeloid leukemia whereas in children, the predominant type is lymphatic. A number of study reports investigating the relation between parental exposure and childhood leukemia were evaluated (Shaw et al., 1984; Shu et al., 1988; Buckley et al., 1989; McKinney et al., 1991; Kaatsch et al., 1998; Shu et al., 1999; Feychting et al., 2001). These studies are summarized in Appendix C.

With respect to maternal exposure, three studies provided some indications for an association of maternal benzene exposure with childhood leukemia (Shu et al., 1988; Buckley et al., 1989; McKinney et al., 1991). Two other studies reported no association at all (Kaatsch et al., 1998; Shu et al., 1999). With respect to paternal exposure, two studies provided some indications for an association of paternal benzene exposure with childhood leukemia (Buckley et al., 1998; McKinney, 1991). Five others reported no association or had an insufficient number of cases (Shaw et al., 1984; Shu et al., 1988, Kaatsch et al., 1998; Shu et al., 1999; Feychting et al., 2001). All of the studies summarised in Appendix C are seriously compromised in one or more ways:

- Exposure levels were not quantified in any of these studies,
- exposure duration was only taken into account in one study,
- most studies probably involve occupational exposure to various chemicals or mixtures,

- most are case-control studies with a high risk of positive recall bias (parents of children with cancer having a higher reporting rate on possible detrimental factors compared to controls) although the level of bias probably differs among the studies,
- most studies have a (too) low number of cases which makes associations less reliable, which is illustrated by relatively higher risks for "non-suspected job categories" such as governmental, administrative, legislative, literary, or artistic work,
- most associations provide indirect (i.e. coupled to a job category with possible exposure to benzene) instead of direct (actually reported benzene exposure) associations; actually in some case a significant OR was only found for a job-category but not for self-reported exposure to benzene,
- Most studies do not specify the leukemia subtypes which might be important because the various subtypes may have different etiologies and, moreover, the most pronounced leukemia type in children (ALL) is not associated with benzene exposure in adults.
- One study indicates an effect for maternal exposure but not parental (e.g. Shu et al., 1988) while another indicates primarily paternal exposure but less maternal (Buckley et al., 1998).

The ways in which parental exposure to benzene might induce childhood leukemia have been indicated to be germ cell mutations prior to conception, transplacental fetal exposure, exposure through breast milk, or direct exposure postnatally to chemicals brought home on clothing. The studies summarized do not support a single mechanism although pre-conceptional benzene exposure of father has been indicated as a risk factor.

In conclusion, although the studies summarized may provide some indications for a possible role of parental benzene exposure, the pattern that emerges from these studies is somewhat inconsistent and does not allow any definitive conclusions on the effects of parental exposure to benzene and possible childhood leukemia. Nevertheless, it remains questionable whether the exposures involved are relevant for setting AEGL values since some form of repeated exposure might be necessary (see e.g. Buckley et al., 1998).

2.4 Genotoxicity

In a recent comprehensive literature review, Zhang et al. (2002) discussed a range of studies which reported chromosomal aberrations (CA), Sister Chromatid Exchanges (SCE), and micronuclei (MN) associated with benzene exposure. For this purpose, studies concerning benzene-associated leukemia cases, studies concerning patients with pre-leukemia or benzene-poisoned states, studies concerning benzene-exposed individuals, and comparisons with other leukomogens were all evaluated. At present, it is not clear what the effect of benzene exposure is on SCE and MN. It was found that results on SCE and MN are much more variable than those obtained for CA. In addition, it was stated that SCE's do not appear to show a significant association with future cancer risk. Therefore, Zhang et al. (2002) concluded that CA is probably the most valid cytogenetic endpoint available for predicting future leukemia risks.

There is no doubt that benzene exposure can results in chromosomal damage in humans. Benzene-associated leukemias are more likely to harbor chromosome abnormalities then de novo leukemias arising in the general population. Aneuploidy and polyploidy have been detected in benzene exposed individuals with trisomy of C-group chromosomes being quite common (Zhang et al., 2002). Structural changes were gaps and breaks but there is insufficient data to indicate any specific pattern of CA to be associated with benzene exposure. However, from G-banding techniques in benzene-exposed investigations it can be observed that translocations occur in 37% of the cases, deletions in 24%, loss or deletion of chromosomes 5 or 7 in 29%, and aneuploidy in 49%. It is hypothesized that this range of genotoxic endpoints can be induced by the fact that benzene produces genomic

instability by causing recombination, double strand breaks and mitotic spindle disruption (Zhang et al., 2002). Although part of the induced abnormalities appear to be reversible (see animal experiments), CA may persist for years after exposure even when hematotoxic effects have recovered and are therefore a marker for possible future cancer risk. A quantitative relation is, however, not available.

Chromosome aberrations and SCE's can be induced already at low exposure levels. Sarto et al. (1984) investigated the occurrence of SCE and CA in 22 benzene exposed workers. Two groups of workers were investigated with a mean age of 41.5 ± 9.6 years and mean time of exposure of 11.4 ± 7.0 years. Most of the exposure time was after 1971 (the time when various procedures were automated providing a substantial reduction in occupational benzene exposure). Environmental benzene exposure was measured using personal samplers (length of measurement not defined) and in addition alveolar air was sampled every 5 or 20 min. (method not specified). A group of matched controls had no exposure (not measured). Environmental exposure was 1.9 ± 1.4 ppm in one group and 5.3 ± 3.0 ppm in the second group (range 0.2 – 12.4 ppm). Alveolar air measurements were 2.2 ± 0.8 and 4.4 ± 2.7 ppm respectively (range 1.1-9.1 ppm). In the exposed workers, no hematological changes or increased numbers of SCE were found. A difference in SCE's was found between the two groups of workers but this was not related to exposure, but more likely to age and smoking habits. In the workers, a statistically increased number of cells with aberrations of the chromosome type was observed (1.1 ± 1.0 % vs. 0.5 ± 0.6 % in controls). The interpretation of the study is hampered by the fact that it cannot be discounted that some workers were exposed to high concentrations of benzene before 1971.

Picciano (1979) investigated the frequency of chromosome aberrations (CA) in 52 benzene exposed workers (sex not specified, mean age 39.3 yr, mean exposure duration 57 months (range 1 month – 26 yr)) and 44 control individuals without previous chemical or radiation exposure (mean age 26.6 yr). The time-weighted-average exposure over a 4 year period was 2.1 ppm, estimated from urinary phenol measurements and stationary area samplers and personnel monitors (not further defined). At the time of blood sampling, no urinary phenol was detected indicating no exposure directly before the sampling. It cannot be ruled out that individual exposure was much higher than 2.1 ppm at intermittent periods. The benzene-exposed group showed no change in chromatid breaks or total percentage of abnormal cells. However, a 2-fold increase in chromosome breaks and a 3-fold increase in marker chromosomes (rings, dicentrics, exchange figures etc..) was observed for benzene exposed workers. Among the controls, 59% had no chromosome breaks and 41% had one or more. Among benzene-exposed workers these values were 27 and 73% respectively. There was no relation between CA's and age. The relation between CA's and exposure length (considering the range of 1 month to 26 years) was not investigated.

Zhang et al. (1996) investigated the occurrence of aneuploidy in circulating lymphocytes of 44 benzene exposed workers (23 males, 21 females; age 35.3 ± 7.8 years). Current personal exposure (expressed at 8h-TWA) was determined using passive dosimetry badges for the full workshift on 5 consecutive days. Two groups were established: one group with an exposure ≤ 31 ppm (n=21), the other with exposure > 31 ppm (n=22) exposure to other aromatics was marginal). A control group of 44 non-exposed workers was used. A significant increase in the incidence of hyperdiploidy was observed in the > 31 ppm group compared to controls but no effect was seen in the ≤ 31 ppm group. Trisomy was the main form detected. It was shown at the individual level that the incidence of hyperdiploidy was strongly correlated with urinary phenol levels and with decreases in circulating lymphocytes. This indicates that hyperdiploidy is directly linked to benzene exposure.

2.5 Carcinogenicity

Benzene has been identified as a human carcinogen. U.S. EPA has placed benzene in carcinogenic category A (EPA, 1997). Within the European Union, benzene is classified as “Carcinogen, category 1” and labeled “T, R45, May cause cancer”. There is sufficient scientific evidence to assume a causal relationship between relatively high levels of repeated benzene exposure and (acute) non-lymphatic leukemia (ANLL) or acute myeloplastic leukemia (AML) (IARC, 1982; EU, 2002). Whether benzene can induce also other types of hematological tumors is still subject to debate, although indications exist for a relationship between benzene exposure and non-Hodgkin’s lymphoma, multiple myeloma, and acute or chronic lymphatic leukemia (ALL) (Goldstein, 2000).

Short history of epidemiological studies

Many epidemiological studies have been performed in relation to the leukomogenic action of benzene in workplace surroundings. With respect to AEGL-development, it is considered to be beyond the scope of the present document to summarize all these individual epidemiological studies. Therefore, the following paragraphs present an overview of the carcinogenic information on humans exposed to benzene with specific attention to 1) the studies relating to the so-called “Pliofilm cohort”, 2) studies on large groups of workers in China, and 3) a recent cohort study from Health Watch Australia.

Although the hematological disorders caused by benzene were known already before 1900 (Snyder, 1987), the leukomogenic activity of benzene became progressively evident in the 20th century. The publications of Vigliani and co-workers can be considered as landmark publications in which they identified a range of fatalities in benzene-workers to be associated with aplastic anemia and leukemia (Snyder, 2002). In Turkey, Aksoy and co-workers examined workers from the shoe industry using benzene-based glues. In a cohort of 28500 workers, 217 cases of bone marrow depression and 26 cases of leukemia were found (Aksoy et al, 1971). In 51 cases of pancytopenia, 13 developed leukemia (Aksoy et al., 1972).

The Pliofilm cohort consist of employees of the rubber industry in Ohio of which individuals were exposed to levels well over 10 ppm. It has a major advantage in that exposure to other chemicals can be largely neglected (EU, 2002). In 1977, Infante et al. published the first results from this group of workers. From 7 cases of leukemia in this cohort Rinsky et al. (1981, 1987) calculated a standard mortality ratio (SMR) of 560 for benzene exposed workers for the total group and of 2100 for workers exposed more than 5 years. They suggested that given an exposure of 40 ppm-years to 400 ppm-years the projected SMR values for an excess of benzene-associated leukemia would range from 109-6637 (Snyder, 2002). As reported in the US EPA Carcinogenicity update (1997), the Rinsky studies reveal relative risk estimates of 1.1 for 0-40 ppm-years exposure, 3.2 for 40-200 ppm-years, 11.9 for 200-400 ppm-years, and 66.4 for > 400 ppm-years.

The Pliofilm studies have been criticized especially with respect to the estimated exposure levels. Were the original publications cited an exposure level of 0 to 15 ppm, others have argued that exposure levels were much higher. Additional publications by Crump and Allen (1981, not available) and Paustenbach et al. (1992) have argued that actual exposure (at least in early years before 1950) may have been much higher due to various reasons. This would indicate that the Rinsky et al. publications would overestimate the risk for developing leukemia. As reviewed by Snyder (2002), the actual exposure estimates for the Pliofilm cohort may be higher than originally proposed, but probably not as high as suggested by Paustenbach et al. (1992).

More recently, a large epidemiological study was performed by Yin et al. (1996) in which 74828 benzene exposed individuals (both man and women) from 781 different factories were followed for a mean period of 10-

12 years. The study period was from 1972-1987 and exposure estimates were established for about 60 different categories of workers over a period of 1949 till 1987. Exposure estimations for this study population were established by Dosimeci et al., 1996) divided into 6 exposure groups: < 1 ppm, 1-5 ppm, 6-10 ppm, 11-25 ppm, 26-50 ppm, and > 50 ppm. However, it should be noted that the reliability of exposure estimates in the lower range is questionable; possibly no difference can be made between < 1 ppm and 1-5 ppm. The mean exposure levels for all factories/categories range from 20.4 ppm in the period from 1949-1950 to 11.5 ppm in the period 1985-1987 (Dosimeci et al., 1996). The results of this large cohort study are presented in the following two tables.

Table 6 Cancer mortality among benzene-exposed workers (Yin et al., 1996)

	Women			Men		
Cause of death	Observed	RR	95% CI	Observed	RR	95% CI
Malignant neoplasms (140-208)	99	0.9	0.6-1.3	425	1.2	1.0-1.5
Nasopharyx (147)	2	∞	0.3-∞	12	2.1	0.7-9.3
Esophagus (150)	2	0.8	0.1-16.7	25	2.0	0.9-5.4
Stomach (151)	14	1.0	0.4-2.8	71	0.9	0.6-1.4
Colon, rectum (153, 154)	10	0.7	0.3-2.0	24	1.1	0.5-2.3
Liver & gall bladder (155, 156)	8	0.4	0.2-1.3	101	1.3	0.9-1.9
Lung, trachea, bronchus (162)	16	1.0	0.4-1.3	109	1.5	1.0-2.2
Malignant Lymphoma (200-202)	5	∞	1.0-∞	12	3.3	0.9-21.6
Leukemia (204-208)	13	2.8	0.8-17.6	25	2.1	1.0-5.3

It was shown that the relative risk (RR) for leukemia-related mortality in benzene-exposed individuals was 2.8 for women and 2.1 for men. In addition, a high RR was observed for malignant lymphoma.

Table 7 Incidence of hematolymphoproliferative disorders among benzene-exposed compared to non-exposed workers (Yin et al., 1996)

Diagnosis	Exposed workers	Non-exposed workers	RR	95% CI
All hematolympho-proliferative malignancies	63	12	2.6	1.5-5.0
Malignant lymphoma	20	3	3.5	1.2-14.9
Non-Hodgkin's lymphoma	17	3	3.0	1.0-13.0
Multiple lymphoma	1	1	0.4	0.0-10.7
All leukemia	42	9	2.6	1.3-5.7
Acute myelogenous leukemia (AML)	23	4	3.1	1.2-10.7
Chronic myelogenous leukemia	9	2	2.6	0.5-16.9
Acute lymphocytic leukemia	5	1	2.8	0.5-54.5
Myelodysplastic syndrome	7	0	∞	1.7-∞
Aplastic anemia	9	0	∞	2.2-∞
Agranulocytosis	2	0	∞	0.3-∞
Total	81	13	3.4	1.9-5.2

With respect to hematolympho-proliferative disorders, significant increased RR were found for malignant lymphoma, non-Hodgkin's lymphoma, and acute myelogenous leukemia. RR for chronic myelogenous leukemia and acute lymphocytic leukemia were increased but not significant. The risk was significantly increased for the

combined grouping of acute myelogenous leukemia and myelodysplastic syndromes since the latter may proceed overt leukemia. Interestingly, a significantly increased risk was also observed for mortality due to lung cancer in men. However, the publication reported no details on smoking habits† and adjustments for confounding variables and, moreover, co-exposure to other industrial (carcinogenic) chemicals in this cohort cannot be ruled out (Yin et al., 1996). However, also the exposure estimates from the Chinese cohort study are critical (Snyder, 2001)

In Australia, a large prospective cohort study investigates the health from employees in the petroleum industry (Health Watch, 2000). In this study 17525 people, working 5 years or longer in the industry are involved. The study revealed a statistically significant increase in the incidence of all leukemias combined. The risk is associated with exposure to total hydrocarbons. The relation between lympho-haematopoietic cancer and exposure to benzene was subject of a subsequent nested case-control study (Monash University, 2001). The case-control study included 79 cases of lympho-haematopoietic cancers, 33 leukemias, 31 non-Hodgkin's lymphomas, and 15 multiple myelomas. The leukemias consisted of 9 AML, 6 CML, 2 ALL, 11 CLL, and 5 other types. Individual exposure estimates were established based on 18 different job-groups. The exposure estimates in this study is probably of higher reliability since the they are based on more actual measurements than previous occupational studies on the leukomogenic action of benzene. Relations between cancers and various types of exposure metrics were established (cumulative exposure, exposure duration, exposure intensity, start date of exposure, influence of "peak" exposures). Lifetime cumulative exposures were low for the majority of subjects (0.005 – 57.3 ppm-years, mean 4.9 ppm-years). Nearly 85% of the subjects had an exposure < 10 ppm-years while only 0.6% had an exposure > 40 ppm-years. Average exposure intensity was less than 1 ppm for 98% of the individuals (range 0.001 to 2.07 ppm). The total incidence of lympho-haematopoietic cancers was strongly associated with total benzene exposure. The exposure group ≥ 8 ppm-years had a mean OR of 3.32. The strongest association was found with exposure between 5 to 15 years prior to diagnosis. Recent exposure within 5 years made only a small contribution. There was no association with the duration of employment nor with the starting date of employment. A very strong association was found for leukemias alone, at exposure ≥ 16 ppm-years an OR was found of about 35. No association was found with employment duration and start period of employment. A strong association was observed for leukemia subtypes AML (or ANLL) and CLL. No associations were found for chronic myeloid leukemia or other leukemia types. When considering the contribution of high "peak" exposures to the leukemia risk, it was found that handling "concentrated benzene" and BTX induced a considerable higher risk (mean OR was 12.5). The latter and the fact that duration of employment was not associated with leukemia risk, led to the suggestion of the authors that a non-linear exposure intensity-risk relationship, with a disproportionate higher risk for leukemia with high exposures (Monash University, 2001).

Linear or non-linear dose-response relationship and peak exposures

Various publications have addressed the issue of linearity in the dose-response relation of benzene-induced hematotoxicity and leukemia (e.g. Lamm et al., 1989; Bailer and Hoel, 1989; Paxton, et al., 1996; Cox, 1996; EPA, 1997; Health Council, 1997; ACGIH, 1997; Goldstein, 2000; Snyder, 2002). Especially for extrapolation to low doses, arguments have been presented for a non-linear, a sub-linear, and a supra-linear dose relationship. In addition, arguments have been presented for epigenetic factors responsible for leukemia induction which lead to the suggestion of a threshold approach. As pointed out in EPA 1997, the various arguments proposed for non-linearity can be counteracted by arguments supporting linearity. Nevertheless, present knowledge is insufficient to support any quantitative deviation from the linear dose-response curve, at least from a regulatory point of view (EPA, 1997; Health Council, 1997; Goldstein, 2000).

† Smoking is male dominant in China.

Because AEGL's will address relatively high exposures, the non-linearity at low doses is not a major issue. Instead, the effect of short-term peak exposures is much more important. Most epidemiological studies have addressed the association between leukemia and cumulative exposure, often expressed in ppm-years. However, few have addressed the risk of periodic high peak exposures. Rushton and Romanuik (1997) studying a group of petroleum workers in the UK, showed that the risk for acute myeloid leukemia was not increased with cumulative exposure, maximum intensity or mean intensity. However, when characteristics of peak exposures (daily or weekly) were introduced a positive association was found, although the number of cases was small. In the nested case-control study of Health Watch in Australia (Monash University, 2001), it was shown that a particular group of individuals having a large chance for peak exposures had a significantly increased risk for leukemia. In addition, exposure duration (employment duration) was often not associated with increased risk (Rushton and Romaniak, 1997; Monash University, 2001). This provides indications that short term peak exposure may be associated with a disproportional higher risk for leukemia. On the other hand, it was shown that recent exposures in an occupational setting (less than 5 years) does not significantly contribute to a higher risk but that especially exposures for 10 to 15 years showed the largest risk estimates (Monash University, 2001). This indicates that besides dose rate some extend of repeated exposure is necessary to increase the risk for leukemia.

Carcinogenic risk assessments

Various publications have addressed the issue of quantitative risk estimates for benzene-associated leukemia. The differences between these risk estimates are largely the consequence of differences in the exposure estimates used in the dose-response modeling (see e.g. ACGIH, 1997).

The IARC evaluated benzene in 1982 and concluded that a relationship between benzene exposure and acute myelogenous leukemia has been established in epidemiological studies. For a quantitative risk assessment, the IARC used the results from the Rinsky et al. study from 1981 from which they calculated 140 to 170 excess cases of leukemia per 1000 exposed population. With regard to exposure levels they concluded that they would vary between 10 and 100 ppm. The conclusion was that a working lifetime exposure to 100 ppm would result in 140-170 cases of leukemia per 1000 exposed workers. Other available studies by Ott, Vigliani, and Aksoy were considered to be supportive for this conclusion (IARC, 1982).

In the Netherlands, RIVM has evaluated benzene in 1987 (Slooff et al., 1987) and concluded that an additional risk of $1{:}10^{-6}$ for lifetime exposure was associated with exposure levels of 0.05 – 0.6 $\mu g/m^3$. For risk assessment, the extrapolation of White et al. (based on the Rinsky et al., 1981 study) was used to calculate a level of 0.12 $\mu g/m^3$ (0.037 ppb) associated with an additional risk of $1{:}10^{-6}$ for lifetime exposure. This value is equivalent to a lifetime unit risk of 2.7×10^{-2} per ppm. Because it was argued that this linear extrapolation drastically overestimated the risk of benzene exposure, the exposure level associated with a $1{:}10^{-6}$ lifetime risk was increased 100-fold to 12 $\mu g/m^3$. In 1997, The Health Council of the Netherlands, re-evaluated the benzene carcinogenicity and re-adopted the value of 12 $\mu g/m^3$. Recently, the EU working group on benzene estimated that a lifetime risk at 10^{-4} was associated with exposure levels of 20-36 $\mu g/m^3$ (EU, 1999).

In 1979, the EPA Carcinogen Assessment Group determined a carcinogenic risk used the studies of Infante, Ott and Aksoy and calculated that a risk of 10^{-5} for a lifetime average exposure would be associated with a level of 4.15×10^{-4} ppm (Marcus, 1990). The latter value can be recalculated into a lifetime risk of 2.4×10^{-2} at 1 ppm exposure.

In 1985, the EPA performed an update of the benzene carcinogenic risk assessment and used the

geometric mean of the different risk estimates calculated by Crump and Allen (1984) for OSHA (using three approaches cumulative, weighted cumulative, and window exposure and two risk models (absolute and relative). The Crump and Allen calculations were based on the studies by Rinsky et al. (1981), Ott et al. (1978, cited in EPA 1997), and Wong et al. (1983, cited in EPA 1997). The resulting quantitative cancer unit risk amounted to 2.6×10^{-2} for a lifetime exposure to 1 ppm (EPA, 1997).

In the most recent EPA update on benzene carcinogenicity from 1997, the 1985 risk estimate was basically reaffirmed after a comprehensive review of the literature (EPA, 1997). It was shown that at 1 ppb lifetime exposure, the various published risk estimates ranged from 8.6×10^{-11} to 4.1×10^{-5} depending on the source data, the exposure estimates, and the extrapolation models used (linear, non-linear, proportional hazard, conditional logistic, relative risk, additive risk). At a level of 1 ppm, the risk estimates ranged from 8.6×10^{-5} to 8.4×10^{-1} (EPA 1997). Using linear assumptions, the risk at 1 ppm ranged from 7.1×10^{-3} to 2.5×10^{-2}. In addition, it is stated that "the Agency is fairly confident that exposure to benzene increases the risk of leukemia at the level of 40 ppm-years of cumulative exposure. However, below 40 ppm-years, the shape of the dose-response curve cannot be determined…". Exposure to 40 ppm-years at the workplace corresponds to a lifetime environmental daily exposure of 120 ppb. Compared to various environmental background exposures, margins of exposure (MOE's) were calculated to be 26 (for mean lifetime environmental exposure), 55 (for background exposure of non-smokers), and 10-15 (for a lifetime spent in a smoke-filled bar).

The National Research Council (NRC, 1986) published the EEGL values for benzene (1h and 24h). In this report the risk of acute exposure to benzene for leukemia was calculated using the 1979 EPA $1{:}10^{-5}$ risk estimate of 2.4×10^{-2} for a lifetime exposure to 1 ppm. The dose yielding a lifetime risk of 10^{-4} was calculated to be 4.2 ppb. Using a correction factor of 70 yr x 365 days and a multistage factor of 2.8, the 24h EEGL for carcinogenicity was 38 ppm. On the basis of Haber's Law, this translates into a 1h EEGL value of 912 ppm (NRC, 1986).

The Spacecraft Maximum Allowable Concentrations (SMAC's) for benzene established by the NRC (1996) are 10, 3, 0.5, 0.1, and 0.07 ppm for time durations of 1h, 24h, 7d, 30d, and 180d respectively. The acceptable concentrations for leukemia are 12 ppm for 24h, 1.7 ppm for 7d, 0.4 ppm for 30d, and 0.07 for 180d. This means that only the SMAC for subchronic duration of 180d are determined by the risk for leukemia. SMAC's for the other time intervals were based on immunotoxic effects (decreased numbers of peripheral lymphocytes).

2.6 Summary of human toxicity data

Lethality

Numerous cases of death due to benzene inhalation have been reported, very often related to vapor exposure in enclosed spaces. However, information on exposure levels and duration is mostly lacking. Flury (1928) provides only an estimate of acute lethality for humans: 20,000 ppm for 5-10 min is lethal, 7500 ppm for 30-60 min is "dangerous". Gerarde (1960) has reported the same levels without reference to primary sources. Various case studies report lethal exposures within minutes (max. 45 min) but no exposure concentrations (Winek et al., 1967; Tauber, 1970; Winek and Collom, 1971; Avis and Hutton, 1993; Barbera et al., 1998). Death normally results from CNS depression with ultimately paralysis of the respiratory center (Hamilton, 1931). However, sudden death has been reported after a short period of euphoria and hyperactivity, suggested to be due to cardiac arrest (Bass, 1970; Reinhardt et al., 1971; Litovitz, 1988). Brain levels of benzene in various lethal cases range from 13.8 – 179 mg/kg.

1
2 Non-lethal effects of benzene exposure can be categorized in: irritation, CNS effects, cardiac
3 sensitization, hematological effects (WBC, RBC), developmental effects, genotoxicity, and carcinogenicity
4 (leukemia).
5
6 *Irritation*
7 Primarily high concentrations of benzene are irritating to mucous membranes of the eyes, nose, and
8 respiratory tract (Gerarde, 1960). In addition, skin burns and blistering may occur after acute exposure to high
9 levels (Avis and Hutton, 1993). Volunteers (n=23) exposed to levels up to 110 ppm for 2h reported no subjective
10 symptoms (Srbova et al., 1950). Other metabolism studies with substantial numbers of humans report exposures
11 of 1 – 76.4 ppm (7h TWA) or 19-125 ppm (up to 6-8h) but no information on signs and symptoms was included
12 (Inoue et al., 1986; Hunter and Blair, 1972). Exposure of 3 volunteers to 3400-4900 ppm for 5-15 min caused
13 airway irritation and some excitement and dizziness after 10 min (Lehmann, 1910).
14
15 Workers exposed to benzene vapors in fuel tanks (exposure > 60 ppm; limited GC measurement indicates
16 that initial levels could have been about 653 ppm as indicated by measurment in a similar tank), report eye, nose
17 and throat irritation and cough (Midzenski et al., 1992). In a recent spill of 'benzene-heartcut' (40-60% benzene,
18 40-60% naphta) in the UK, workmen reported a benzene-like odor and burning sensation in the nose and some
19 skin irritation. Limited information on exposure shows 50 ppm in open air and 100 ppm indoors (A. Keddie,
20 UK/HSE, personal comm.). In long term benzene exposed workers, signs of irritation were observed at average
21 exposure levels of 1 - 40 ppm (Yin et al., 1987, peaks unknown), or at 'peak' levels of 30-300 ppm (only 4
22 limited measurements, Kraut et al., 1988). In the latter studies, however, co-exposure to other substances cannot
23 be excluded.
24
25 *CNS effects*
26 One of the primary effects of acute benzene exposure is CNS depression, similar to many other solvents.
27 Although thesed effects are (qualitatively) well known for over 100 years, detailed exposure data for this endpoint
28 are limited. Gerarde (1960) states that 50-150 ppm for 5h results in headache, lassitude, and weariness while 500
29 ppm for 1h induces symptoms of illness (exposure levels probably estimated, no reference to primary data).
30 Fishbein (1984) states that CNS effects would be initially seen at exposures above 250 ppm. In a case study, one
31 out of four male workers showed major CNS effects after working in a confined space for five intermittent
32 periods of 2-3h spread over 2 days. Area levels up to 600-1500 ppm were measured in a simulation experiment
33 with the door closed (Drozd and Bockowski, 1967). These levels are relevant primarily for the first period of 2h
34 work because the workspace was closed during that period. However, personal exposure might actually have been
35 higher. It was reported that the men left the room occasionally when the benzene vapors became annoying but this
36 condition (2h exposure at 600-1500 ppm) did not appear to result in an impaired ability to escape. Workers
37 exposed to benzene vapors in fuel tanks for 1 day to 3 weeks (exposure > 60 ppm; limited GC measurement
38 indicates that initial levels could have been about 653 ppm as indicated by measurment in a similar tank), report
39 dizziness / light-headedness, nausea, headache, and drowsiness (Midzenski et al., 1992). Long-term exposure of
40 workers to benzene revealed changes in EEG patterns (short-term (1-2h) personal sampling 45-154 ppm
41 (sometimes 308 ppm), Kellerova, 1985), dizziness and headache (exposure at 1-40 ppm, Yin et al., 1987), or
42 fatigue, lightheadedness, increased sleep requirements, and headache (limited exposure determinations 30-300
43 ppm (Kraut et al., 1988) or within a range of 11-1060 ppm (Greenburg 1939). However, in most of these
44 occupational studies, benzene may have accumulated in fat tissues while co-exposure to other substances cannot
45 be excluded.
46

Occupational exposure to benzene has progressively decreased over the last century. So, the highest exposures are reported from the 1920-1930's and partly from the 1960-1970's. In addition, recent measurements from workplaces in China are available (Wong, 2002). Genereally *mean* workplace levels have been reported between 100-500 on a regular basis in the past. Frequently, however, also concentrations of 1000 – 1500 ppm were observed. In most cases no details about sampling strategy, duration of sampling and analytical methods were provided although the 'old' papers mostly deal with area sampling. The massive amount of data that fit the general picture provides sufficient confidence in the exposure ranges. Unfortunately, acute occuring health effects were almost never reported and at least not coupled to exposure data on the individual level. Most studies focussed on hematological changes and carcniogenicity when studying health effects. However, one must conclude from the large extent of people involved in these occupational exposures, and the fact that these exposure levels occurred on a regular basis that those conditions were not severely adverse with respect to acute exposure and will not have resulted in an impaired ability to escape, although some occurrence of health effects such as (self-)reported headache, dizziness, and irritation may occur at these levels.

Cardiac sensitization

Benzene has been claimed to be a potent inducer of cardiac sensitization (Bass, 1970; Reinhardt, et al., 1971; Litovitz, 1988). However, no exposure-time-effect relationship can be established for humans.

Hematological effects

Repeated benzene exposure causes bone marrow toxicity resulting in a dose dependent destruction of bone marrow precursor cells that are responsible for the production of mature red blood cells, platelets, and granulocytic and lymphocytic white blood cells (Goldstein, 2001). This may result in decreased WBC counts, decreased RBC counts, and in more severe forms anemia or pancytopenia. Symptomatic effects as a result of these effects are increased risk of infections and increased risk for hemorrhage. It should be noted that most of the hematological effects are in principle reversible (e.g. changes in circulating RBC and WBC). Only a loss of pluritpotent stem cells within the bone marrow is truely irreversible.

Although these effects are well characterized, a definitive no-response-level for humans cannot be derived from the available human data. The available data do not allow definition of a single dose exposure level which would or would not induce hematological effects. The only short-term exposure study available is Midzenski et al. (1992) in which workers were exposed to benzene vapors in fuel tanks for 1day – 3 weeks (exposure > 60 ppm; max level probably 653 ppm as indicated by measurement in a similar tank). These workers showed no consistent hematological abnormalities (Midzenski et al., 1992).

Developmental effects

Benzene exposure has been suggested to be associated with menstrual disorders and decreased female fertility. However, these studies all suffered from major shortcomings. No association was found between spontaneous abortions and parental benzene exposure (ATSDR, 1997; ACGIH, 1997).

Another series of studies has investigated a possible relation between parental exposure and childhood leukemia. Although these studies may provide some indications for a possible role of parental benzene exposure, the pattern that emerges from these studies is inconsistent and does not allow any definitive conclusions on the effects of parental exposure to benzene and possible childhood leukemia. Nevertheless, it remains questionable whether the exposures involved are relevant for setting AEGL values since some form of repeated exposure might be necessary.

Genotoxicity

Benzene is a genotoxic – clastogenic – agent that induces CA's, SCE's, and MN's (review by Zhang et al., 2002). Because SCE's do not appear to show a significant association with future cancer risk, Zhang et al. (2002) concluded that CA is probably the most valid cytogenetic endpoint available for predicting future leukemia risks. However, benzene does not induce a specific pattern of CA's but may result in genomic instability by causing recombination, double strand breaks and mitotic spindle disruption (Zhang et al., 2002). In occupational studies involving repeated low dose exposure, CA and SCE may occur already at low exposure levels at around 2 ppm (Sarto et al., 1984; Picciano 1979; Zhang et al., 1996). Only one study investigates the occurrence of CA and SCE in workers exposed to a single peak of benzene vapor (brief exposure due to spillage, concentration and duration not defined). CA were not increased. SCE showed a slight increase compared to a control group and the number of SCE's were correlated to the urinary phenol level.

Carcinogenicity

Benzene has been identified as a human leukomogen. Sufficient information is available to suggest a causal relationship between benzene exposure and acute myelogenous leukemia (AML) or related forms of acute non-lymphocytic leukemia (ANLL). Whether benzene also causes other hematological tumors is still subject to debate although indications exist for a relationship between benzene exposure and non-Hodgkin lymphoma, multiple myeloma, and acute or chronic lymphocytic leukemia (Goldstein, 2000).

Various cancer risk estimates exist. In the most recent EPA update, the risk at 1 ppm ranged from 7.1 x 10^{-3} to 2.5 x 10^{-2} using linear dose assumptions. In 1986, the NRC defined a 1h EEGL of 912 ppm and a 24h EEGL of 38 ppm. In 1996, the NRC defined SMAC's based on leukemia: the 24h value was 12 ppm.

3 ANIMAL TOXICITY DATA

3.1 Acute Lethality

3.1.1 Rabbits

In a study on the toxicity of butadiene, Carpenter et al. (1944) also compared the narcotic properties of this substance to that of benzene. In a limited study, 10 rabbits (strain not specified) were exposed - nose-only - to a nominal concentration of 35,000 – 45,000 ppm benzene until death. The authors recorded the average time for the occurrence of clinical signs and death. In Table 8, the results are shown.

Table 8 Experimental outcomes on acute benzene exposure in rabbits (Carpenter et al., 1944)

Symptom or reflex	Average time of occurrence (min)
Light anesthesia, relaxed	3.7
Excitation, running movements, tremors, chewing	5.0
Loss of pupillary reflex to strong light	6.5
Loss of blink reflexes to tactual stimulus	11.4
Pupillary contraction	12.0
Involuntary blinking	15.6
Death	36.2 (range 22.5 – 71 min)

In 6 rabbits exposed to this concentration, arterial and venous blood samples were analyzed for benzene. Benzene levels after 15 min of inhalation were 0.18 mg/ml in femoral arterial and 0.16 mg/ml in femoral venous

blood. At 22.5 min, benzene levels were 0.27 mg/ml in the abdominal aorta and 0.16 mg/ml in the postcaval vein.

Rabbits exposed to 20,000 ppm benzene vapor (tracheal intubation) showed increased respiratory and heart rates and decreased blood pressure (Kujime, 1990; Japanese article - abstract in English). After 17 min, grand mal seizures and seizure waves were observed in the CNS. No animals died for 3h exposure at this concentration. At 80,000 ppm, seizures already begun after 30 sec. All rabbits had respiratory paralysis and died within 13 minutes. Benzene levels measured in brain and blood (see Table 9) showed higher levels in brain (especially spinal cord, medulla oblongata, pons) than in blood.

Table 9 Benzene levels in rabbits exposed to benzene vapor (Kuijme, 1990)

	Blood	Spinal Cord	Medulla Oblongata	Cortex	Pons	Fat	Lung	Liver	Heart
20,000 ppm 3h	1.8	5.3	5.9	2.5	6.3	152	1.4	4.0	3.1
80,000 ppm 13 min	3.8	10.9	16.3	8.6	16.3	0.54	2.7	5.5	15.5

In a limited study by Robinson and Climenko (1941), hrabbits were exposed to high levels of benzene vapor (approximately 10,000 ppm nominal) for 2h after blood sampling by heart puncture. Benzene levels in blood were stated to be 250-300 mg/L. Directly at the end of the exposure RBC values were decreased (82% of the animals showed a decreased of 5 to 25%). RBC values progressively decreased over a few days and returned to normal within 20 days. It was stated that ‘a few lethalities’ occured on a total number of at least 34 rabbits. It cannot be excluded that heart puncture just before exposure contributes to the observed deaths.

3.1.2 Rats

In a limited study, Carpenter et al. (1949) exposed a group of 6 Sherman rats to a nominal concentration of 16,000 ppm benzene for 4h. The only statement made, is that this exposure kills 2/6, 3/6 or 4/6 rats within a 14 days observation period. No details were provided on the number of death animal or the time to death.

In a later study, the group of Carpenter published a summary on their findings of acute lethality for a large number of substances (Smyth et al., 1962). In this paper, an acute inhalation toxicity study with rats was tabulated with the following results. A group of 6 albino rats (sex not specified) was exposed to a nominal concentration of 16,000 ppm benzene for 4h. Within an observation period of 14 days, 4 out of 6 rats died. It is likely but not certain, that this study is identical to the study published by Carpenter et al. in 1949. In addition to the dynamic inhalation experiment, the paper of Smyth et al. (1962) states that rats would tolerate a concentrated vapor of benzene for maximally 5 minutes.

Drew and Fouts (1974) investigated the effect of pretreatment with Phenobarbital and chlorpromazine on the acute toxicity and metabolism of benzene. From the description of experiments it can be concluded that the total group size for calculating LC50 values was 48 rats/group. Female Sprague Dawley rats were exposed to benzene for 4h. Benzene vapors were prepared by diluting saturated benzene vapor with filtered air. Concentrations were measured at 30 min intervals by drawing a known amount of air through methanol and the

benzene absorbed in methanol (efficiency > 95%) was determined spectrophotometrically. Unfortunately, the concentrations used in the experiments as well as the results for each concentration separately were not reported. Based on a total group size of 48 rats, the LC50 for a 4h exposure was reported to be 13,700 ppm (44,388 mg/m^3). (Pre)-Treatment with Phenobarbital or chlorpromazine did not significantly alter the LC50.

Bonnet et al. (1982) exposed male Sprague-Dawley rats to benzene (purity 99.5%) concentrations for 6h (whole body, dynamic concentrations). From the limited description of the experiment, it is concluded that concentrations reported are analytical values (gas chromatography) and that 12 animals were tested at each concentration. Eleven exposure concentrations between 7000 and 15,000 ppm were investigated (not specified). During exposure, animals showed stereotypic behavior, somnolence, tremor, and muscular twitching. Animals were observed for 14 days after exposure. The LC50 value determined by the method of Bliss was 9536 ppm (95% CL 9105-9928 ppm).

In a review of the Russian literature (IRPTC, 1985) it is reported that the LC50 for rats in a 2h exposure is 34,000 mg/m^3 (10,472 ppm) and 66,700 mg/m^3 (20,544 ppm) in two separate studies. A 4h LC50 for rats was reported to be 65000 mg/m^3 (20,200 ppm). The original Russian papers were not available and further details were not provided.

Studies with non-inhalation exposure

Using intraperitoneal injection of benzene in corn oil in dosages of 1,2, 3, and 4 g/kg bw (n=8 rats/dose), Drew and Fouts (1974) reported an i.p. LD50 of 2890 mg/kg bw (corn oil pretreatment) or 2940 (saline pretreatment) in Sprague-Dawley rats.

Kimura et al. (1971) showed oral LD50 values for analytical grade benzene of 3.4 ml/kg (95% CL 2.0-5.7) for young rats (14days), 3.8 ml/kg (2.9-4.8) for young adult rats, and 5.6 (4.0-7.8) for older rats (only male Sprague-Dawley rats, using oral intubation). Newborn rats were more sensitive (no value given).

3.1.3 Guinea pigs

No studies available.

3.1.4 Mice

Lazarew (1929) exposed mice (strain and sex not specified) to benzene vapor in a ‘closed’ system (likely static conditions, method of creating or measuring vapors not defined). In 2h experiments, the minimal concentration needed to induce mice to lay on their sides, to loose reflexes, or to die were determined (methods not specified). Laying at their site occurred when the concentration was 15 mg/L (equivalent to 15,000 mg/m^3 or 4620 ppm) while lethality occurred from 45 mg/L (equivalent to 45,000 mg/m^3 or 13,860 ppm). Because only a 10 L vessel was used while the number of animals in the vessel was not specified, the occurrence of hypoxia and hypercapnia cannot be excluded.

Svirbely et al. (1943) exposed groups of 16-20 Swiss mice (sex not specified) to benzene (purity 99.5%) for 7h to concentrations ranging from 4980 to 14,600 ppm. Concentrations reported were calculated nominally as well as measured by an interference refractometer. Maximal concentrations were reached within 45 min. after the start of exposure. Observations were continued up till 4 weeks after the exposure. Clinical signs were recorded and histopathology was performed on various organs of mice (not specified which mice, or at what time-point tissues were obtained) In Table 10, the mortality of mice is presented for both an 8 hour (7h exposure + 1h observation) as well as a 4 week period.

1
2 Exposure to 4980 ppm benzene for 7h did not result in any mortality of mice while exposure to 14600
3 ppm resulted in 100% mortality within 8h. The LC50 for 7h exposure reported by the study authors was 9980
4 ppm (95% CL 9180-10,860 ppm) calculated by the method of Bliss. Clinical signs included (in progressive order)
5 restlessness, muscular twitching, S-shaped curve in tail, changes in respiration, incoordination, and narcosis.
6 Histopathology showed slight to moderate congestion of alveolar capillaries associated with extravasation of
7 erythrocytes in the alveolar space. Pathological lesions in kidney, liver, spleen and heart were negligible.
8

9 **Table 10 Mortality rates from Svirbely et al. (1943): Inhalation of benzene by mice for 7h.**

Number of mice	Concentration in mg/l	Concentration in ppm	Mortality within 8h [a] (% mice dead)	Mortality within 4 wk (% mice dead)
18	46.5	14,600	100	100
20	39.6	12,430	75	75
18	36.8	11,540	89	89
20	34.9	10,950	45	45
16	33.3	10,450	50	56.3
16	32.2	10,200	75	75
20	29.6	9280	35	35
18	26.6	8330	16.7	27.8
18	23.9	7490	11.1	16.7
18	15.9	4980	0	0

10 *a) This involves a 7 hour exposure period + 1 hour observation.*
11
12 Using the EPA Bench Mark Dose Software version 1.3.1, the data of Svirbely et al. (1943) were used to
13 calculate the 5% lethality level in its lower 95% confidence limit by the Probit model. The BMC_{05} was calculated
14 to be 6039 ppm with a 95% lower confidence limit of 4751 ppm. The latter value is very close to the observed
15 NOEL by Svirebely et al. being 4980 ppm.
16
17 Bonnet et al. (1982) using the same experimental set-up as for rats (see above), reported a 6h LC50 value
18 for mice (strain not specified) of 14,122 ppm (95% CL 13,744-14,591 ppm).
19
20 In a review of the Russian literature (IRPTC, 1985) it is reported that the LC50 for mice in a 2h exposure
21 is 45,000 mg/m^3 (13,860 ppm) and 24,000 mg/m^3 (7392 ppm) in two separate studies. The original Russian
22 papers were not available and no further details were provided.
23

1 **Table 11 Summary of lethality data in animals**

Species	Exposure levels (ppm)	Exposure duration	LC50 (ppm)	Remarks	Reference
Rabbit	35,000-49,000 (nominal) nose-only	< 71 min	Not determined	Time to death 36.2 min on average (range 22.5 – 71 min)	Carpenter et al., 1944
Rat	16,000 (nominal)	4h	?	This exposure resulted in 2/6, 3/6 or 4/6 death in a 14d obs. period	Carpenter et al., 1949
Rat	16,000 (nominal)	4h	Not determined	This exposure resulted in 66% mortality (4/6)	Smyth et al., 1962
Rat SD	-	4h	13,700	Total 48 rats	Drew and Fouts 1974
Rat SD	± 7000 – 15,000	6h	9536		Bonnet et al., 1982
Mouse	4620 or 13,860 (no further information, likely static conditions	2h	Not determined	Loss of control at 4620 ppm, lethality at 13860 ppm	Lazarew, 1929
Mouse	± 7000 – 15,000	6h	14,122		Bonnet et al., 1982
Mouse Swiss	4980-14,600	7h	9980	16-20 animals per dose	Svirbely et al., 1943

2
3
4 **3.2 Nonlethal Toxicity**
5
6 **3.2.1 Monkeys**
7 Nahum and Hoff (1934) exposed 2 monkeys (Macaca mulatta) and 10 cats briefly to benzene vapor. The
8 animals were fitted with trachea cannula's (under amytal anesthesia) connected to a wash bottle containing
9 benzene: air drawn into the lungs of the animals passed the surface of the benzene liquid. Concentrations were not
10 provided and could not be estimated based on this description but are likely to be high based on the description of
11 the set-up. Exposure duration was probably ± 10 minutes but sometimes repeated exposures with intermittent
12 recovery periods were used. Experiments were conducted about 30 to 90 minutes after cannulation. The main
13 results of this study were that ventricular extrasystoles became evident already after 1 minute of exposure to
14 benzene associated with stimulation from various foci outside the pacemaker region (ECG determinations). After
15 narcosis deepened extrasystoles disappeared but when inhalation was continued an increase in heart rate was
16 observed which could terminate in ventricular fibrillation. After 5 to 10 minutes, respiration ceased. Experiments
17 done after adrenolectomy and removal of the stellate ganglia, showed a reduction in ventricular extrasystoles.

1 Subcutaneous injection of adrenaline resulted in a return of the aberrant ventricular rhythms. It should be
2 remarked that only 1 monkey was tested with and 1 without stellate ganglion).
3
4 **3.2.2 Rabbits & Guinea Pigs**
5 In the review by Von Oettingen (1940), a study by Lobeck was cited which showed cornea damage in
6 rabbits exposed to about 12000 ppm for 1h/day for several days. Grayish white turbidity of the cornea was
7 apparent after 6-8 days but first cahnges may be noted already after 25 min of exposure (fine punctated turbidity).
8
9 Engelhardt and Estler (1935) exposed rabbits and cats (4-5/group) to benzene vapors at concentrations of
10 25, 50, 75, and 100 mg/L (dynamic conditions, no details on concentrations, most likely nominal). Concentrations
11 are equivalent to 25,000 (7700), 50,000 (15,400), 75,000 (23,100), and 100,000 (30,800) mg/m^3 (ppm). Animals
12 were exposed one or two at a time for a maximal duration of 6h. However, when deep narcosis was reached
13 animals were removed from the inhalation chamber and rectal temperature was measured. One cat died after 12
14 min at 30,800 ppm. The main results are summarized in Table 12. A concentration of 7700 ppm can be tolerated
15 for 4 to 6h without occurrence of deep narcosis.
16
17 **Table 12 Non-lethal effects of benzene exposure in rabbits and cats (Engelhardt and Estler, 1935).**

	7700 ppm 25,000 mg/m^3	15,400 ppm 50,000 mg/m^3	23,100 ppm 75,000 mg/m^3	30,800 ppm 100,000 mg/m^3
Rabbits				
Time to laying down (min)	> 75	60 ± 49	20 ± 11	17 ± 6
Time to light narcosis (min)	> 60	> 29	34 ± 24	24 ± 8
Time to deep narcosis (min)	-	> 229	42 ± 19	32 ± 5
Mean rectal temp. (°C)	36.05	36.90	39.23	39.08
Cats				
Time to laying down (min)	> 30	21 ± 7	17 ± 6	18 ± 11
Time to light narcosis (min)	> 50	> 18	30 ± 23	22 ± 9
Time to deep narcosis (min)	> 240	> 37	44 ± 18	30 ± 7
Mean rectal temp. (°C)	36.60	37.67	38.00	38.50

18 *When not all animals in a certain treatment group showed the specific endpoint, only the shortest time is given of a single*
19 *animal (> x minutes). Other animals showed the specific effect at a later stage or did not show it at alll.*
20
21 Weiskotten et al. (1920) performed experiments with rabbits. Animals were exposed for 10h/day (n=6) or
22 24h/day (n=5) for 24 days. The paper states that on average 16.6 cm^3 of benzene were evaporized per hour with
23 an air rate of 71.4 L/h. This correlates to a nominal concentration of about 62,000 ppm. Because rabbits died
24 within 30-40 min at levels up to 49,000 ppm (Carpenter 1944), the calculated value is probably not realistic but
25 exposure values are very high. Two animals died on the first day, one on day 2, and one on day 3. Other animals
26 died later, two survived. All animals showed a gradual weight loss. It was shown that the level of circulating
27 leucocytes started to decrease within 2 days. No difference was noted between animals exposed for 10 and 24h,
28 indicating that the response was maximal. RBC counts decreased only gradually reaching a low level after 6-16
29 days.
30
31 In the review by Von Oettingen (1940) a metabolism study with guinea pigs was described using
32 exposure levels of 15,000 and 6000 ppm for up to 20 minutes without mortality.
33

Groups of guinea pigs (sex not specified) were exposed (6h/day, 6 days/week, 1 to 5 weeks) to benzene vapor at a concentration of 4 mg/L, equivalent to 4000 mg/m^3 or 1232 ppm (Rotter, 1975). Because no statement was included on actual measurements, it must be assumed that this represents a nominal concentration. The control group consisted of 31 animals, while five exposed groups consisted of 5 animals each (1,2,3,4, or 5 week exposure). After exsanguination, blood was analyzed for ASAT, ALAT, Aldolase, ChE, prothrombin time and total protein and protein fractions.

Because of mortality observed after 3 weeks of exposure, daily exposure were shortened to 5h/day. This indicates that inhalation of benzene at 1232 for 6h/day up to at least 2 weeks did not induce mortality. Changes observed on blood parameters were generally most pronounced in week 2 and week 5. Total protein was decreased over the whole experimental period (significant only in week 1 and 5) associated mainly with decreases in albumins, ß-globulins, and γ-globulins. α1-Globulins were decreased only in week 1. ASAT was significantly increased in week 2 and 5 (max. + 43%) while ALAT was significantly increased in week 1, 2, and 5 (max. + 77%). Prothrombin time was increased in week 2,3 and 5. Cholinesterase activity (not specified) was decreased only in week 1 (-21%) but this is probably of minor toxicological significance. This study indicates the induction of liver damage during benzene inhalation.

3.2.3 Rats

In the review by Von Oettingen (1940) a study by Batchelor was cited showing that exposure of rats to 1000-2440 ppm (duration not provided) resulted in irritation of the mucous membranes whereas even lower concentrations may prove irritant.

Morvai et al. (1976) exposed 5 male CFY rats to an unknown concentration of benzene by inhalation (after anesthetization with pentobarbital) until death. ECG recordings were made every 2 minutes. Benzene inhalation induced moderate repolarization disturbances and occasionally arrhythmia at the beginning of anesthesia. On continuing anesthesia respiratory paralysis developed concurrent with atrial and ventricular fibrillation. In the same paper also acute experiments with i.p. injection of benzene were described showing that at 0.2 ml/100 g bw (equivalent to 1756 mg/kg bw) the majority of animals was killed without changes in the ECG but that at 0.4 ml/100 g bw (equivalent to 3512 mg/kg bw) repolarization disorders, ventricular extrasystoles and ventricular fibrillation occurred in all animals.

Tripathi and Thomas (1986) described an animal model for induction of ventricular tachycardia in the Wistar rat (n=25) and albino guinea pig (n=20) of both sexes. All animals were anesthetized with urethane and the trachea was cannulated. The jugular vein was cannulated for adrenaline injection and ECG recordings were obtained by lead II. The animals were artificially respirated at a stroke volume of 1 ml/100 g bw. After a 15 min stabilization period after cannulation, benzene vapor was administered through the artificial respiration (by bubbling the air through benzene liquid). The concentration of this vapor is not determined but based on the description of the set-up, the levels will have been very high. After 1,5 min of inhalation, adrenaline was infused over 30 sec. After which the exposure was stopped. Total benzene exposure was therefore only 2 min. Heart rate, time for onset of tachycardia, and duration of tachycardia were then recorded. Inhalation of benzene for 2 min induced an increase in heart rate of 74% in rats and 46% in guinea pigs with about 95% of the animals responding. The onset until tachycardia was 9.8 ± 1.2 sec in rats and 3.0 ±1.0 sec in guinea pigs. The duration of ventricular tachycardia was about 50 to 60 sec after which normal ECG reappeared.

Vidrio et al. (1986) exposed male wistar rats to toluene or benzene in a desiccator after anaesthetisation with chloralose. Solvents were added by syringe (0.8 ml toluene, 0.2 ml benzene) which was calculated to result

1 in nominal levels of 66272 ppm toluene and 19860 ppm benzene (see below also). ECG's were recorded for up to
2 30 min. After 10 min, heart rate was significantly increased in benzene exposed animals compared to controls.
3 The P-interval decreased after 25 min inhalation of benzene but no other changes were apparent from the ECG
4 recording. Injection of adrenline significantly increased the number of ectopic beats in benzene exposed animals.
5 In a further study by the same group, Magos et al. (1990) investigated the influence of toluene and benzene
6 inhalation on ventricular arrhythmias. Male Wistar rats (6/group) were individually placed in a glass desiccator
7 containing 0, 0.05, 0.10, or 0.20 ml benzene at the bottom and exposed to the resulting benzene vapor for 15 min
8 (static conditions). Measurements of the vapor concentrations showed that equilibrium was reached within 4-8
9 min. The measured mean concentrations between 8 and 15 min were 1299-1879 ppm, 2743-3119 ppm, and 7332-
10 8224 ppm for the 0.05, 0.1, and 0.2 ml benzene experiments respectively. It should be noted that, using an
11 identical experimental set-up, there is a large difference between the calculated nominal value of 19,860 ppm
12 above by Vidrio et al. (1986) and the measured value of 7332-8224 ppm by Magos et al. (1990). After removal
13 from the inhalation jar, animals were allowed to recover from the CNS depressing effects for 10 min because
14 otherwise anesthetisation with pentobarbital was not possible. Then they were anesthetized with pentobarbital,
15 cannulated in the trachea and artificially respirated. ECG recordings were made from lead III for 30 minutes.
16 Cardiac arrhythmias were induced by ligation of the left anterior coronary artery or by i.v. injection of 10 or 20
17 µg/kg bw aconitine.
18
19 Exposure of rats to benzene itself did not induce any changes in heart rates or ECG compared to controls.
20 When coronary ligation (i.e. inducing ischemia) was employed, the total number of ectopic beats induced by this
21 procedure was significantly higher in benzene exposed animals (0.2 ml) compared to the increase in control
22 animals (3885 ± 43 vs. 2024 ± 324 ectopic beats in 30 min). The increase in the total number of ectopic beats due
23 to injection of 10 µg/kg bw aconitine (a K-channel blocker), was somewaht larger in benzene exposed animals
24 (3466 ± 358 vs. 3073 ± 300 beats in 30 min for 0.05 ml benzene and 5043 ± 647 vs. 3073 ± 358 beats in 30 min
25 for 0.1 ml benzene) but did not reach statistical significance compared to controls. Injection of a higher dose of 20
26 µg/kg bw aconitine after inhalation at 0.2 ml benzene induced severe effects between minute 4 and 16 (ventricular
27 fibrillation, asystole and death).
28
29 Furnas and Hine (1958) performed a series of acute and subacute inhalation experiments with aromatic
30 hydrocarbons. In all experiments male Long Evans rats were used. Animals (n=2) exposed to 10,000 ppm
31 benzene (nominal concentration) showed dyspnea, twitching, ataxia, and hyperreactivity to auditory stimuli after
32 30 min of exposure. Violent twitching appeared by 40 min and the pattern of clinical signs remained unchanged
33 until 2h of exposure. Animals exposed to 20,000 or 40,000 ppm benzene showed the same pattern of clinical
34 signs, developing more rapidly and some animals died within a 2h exposure.
35
36 Additionally, groups of 8 animals were exposed to 40,000 ppm benzene for 20, 20, 27, 30, or 35 min (5
37 separate experiments). Clinical signs included local irritation of the respiratory tract, depression, twitching.
38 Although the presentation of the data is not very clear, the present reviewer concludes that 3 out of 8 animals died
39 in the 27 and 35 min exposure experiments. For the other experiments, no mortality was reported. Survivors were
40 anesthetized by chloroform 24h after exposure and the brain, spinal cord, and sciatic nerve were examined
41 histologically (techniques not explained). No gross lesions (except irritation of respiratory tract) and no specific
42 damage to the central nervous system were observed.
43
44 EEG measurements were performed on immobilized rats using 4 needle electrodes (above the eyes and in
45 front of the ears). At 10,000 ppm no change in EEG recordings was observed for 1h exposure. Increasing the
46 concentration thereafter to 25,000 ppm resulted in high-voltage slowing (frequency 3/s at potentials of 200 µV

1 compared to 5/s at 75 µV at rest). After cessation of exposure, EEG recordings were normal in about 30 min. At
2 30000 ppm, convulsive twitching was observed in 10 min.
3
4 A group of ten Long Evans rats was exposed repeatedly to 10,000 ppm benzene (nominal) over a period
5 of 17 days. The exposure period was increased from 12.5 min on day 1 to 22 min on day 5 and 2 x 30 min from
6 day 6 to 17 (Furnas and Hine, 1958). A group of 6 rats served as control (non-exposed). In the exposed group 2
7 rats died at day 12 while mean body weight gain was decreased (20 g vs. 50 g in controls). After 17 days,
8 microscopically no changes were observed in the nervous tissues of the exposed rats (brain, spinal cord, and
9 sciatic nerve; see also above).
10
11 Molnar et al. (1986) exposed groups of 8 rats to measured benzene (purity >99%) concentrations of 250,
12 500, 800, 1500, 2000, 4000, 5940 ppm (levels estimated from figures in paper) for 1, 2, 3, or 4 hours. In this
13 dynamic set-up general locomotor activity within the inhalation chamber was measured during inhalation using an
14 automated counting system. At 2000 ppm, group locomotor activity was increased for exposures of 3 and 4h, but
15 not at 1 or 2h. At 4000 ppm, group locomotor activity was increased for all exposure times while activity for all
16 groups was about 2-3-fold higher than at 2000 ppm. At 5940 ppm, a decreased activity for all exposure times was
17 observed indicating that the narcotic threshold has been reached. At the levels which produced increased motility,
18 incoordination and tremor were reported to occur but no data were provided. The NOEL for changes in activity
19 was 1500 ppm for 3 and 4h exposures, and 2000 ppm for 1 and 2h exposures.
20
21 Frantik et al. (1994) investigated the relative neurotoxicity of a large series of solvents. Rats (male adult
22 albino SPF, n=4 per group) and mice (female H strain, n=8 per group) were exposed to at least three
23 concentrations of benzene (analytical purity) or ambient air. Inhalation was performed in a dynamic system for 4h
24 (rats) or 2h (mice) and concentrations were measured by GC. The concentrations of benzene used were not
25 defined. Most animals were used three or four times with intervals of 3 weeks. Directly after an inhalation period
26 the animals received a short electrical pulse through ear electrodes. In rats, the duration of subsequent tonic
27 extension of the hindlimbs was determined while in mice the velocity of tonic extension was determined. These
28 parameters were shown to be the most sensitive and consistent. The study authors calculated the concentration
29 needed to induce a 30% change in the neurological response, i.e. decrease in duration or velocity of the toxic
30 extension. For benzene, an 30%-effect dose of 929 ppm was reported with a 90% confidence interval of 98 ppm
31 (range 831-1027) in rats and a dose of 856 ppm (range 636-1076) for mice. This 30% response level corresponds,
32 according to the study authors, to a concentration that does not influence normal locomotor activity or induces
33 behavioral excitation (e.g. for the aromatics). So, this 30% effect level is a quite sensitive neurological endpoint.
34
35 In a study from Hazleton Laboratories (Coate, 1983), Sprague-Dawley rats (10/sex/dose) and CD-1 mice
36 (30/sex/dose) were (whole body) exposed to benzene vapor 6.5 h/day for 5 consecutive days at 0 or 2000 ppm.
37 Analytical concentrations were within 0.3% of the target concentration and the exposure was in a dynamic
38 condition. An additional group of animals remained non-exposed. Body weight determination, a full
39 hematological analysis, a limited clinical biochemistry analysis, and a gross pathology were performed at day 0
40 and at day 6. All benzene-exposed groups showed a slight weight loss at day 6 compared to air exposed animals.
41 In both rats and mice, in both males and females, a significant decrease of WBC was observed in benzene
42 exposed animals (about 50-60% in rats, 50-70% in mice). In female mice, but not in males, a slight but significant
43 decrease in Hb and RBC levels was also observed. No other toxicological relevant effects were observed.
44
45
46

1 ***Relevant studies with non-inhalation exposure***
2 At birth, newborn rat pups of Fischer 344 rats were sexed and randomly assigned to a foster mother, each
3 litter consisting of 4 male and 4 female pups (Tilson et al., 1980). On days 9, 11 and 13 pups were s.c. injected
4 with corn oil or with a 30% solution of benzene in corn oil, equal to a dose of 550 mg benzene/kg bw per
5 injection. Rats were weaned at day 21 and housed 2-4 per sex until an age of about 160 days. Observations
6 included body weight gain and neurobehavioral tests on day 45, 65, and 100 of age (fore- and hindlimb grip
7 strength, startle responsiveness, latency to orient to a vertical position). Between 100-130 days of age all rats were
8 assessed for responsiveness to d-amphetamine at 0, 0.2, 1, and 3.0 mg/kg bw (dosages tested on each animal in
9 random order, separated by a week recovery) and their locomotor activity was tested in plastic cages. At 160 days
10 of age the animals were tested for exploratory behavior in an operant chamber including nose poke and foot lever
11 challenges.
12
13 Subcutaneous injection of benzene at day 9, 11, and 13 of age did not induce any effects on body weight
14 gain, hind- and forelimb grip strength, startle responsiveness, or latency to orient to a horizontal position. When
15 tested for locomotor activity between day 100 and 130, benzene exposed rats had a significantly higher
16 background activity (males and females both +25%). Responsiveness to d-amphetamine (which increases motor
17 activity) was decreased, however, in benzene exposed rats (significant only for 3 mg amphetamine/kg bw). In the
18 exploratory test at 160 days of age, only the number of wall touches of female benzene exposed mice was slightly
19 lower compared to controls but no changes were observed for lever touches, nose pokes or total responses. This
20 study shows that subacute exposure to benzene early in development (during the phase of rapid brain growth),
21 may induce behavioral changes in adulthood. However, the toxicological significance and relevance for man of
22 these results is unclear.
23
24 Male Sprague-Dawley rats (n=10) receiving single i.p. injections of analytical purity benzene at a dose of
25 39 mg/kg bw/day for 3 consecutive days (Varona, et al.,1998) showed decreased membrane bound tyrosine-
26 aminopeptidase (the enzyme responsible for breakdown of enkephalins) in the hypothalamus (-25%), the
27 brainstem (-20%) and the thalamus (-18%). Enkephalin immunostaining was increased in brainstem nuclei, the
28 substantia nigra and the tegmental nuclei but not in a variety of other brain areas. Benzene exposure also induced
29 a significant analgesic effect (tail flick latency was increased from 2 to about 4 sec.).
30
31

32 ### 3.2.4 Mice

33 Nielsen and Alarie (1982) investigated the sensory and pulmonary irritation effects of benzene. Male
34 Swiss-Webster mice (4 per group) were exposed for 30 min to nominal concentrations of 2000, 3570, 5800, 7600,
35 and 8300 ppm (analytical concentrations were within ±10% of nominal). Exposure was nose-only because the
36 bodies were placed in plesmographs. No pulmonary irritation was observed at any dose level (no RD50 could be
37 calculated). In contrast, a dose related increase in respiratory rate was observed (at 2000 and 3570 ppm to 120-
38 130% of normal, at 8300 up to about 200% of normal). The respiratory rate declined rapidly to control levels after
39 cessation of exposure. The study authors state that individual analysis of the animal data shows a transitory
40 sensory irritation during 20-30 seconds at the start of the exposure (Nielsen and Alarie, 1982).
41
42 Male Swiss mice (n=12) were exposed to a benzene concentration of 60 mg/L, equivalent to 60,000
43 mg/m^3 or 18,480 ppm, for 40 min in a glass chamber (Jonek et al., 1965). A group of 4 mice served as controls.
44 Directly after exposure, all animals were decapitated and sections of the spinal cord from the lumbar region were
45 prepared for histochemical investigation of enzyme activities: SDH, $NADH_2$-diaphorase, ALP, 5-nucleotidase,

1 Acid Phosphatase, desoxyribonuclease II, and thiolacetic esterase. Differences between control and experimental
2 animals were investigated qualitatively by light microscopy.
3 The main effects were observed for SDH and $NADH_2$-diaphorase. In some cells of benzene exposed
4 animals, SDH was increased while it was decreased in cells of the motor nucleus. $NADH_2$-diaphorase was mainly
5 decreased in the grey matter of spinal cord. According to the study authors, this differential picture indicates a
6 selective influence of benzene on the oxidation in neurons. Other enzyme activities were mostly decreased in the
7 spinal cord of benzene exposed animals compared to controls. However, it should be remarked that the control
8 group was quite small (n=4).
9
10 Adult male Swiss mice were exposed to 12 mg/L benzene in a 22L glass chamber for 40 min, equivalent
11 to 12,000 mg/m^3 or 3696 ppm (Kaminski et al., 1979). No details about the inhalation set-up or the generation of
12 vapors was included. Groups of 6 animals each were killed directly after exposure, after 1h, after 3h, or after 24h.
13 A control group of 6 animals was included. Sections of the kidney were histochemically investigated for the
14 expression of SDH, $NADH_2$-diaphorase, ALP, Acid Phosphatase, and ATPase. The results showed that the
15 activity of most of these enzymes was increased directly after exposure (0 and 1h) but decreased 3h after
16 exposure. At 24h, their activity was slightly increased or at control level.
17
18 Adult male CFW mice (n=8 per group) were exposed to 2000, 4000, or 8000 ppm benzene (and various
19 other alkylbenzenes including toluene) for 20 min (Tegeris and Balster, 1994). Exposures were performed under
20 static conditions in cylindrical glass jars and it was stated that concentrations were confirmed by infrared
21 spectrometry (no values provided). The animals were investigated using an adapted FOB screen for mice. Dose
22 related effects were noted on rearing (↓), righting reflex (↓), motor coordination (↓), forelimb grip strength (↓),
23 hindlimb foot splay (↑). At 2000 ppm, a significant decrease was noted for rearing but not for motor coordination.
24 The latter was significantly depressed to below 25% at 4000 and 8000 ppm.
25
26 Mice (strain and sex not specified, 10/group) were exposed to benzene vapor at 5 and 10 mg/L for 7h/day
27 during 6 days, equal to 1540 and 3080 ppm respectively (Estler, 1935). The experiment consisted of dynamic
28 conditions but no details were provided (concentrations likely to be nominal). At 1540 ppm, hyperactivity and
29 rearing of mice was observed at the beginning of the experiment. One mouse showed some ataxia during the first
30 day. At 3080 ppm, hyperactivity was observed every day at the start of the inhalation followed by hypoactivity.
31 Slight ataxia of the hindlimbs was observed on the first day. The tails showed a characteristic S-shape and only 2
32 animals lost equilibrium at 6h on the first day. No controls were included.
33
34 Adult male mice of both the CD1 and C57BL/6J strains were exposed to 0, 300, or 900 ppm benzene
35 (analytical concentrations ± 10%, 60 animals per group) for 6h/day for 5 days, followed by 2 weeks without
36 exposure (Evans et al., 1981). Mice were exposed in a dynamic inhalation chamber while being placed in a
37 restraining cage (not defined whether exposure was whole body or nose-only). After the 6h exposure, mice were
38 returned to their home cage (5 per cage) and after 30 and 75 min they were observed for behavioral changes
39 during 15 seconds. Scoring was performed for the following categories: stereotypic behavior, sleeping, resting,
40 grooming, eating, locomotion, and fighting. The percentage of animals in each category was noted on days after
41 exposure and compared to days without exposure (not defined whether this represents days before the start of the
42 experiment or days within the 2-week 'recovery' period).
43
44 The results showed that no differences in behavior were observed between benzene or control groups, or
45 between the two strains, on days without exposure. On days with exposure, the percentage of mice being inactive
46 (sleeping or resting) was decreased in the benzene exposed groups (at 75 min post exposure) compared to air-

exposed controls (± 70%, ± 25%, and ± 45% for 0, 300, and 900 ppm groups respectively). The percentage of mice eating and grooming was increased (± 15%, ± 40%, 25-40% for 0, 300, and 900 ppm groups respectively). Other categories of behavior were not significantly changed. The 900 ppm group shows intermediate results because it is likely that 900 ppm approaches the narcotic threshold (Evans et al., 1981). Benzene inhalation induced a significant change in behavior directly after exposure, i.e. an increase in activity. However, it is not clear whether this result is the mean outcome of 5 days of exposure or also representative for a single 6h exposure. Furthermore, the relevance of an increased activity for deriving AEGL's is questionable.

Li et al. (1992) exposed Kunming male mice to analytical concentrations of 0, 0.78, 3.13, or 12.52 ppm benzene vapor for 2h/day for 30 days. Four limb grip strength, avoidance response, and locomotor activity were measured as behavioral endpoints. In addition, blood and brain AChE activity, relative organ weights, and differential bone marrow cell counts were determined. The methods and time points of measurements were not clearly reported while the results are inadequately described‡. At 12.52 ppm, various significant effects were observed (decreased grip strength, decreased avoidance response, some decreased locomotor activity, decreased brain AChE activity, decreased liver and spleen weight, decreased number of precursor cells in bone marrow). At 3.13 ppm, only a decreased avoidance response was observed and an increased grip strength. The study authors report that "behavioral function changes were observed on the first 1 or 2 days of exposure" without providing any indications for the temporal pattern over the 30 days of exposure. It is not clear whether the tabulated results of behavioral effects represent the maximal response at day 1 or 2 or represent the effects at the end of the 30 day exposure period.

Adult male C57BL/6J mice were exposed to various regimes of benzene inhalation providing the same Ct-product (Dempster et al., 1984). Exposures consisted of sessions of 6h/day for 5 days/week at analytical concentrations of 100, 300, 1000, and 3000 ppm benzene (chromatographical purity). Group 1 (n=45) was exposed to 100 ppm benzene for 30 days (30 x 6h), group 2 (n=30) was exposed to 300 ppm for 10 days (10 x 6h), group 3 (n=40) was exposed to 1000 ppm for 3 days (3 x 6h), and group 4 (n=30) was exposed to 3000 ppm for a single day (6h), all providing a total exposure of 3000 ppm-days. All groups had concurrent controls, exposed to filtered air only. Baseline values for all parameters were obtained before the start of the experiments. After removal from the inhalation chambers, behavioral test were performed (individually determined milk-licking and locomotor activity in an automated set-up for 15 min; hindlimb grip strength) after which blood samples were taken from the tail vein (n=10 per time point). Total WBC and RBC were counted and differential white cell counts were analyzed in blood smears. These measurements were performed at frequent intervals during the study but at variable times for the different groups. However, for most groups measurements were performed also directly after the first or second exposure. Because the data from this study were plotted against time for all parameters and groups, the resulted summarized below represent those obtained after the first 6h of exposure (100, 1000, and 3000 ppm) or the second day of 6h exposure (300 ppm).

After a single 6h exposure, WBC counts were decreased to 50% of controls for the 3000 ppm group and to 65% of controls for the 1000 ppm group whereas at 100 ppm no effect was observed. At day 2, WBC were decreased to 50% at 300 ppm. Prolonged exposure at 100 or 300 ppm showed that WBC levels reached a plateau at 45% of the control level within 4 to 8 days. When the maximal responses for WBC in each group were compared, maximal lymphocytopenia showed to be constant for the given Ct-product (decrease of about 50%).

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‡ For example: The paper states that benzene inhalation was performed by *'the static exposure method using a 300 m^3 (?!) plexiglass chamber'* without further description. Exposure in such a chamber was done for 2 hours but concentrations were reported with two decimals (0.78, 3.1.3, 12.52 ppm). In addition, the effects reported at these exposure levels do not fit into the general picture of effects in rodents. Therefore, the validity of the study is questionable.

1 No consistent effects on RBC counts were observed at any concentration after the first 1 to 3 exposures.
2 Differential WBC counts showed no consistent changes in leukocytes. Milk-licking activity directly after
3 exposure was increased at 100, 300 and 1000 ppm but not at a single 6h exposure to 3000 ppm. Hindlimb grip
4 strength was decreased by about 10% after a single 6h exposure to 1000 or 3000 ppm but not at 300 ppm. Food
5 intake and body weight changes showed no toxicological relevant changes. For acute effects on hematological
6 parameters, 6h exposure to 100 ppm seems to be an NOAEL. However, it should be remarked that changes in
7 circulating cells are in principle reversible.
8
9 Uyeki et al. (1977) exposed female BDF_1 mice to a mean (analytical) concentration of 16.3 mg/L,
10 equivalent to 16,300 mg/m^3 or 5020 ppm, for 8h. Mice (4/group) were sacrificed 1, 4 or 7 days after the exposure
11 and femoral and spleen the number of colony forming cells (CFC) were measured in vitro. The total number of
12 cells obtained from the femur did not change but the number of CFC's was decreased to 39% of controls at day 1
13 and recovered thereafter. Expressed as $CFC/10^5$ cells, the decrease was still 77% of the control value at day 7. In
14 contrast benzene exposure had no effect on CFC activity in spleen cells but the number of spleen cells as well as
15 spleen weight was increased significantly (no change on day 1, + 40% at day 4, + 25% at day 7). Hematocrit
16 values were decreased to 84 and 89% at day 4 and 7 respectively. Multiple dosing (inhalation 8h/day for 3
17 consecutive days, sampled at day 4) showed a significant decrease in femoral CFC's (45% of control) while the
18 number of femoral cells was unchanged (n=4 animals). Spleen weight (as well as body weight) was significantly
19 decreased. Relative spleen weight, as calculated by the present reviewer, was however similar (0.41% in controls,
20 0.39% in exposed animals). Exposure on day 4 for another 4h (after 3 x 8h) exacerbated the observed findings:
21 femoral CFC was decreased to below 20% of controls and the number of femoral cells was decreased to 68%.
22 Spleen and body weights were decreased significantly and under these conditions the relative spleen weight was
23 also decreased (68% of controls). When bone marrow cells of 3 x 8h exposed mice were injected in X-radiated
24 mice, the number of colony forming units (CFU) in the spleen measured 10 days later was only 41% when
25 compared to the number of CFU's when X-radiated mice were injected with control bone marrow cells. This
26 study shows that a single inhalation exposure to benzene (5020 ppm for 8h) has a significant impact on femoral
27 cell function.
28
29 Rozen et al. (1984) exposed C57B1 mice to benzene at 0, 1, 10, 30, 100, or 300 ppm (whole body,
30 analytical concentrations) for 6h/day for 6 consecutive days. Circulating lymphocytes were significantly decreased
31 after 6 days at all dose levels while RBC counts were only significantly depressed at ≥ 100 ppm. At all dose
32 levels, mitogen induced femoral B-cell proliferation was significantly reduced to about 40% of control levels but
33 total femoral B-cells were decreased only at ≥ 100 ppm. For splenic T-cells a similar picture was obtained:
34 mitogen stimulated proliferation was decreased at ≥ 30 ppm but splenic T-cell number were reduced only at ≥ 100
35 ppm.
36
37 Female CD1 mice were exposed to benzene vapor at a measured concentration of 10 ppm (32.4 mg/m^3)
38 for a single session of 3h, or for repeated exposure for 5 days (3h/day)(Aranyi et al., 1986). Exposed and control
39 mice received thereafter 5 challenges of an aerosol of viable *Streptocuccus zooepidemicus* and ensuing deaths
40 were recorded for 14 days thereafter. A separate group of mice received an aerosol containing ^{35}S-labelled
41 *Klebsiella pneumonia* for analysis of bacterial clearance by alveolar mocrophages. Benzene exposure did not
42 induce a change in the mortality rates after Streptococcus infection in neither the single dose (n=39) or the
43 repeated dose group (n=23) when compared to non-exposed controls. Bacterial clearance of Klebsiella was
44 slightly but significantly increased (+3%) after a single 3h exposure to benzene but decreased by 16% after 5 days
45 of exposure (Aranyi et al., 1986). These results show that a single 3h exposure to benzene at 10 ppm does not
46 induce an adverse change in the host defense capacity.

1
2
3 ### 3.2.5 Other Species
4 Cardiac sensitization was studied in dogs by both Wegria et al. (1943) and Chenoweth (1946). Both
5 publications state that benzene is a potent inducer of cardiac sensitization to adrenaline. Experiments were
6 performed with trachea cannulated dogs under Phenobarbital anesthesia. Unfortunately, any quantitative
7 assessment of the exposure levels or duration to the benzene vapor was lacking. Chenoweth (1946) reports that
8 for benzene a concentration of "roughly estimated at 5% of the inhaled air" permitted the induction of ventricular
9 fibrillation (this corresponds to 50,000 ppm).
10
11 Johnston et al. (1979) exposed Duroc-Jersey pigs (n=4 per group) to measured benzene concentrations of
12 20, 100, or 500 ppm (equivalent to 64.8, 324, or 1620 mg/m^3) for 3 weeks (6h/d, 5d/wk). Urinary phenol levels
13 were increased in a dose-related manner. Weight gain was not affected. Total WBC, lymphocytes, and rosette
14 forming lymphocytes (as tested in a Sheep Red Blood Cell assay) were all significantly decreased to about 15-
15 25% of pre-exposure values at 500 ppm. The decreases were already present after 1 wk of exposure, were
16 maximal at the end of the 3-wk exposure period, but recovered thereafter over a 16 wk recovery period. At 100
17 ppm, these effects were also present but less pronounced. In the bone marrow differential cell count a shift
18 towards erythroid cells was observed at 100 and 500 ppm (significant only at 500 ppm). RBC counts were not
19 consistently changed. An important observation was the increase in multinucleated cells in the bone marrow: 0,
20 0.25, 2.5, and 3.9 per 5000 cells, in the control and exposed groups respectively). In this study, 20 ppm is
21 considered to a NOAEL for subacute inhalation in pigs. In the same study a group of rats was exposed to (only)
22 500 ppm at the same schedule showing a similar pattern in all investigated parameters.
23
24 In the review by Von Oettingen (1940) studies by Lehmann and associates were described on the narcotic
25 action of benzene in cats. A rather extensive experiment with various exposure times (3 min – 7 hours) and
26 exposure concentrations (10-170 mg/l, equivalent to 3080 – 52,360 ppm) was performed in order to determine a
27 time-concentration-response pattern for light narcosis and deep narcosis. In the following table the results are
28 presented (no details on exposure set-up etc.).
29
30

Slight depression			Light Narcosis			Deep narcosis		
Time (min)	Conc (ppm)	C x T / 1000	Time (min)	Conc (ppm)	C x T / 1000	Time (min)	Conc (ppm)	C x T / 1000
3-5	36900	147	24	52360	1256	68	52360	3560
7-9	13860	110	60	38800	2328	124	39110	4850
36	11700	421	70	30490	2134	144	30200	4349
70	7700	539	92	23400	2153	194	18480	3585
110	6776	745	112	18480	2254	326	11100	3619
			134	13552	1816			
			150	12000	1802			
			194	11100	2151			
			360	7700	2772			

31
32 From this experiment it is shown that for slight CNS depression, the exposure concentration is more
33 important than exposure duration determinant since the C x T product becomes greater with increasing duration of
34 exposure. When these results are fitted by $C^n \times T$ = constant, the time-concentration-response curve for slight

1 CNS depression yields an n-value of 2.2, light narcosis yields a N-value of 1.08 whereas the the curve for deep
2 narcosis yields a n-value of 0.93. Together, these results indicate that the CNS depressing effects of benzene
3 within a time frame of 3 min to 7h and concentrations up about 50,000 ppm can be described by using n=1.
4
5 **3.2.6 Repeated inhalation experiments with various species (subchronic – chronic)**
6 Numerous studies with repeated inhalation exposure are published. Most of these studies have been
7 reviewed already in various existing papers or monographs (e.g. EHC 1993, ATSDR, 1997). Although it is
8 beyond the scope of this AEGL-document to review all these studies, a number of representative studies are
9 summarized below, especially those with interim results.
10
11 In a limited study by Svirbely et al. (1944), rats (10/sex/group) and dogs (3 females per group) were
12 exposed to 1000 ppm benzene for 7h/d, 5d/wk, for 28 weeks. Hematological determinations were performed at
13 various intervals during the study. The graphics for rats show a decrease in mononuclear cells, total lymphocytes,
14 a decrease in Hb levels in the second half of the study period without apparent decreases in RBC, and a slight
15 increase in reticulocytes. Most of these effects showed a differential pattern over time compared to the concurrent
16 controls. In dogs, the effects appear to be less pronounced: a lowering in mononuclear cells and some fluctuations
17 in leukocyte counts. Pathological analysis showed hemosiderosis in the spleen of benzene exposed rats.
18
19 In a 12-week inhalation study of Bio/dynamics (Thackara et al., 1979) 7 different species were exposed to
20 benzene vapor for 12 weeks (6h/day, 5d/wk). Mice (Black New Zealand,10m), rats (Sprague-Dawley, 5m/5f),
21 guinea pigs (Hartley, 2m/2f), and rabbits (New Zealand White, 2m/2f) were exposed to 388 ppm, cats (domestic
22 shorthair, 2m/2f) to 380 ppm, dogs (Beagle, 2m/2f) to 379 ppm, and monkeys (Cynomolgus, 2m/2f) to 361 ppm
23 (all averages of analytical concentrations over 12 weeks). Because no control groups were included in this
24 experiment, the results can be only be interpreted in a limited way. No exposure related mortality occurred. No
25 toxicologically related clinical signs were noted in rats, dogs, guinea pigs, rabbits, or monkeys. Mice showed
26 ocular distress (lacrimation, chromodacryorrhea, chemosis, corneal opacity) after 4 weeks of exposure. The male
27 cats showed some respiratory problems already before exposure commenced which lasted up to week 11 and 12.
28 Due to the lack of a control group, body weight changes cannot be interpreted. Hematological changes were
29 evident in mean RBC, WBC and lymphocyte counts and are presented in Table 13.
30
31 **Table 13 Hematological effects in various species after repeated benzene inhalation (Thackara et al., 1979).**

	RBC (10^6 / mm^3)				WBC (10^3 / mm^3)				Lymphocytes (% of total WBC)			
	pretest	Wk 4	Wk 8	Wk 12	pretest	Wk 4	Wk 8	Wk 12	pretest	Wk 4	Wk 8	Wk 12
Mouse (m)	8.72	6.91	6.71	6.19	17.06	6.32	3.98	5.57	76	48	37	23
Rat (m)	5.78	7.17	8.10	8.15	14.12	14.90	13.58	13.90	90	88	88	80
Rat (f)	5.80	6.64	7.04	7.73	11.50	9.52	7.88	9.76	82	87	92	91
Guinea pig (mf)	4.75	5.12	5.77	5.45	12.68	14.65	15.63	16.33	62	58	80	79
Rabbit (mf)	5.75	5.74	6.36	5.37	11.95	10.45	10.60	10.65	67	68	65	62
Dog (mf)	5.78	6.64	6.43	6.76	13.08	12.53	14.23	10.78	32	38	30	33
Cat (mf)	7.85	9.13	7.66	9.46	27.35	22.90	22.63	17.15	42	38	46	60
Monkey (mf)	6.17	6.56	6.42	6.08	14.03	12.30	13.25	11.23	57	58	63	65

32
33 A decreased RBC count is observed in mice only. In other species a gradual increase or no change was
34 observed. In mice and female rats (less pronounced), a clear decrease in total WBC was observed. In male rats,

rabbits, and dogs no effect is seen while in guinea pig an increase is noted over time. In mice, the decrease in WBC is accompanied by a percent decrease in lymphocytes whereas in female rats the relative amount of lymphocytes in increased. In cats and monkeys, a decrease in WBC (and some increase in % lymphocytes) is observed only in week 8 and/or 12.

In addition, bone marrow cellularity counts in mice indicates a lower myeloid:erythroid ratio 4 weeks after exposure compared to week 12, which could indicate a hyperplastic erythroid response. These changes over time are difficult to interpret due to the lack of concurrent controls. However, from this study it is suggested that mice are the most susceptible species with respect to hematological changes.

In a 90-day inhalation study with mice and rats performed by Hazleton Laboratories and the Medical College of Virginia (White, 1983), the effects of benzene exposure on circulating immunoglobulin levels were investigated. CD-1 mice (6-10/sex/dose/timepoint) and CD rats (9-10/sex/dose/timepoint) were exposed to benzene vapor at 0, 1, 10, 30, or 300 ppm. Exposure duration per day and exposure generation (nominal/analytical concentrations) were not described. Within the framework of AEGL development, only the results from the first timepoint (7 days of inhalation) are summarized. In rats, no toxicological relevant changes in immunoglobulin levels (IgM and IgG) were observed. In female mice, significantly decreased IgA levels (± 50%) were observed after 7 days of exposure at 30 and 300 ppm compared to 0 ppm. A similar change was observed for IgG2A levels at 30 and 300 ppm. IgG1 and IgG2B levels were quite variable, resulting a in significant increase at 10 ppm but not at other levels. This change is considered toxicologically not relevant. In male mice, no clear changes were observed in any of the immunoglobulin classes.

Green and co-workers reported the effects of repeated inhalation benzene exposure from a series of experiments (Green et al., 1981a; Green et al., 1981b). The following experiments were performed.
Experiment 1: 5 days exposure 6h/day at measured levels of 1.1, 9.9, 103, 306, 603, 1276, 2416 or 4862 ppm (estimated 12 – 20 animals per group).
Experiment 2: 10 weeks exposure, 5d/wk, 6h/d at a measured level of 9.6 ppm (12 animals per group)
Experiment 3: 26 weeks exposure, 5d/wk, 6h/d at a measured level of 302 ppm (12 animals per group).
In experiment 1, exposure to 1.1 or 9.9 ppm did not induce any effects on BW gain, spleen weight, peripheral blood cell counts, femoral cell counts or splenic cell counts with the exception of splenic granulocytes. The latter was significantly decreased at all concentrations (about 50% of controls with maximal effects at 306 and 603 ppm). At levels of 103 ppm and above, benzene exposure for 5 days resulted in marked decreases of circulating WBC (circulating lymphocytes and PMN's showing a similar pattern) and femoral and splenic total nucleated cells, granulocytes and lymphocytes. Blood RBC counts were only decreased at ≥ 2416 ppm but nucleated red cells in femur and spleen were already decreased at 103 ppm. Spleen weight was significantly decreased at ≥ 103 ppm (Green et al., 1981a). The number of colony forming units (hematopoietic stem cells) and granulocyte/macrophage progenitor cells (GM-CFU) following a similar pattern: i.e. no effects at 1.1 or 9.9 ppm but significant decreases at ≥ 103 ppm (Green et al., 1981b). So, except for the splenic granulocytes, no depression of cell types in blood, femur, and spleen was observed after 5 x 6h inhalation exposure to 9.9 ppm.
In experiment 2, 10 weeks exposure to 9.6 ppm, no reductions were seen in any parameter. However, total nucleated cells/spleen, nucleated RBC/spleen, spleen weight, and CFU/spleen were increased significantly.
In experiment 3, 26 weeks exposure to 302 ppm, marked effects were observed in almost all circulating, femoral and splenic cell parameters. Total WBC, lymphocytes, RBC and Hct were decreased in circulating blood, but not PMN's. In addition, total nucleated cells, granulocytes, nucleated RBC, lymphocytes and CFU were all suppressed in both femur and spleen (Green et al., 1981ab).

1 Prolonged exposure of C57B1 mice to 300 ppm (6h/day for 6 days, for 30 days (5d/wk, 6 wks), or for
2 115 days (5d/wk, 23 wks) resulted in a marked time dependent exaggeration of some effects (Rozen and Snyder,
3 1985). Circulating RBC's and lymphocytes progressively declined with prolonged exposure. Spleen and thymus
4 weights were markedly reduced by respectively 27-50% and 65-80% but the maximal response was observed after
5 6 days. Cellularity in bone marrow and thymus (but less in spleen) was increased significantly during exposure
6 indicating enhanced proliferation. Number of B-cells in spleen however progressively declined to almost zero at
7 115 exposures whereas in bone marrow B-cell number increased with time. Mitogen stimulated B-cell
8 proliferation progressively declined to almost no response after 115 responses whereas mitogen induced T-cell
9 proliferation showed no consistent time dependent response.
10
11 In a 13-wk subchronic inhalation study by Ward et al (1985) with mice and rats using exposure of 1.15,
12 11.7, 30.4, and 307 ppm (6h/d, 5d/wk, analytical concentrations), clear hematotoxicity was evident only at 307
13 ppm. Observations were done at 7, 14, 28, 56, and 91 days. In mice, decreases in Hct, Hb, RBC, WBC, platelets,
14 percentage lymphocytes, and myeloid/erythroid ratios were observed in blood with increases in MCV, MCH, and
15 glycerol lysis time. At 307 ppm, also testes atrophy and ovarian cysts were observed. These changes in mice
16 appeared first on day 14 (not day 7). A slight-moderate thymus atrophy and slight-moderate hypoplasia of femoral
17 bone marrow were observed from day 7 onwards. In rats, the only effects observed were decreased WBC and
18 lymphocytes and an increased in relative neutrophil counts at 307 ppm. At this dose, also a decreased femoral
19 bone marrow cellularity was observed from day 7 onwards. At 30.4 ppm, minor changes in the same parameters
20 were observed in mice but none were statistically significant. In general, mice were substantially more sensitive to
21 benzene hematotoxicity compared to rats, with male rats being more sensitive than females.
22
23 Cronkite et al. (1989) published the results of inhalation experiments with various durations and
24 concentrations in mice (various strains): 10, 25, 100, 300, 400, and 3000 ppm for up to 16 weeks (measured
25 concentrations, not further specified in this paper). However, not all observations were performed at all
26 concentrations which limits the value of this study. Generally, repeated exposures to 100 ppm and above
27 produced decreases in blood lymphocytes, bone marrow cellularity, and CFU-S and an increase in the CFU-S
28 fraction in DNA synthesis. The change in CFU-S was observed after 5 days exposure to 400 ppm. Changes in
29 CFU-E and CFU-GM were observed after 29 days exposure to 400 ppm but not after 1 or 4 days exposure.
30 Decreased macrophage aggregates in blood could be seen already after 1 (about 75% of control) and 4 days
31 exposure (about 58% of control) with an almost complete disappearance at 29 days.
32
33 The same paper also investigates the effect of dosed rate: 2 days exposure to 3000 ppm compared to 19
34 days exposure to 316 ppm (both resulting in a total cumulative dose of about 6000 ppm). It was shown that 19
35 days exposure to 316 ppm had a significantly higher effect on blood lymphocytes and neutrophils, femoral bone
36 marrow cellularity, and femoral CFU-S. While the effect of 2 day 3000 ppm exposure generally results in a 50%
37 decrease in most parameters 1 day after the exposure, 19 day exposure to 316 ppm often results in decreases of
38 about 10-30%. Furthermore, recovery of these effects was normally more rapid after the 2 day exposure.
39 Comparison of 8 exposures to 3000 ppm with 80 exposures to 300 ppm showed similar trends. This indicates that
40 the hematotoxic effects of benzene are primarily important after repeated exposure but are much less severe after
41 subacute exposure.
42
43 Farris et al. (1997) exposed male B6C3F1 mice to 0, 1, 10, 100, or 200 ppm for 8 weeks or to 0, 1, 5, or
44 10 ppm for 4 weeks (analytical concentrations were kept close to the target concentration). Exposure lasted 6h/d
45 and 5d/wk. The study primarily investigated hematotoxic effects of benzene. Repeated exposures to 10 ppm or
46 less did not induce any effects. Repeated exposure to 100 or 200 ppm induced a range of effect in both bone

1 marrow and peripheral blood. At 200 ppm decreased bone marrow cellularity, CFU-HPP (equivalent to CFU-S),
2 CFU-E, CFU-GM, and other stadia of erythrocytic or granulocytic cells were observed in bone marrow. Most
3 changes were evident already at 1 week of exposure progressing during the 8 wk exposure. In blood, decreased
4 RBC, % PCE, WBC, and platelets were found during 200 ppm exposure. At 100 ppm, an identical pattern of
5 effects was found but the effects were generally less severe, became evident later during exposure (e.g. at wk 2,
6 but not wk 1), and sometime showed some recovery already at wk 8. Thus, repeated exposures for 1 to 8 weeks to
7 100 ppm and higher produced clear hematotoxicity in male mice.
8

9 **Table 14 Summary of data on non-lethal toxicity in animals**

SUMMARY OF NON-LETHAL EFFECTS IN ANIMALS AFTER INHALATION EXPOSURE				
Species	**Concentration (ppm)**	**Exposure duration**	**Major effect and comments**	**Reference**
Monkey	unknown	± 10 min	Cardiac arrhythmias	Nahum and Hoff, 1934
Rabbit	± 12,000	1h/d, several days	Eye irritation after 25 min	Von Oettingen, 1940
Rabbit	7700-15,400-23,100-30800	Max. 6h	Mean time to light narcosis > 60 min, > 29 min, 34 ± 24, 24 ± 8 at 4 concentrations resp.	Engelhardt and Estler, 1935
Cat	7700-15,400-23,100-30800	Max. 6h	Mean time to light narcosis > 50 min, > 18 min, 30 ± 23, 22 ± 9 at 4 concentrations resp.	Engelhardt and Estler, 1935
Cat	6776 – 52,360	3 min – 7 h	Light narcosis and deep narcosis time-conc curves can be described by N=1. 3-5 min at 36900 ppm showed slight CNS depression, 24 min at 52360 showed light narcosis	Von Oettingen, 1940
Guinea pig	1232	6h/d, 6d/wk, 1-5 wks	No mortality for 3 wks, protein and globulin changes indicate of liver damage	Rotter, 1975
Rat	1000-2440	unkown	Irritation opf mucous membranes	Von Oettingen, 1940
Rat	unknown	minutes	Cardiac arrhythmias	Morvai et al., 1976
Rat	Saturated vapor ?	2 min	(Artificial respiration) Increased heart rate, recovery after 50-60 sec.	Tripathi and Thomas, 1986
Rat	unknown	30 min	Heart rate ↑; after injection of adrenalin no. of ectopic beats ↑.	Vidrio et al., 1986
Rat	1299-1879, 2743-3119, 7332-8224	15 min	Heart rats were normal 10 min after inhalation. After coronary ligation or injection of K-channel blocker: ectopic beats ↑ in highest dose.	Magos et al., 1990
Rat	10,000	Up to 2h	Dyspnea, twitching, ataxia, hyperreactivity to audiotry stimuli after 30 min. Exposure at 20,000 or 40,000 showed similar signs but more progressive and some animals died.	Furnas and HIne, 1958
Rat	40,000	20 to 35 min	Irritation of the respiratory tract, depression, twitching	Furnas and HIne, 1958

SUMMARY OF NON-LETHAL EFFECTS IN ANIMALS AFTER INHALATION EXPOSURE				
Species	**Concentration (ppm)**	**Exposure duration**	**Major effect and comments**	**Reference**
Rat	250, 500, 800, 1500, 2000, 4000, 5940 (actual)	1, 2, 3, or 4 h	No mortality. At 2000 and 4000 ppm locomotor activity ↑, at 5940 locomotor activity ↓	Molnar et al., 1986
Rat	Not defined	4h	30% ↓ duration tonic extension hinlimb after electrical stimulation at 929 ppm	Frantik et al., 1994
Rat	2000 (actual)	6.5h/d, 5 days	BW ↓, WBC ↓	Coate, 1983
Mice	2000, 3570, 5800, 7600, 8300 (actual)	30 min	Nose only exposure. Respiratory rate ↑, no RD50 could be calculated	Nielsen and Alarie, 1982
Mice	18,480	40 min	Static condition. Changes in enzyme activity of SDH and $NADH_2$-diaphorase in spinal cord	Jonek et al., 1965
Mice	3696	40 min	Static condition. Enzyme activities in kidney ↑ at 1 after exposure but decreased at 3h postexposure.	Kaminski et al., 1979
Mice	2000, 4000, 8000 (actual)	20 min	Dose dependent effects on rearing ↓, righting relfex ↓, motor coordination ↓, forelimb grip strength ↓, hindlimb footsplay ↑. Motor activity ↓ at 4000 and 8000 ppm.	
mice	Not defined	2h	30% ↓ velocity tonic extension hindlimb after electrical stimulation at 856 ppm	Frantik et al., 1994
Mice	1540, 3080	7h/d, 6 days	At 1540 ppm: hyperactivity and rearing at begining of experiment. At 3080 ppm: hyperactivity observed every day followed by hypoactivity, axtaxia of hindlimbs. Loss of equilibrium after 6h.	Estler, 1935
Mice	300, 900 (actual)	6h/d, 5 days	Retraint exposure. Directly after exposure (75 min) mice were more active. At 900 ppm, intermediate results.	Evans et al., 1981
Mice	100, 300, 1000, 3000 (actual)	6h/d, 5d/wk	After single 6h exposure, WBC ↓ at 1000 and 3000 ppm. No effect at 100 ppm. Hindlimb griop strength 10% ↓ at 1000 and 3000 ppm. No effect at 300 ppm.	Dempster et al., 1984
Mice	5020 (actual)	8h (1 or 3 days)	Animals showed prostration. CFC in femur ↓, CFC in spleen not affected but splenic cellularity and spleen weight ↑. Hct ↓ at single 8h exposure. Three day exposure: femoral CFC ↓, spleen weight ↓, CFU from spleen ↓.	Uyeki et al., 1977

1

This table includes only the short term experiments (sinlge exposures or repeated exposure for a few days) or experiments with results reported for the first exposure. Repeated inhalation experiments are not included. See also the summary on animal toxicity in section 3.6.

3.3 Developmental/Reproductive Toxicity

Animal developmental toxicity was reviewed by Schwetz (1983) which included most of the studies summarised below. Schwetz concluded that there was little or no evidence for a teratogenic effect of benzene. At maternally toxic dosages, a common decrease in fetal body weight and evidence of retarded fetal development (delayed ossification) was observed.

3.3.1 Rabbits

No studies available.

3.3.2 Rats

Green et al. (1978) exposed pregnant Sprague-Dawley rats to benzene vapor at nominal concentrations of 0, 100, 300, and 2200 ppm for 6h/day from gestation day (GD) 6 to 15. Concentrations in the inhalation chamber were monitored but not reported in the paper. The study included three control groups (concurrent with the three experimental exposures ?). Dams were sacrificed at GD 21 and fetuses were investigated. Dams exposed at 2200 ppm became lethargic during the 6h exposure period and showed a significant decrease in body weight (gain) starting after the first day of exposure. Dams exposed to 100 or 300 ppm showed no abnormalities. No effects were found on litter size, implantations, resorptions, or the sex ratio of the fetuses. Fetal body weight and fetal body length were significantly decreased at 2200 ppm. At 300 and 2200 ppm a slight increase in fetuses with localized hemorrhages was observed. A significant increase in delayed ossification of the sternebrae was observed in female fetuses at 300 and 2200 ppm (not in males) associated with a significant increase in female fetuses with missing sternebrae at 2200 ppm. In this study the NOAEL for maternal toxicity is 300 ppm while the NOAEL for embryo/fetotoxicity is 100 ppm.

Pregnant CFY rats were exposed on GD 7 to 14 continuously to benzene vapor at concentrations of 0, 50, 150, 500, or 1000 ppm for 24h/day (Tatrai et al., 1980). Actual exposure concentrations were measured but not reported. Dams were sacrificed at GD 21 and the fetuses examined. Experimental groups consisted of 20-22 animals whereas the control group had 48 animals. Animals were sacrificed at GD 21. Mortality of dams was observed at ≥ 150 ppm while a considerable fraction of dams appeared to be non pregnant or to show total resorption (25, 37, 27 % at 150, 500, and 1000 ppm respectively). Maternal weight gain was significantly decreased in all dose-groups and absolute and relative liver weights were significantly increased at ≥ 150 ppm. Placental weight was dose relatedly decreased at all dose levels. The number of dead fetuses was increased at the three dose levels ≥ 150 ppm (6.3, 5.4, 4.7% respectively vs. 0% in controls). Mean number of implantations per dam was not affected. Post-implantation loss was significantly increased at three dose levels ≥ 150 ppm (42.2, 32.5, 28.9 % vs. 6.4% in controls). Investigation of the fetuses showed clear signs of retarded development. Mean fetal weight was significantly decreased at all dose levels while the number of small fetuses was significantly increased at ≥ 150 ppm. In addition, signs of skeletal retardation were evident (delayed ossification, bipartite vertebral centers, shortened 13th rib). A slight but non significant increase in the number of fetuses showing hydronephrosis was observed at ≥ 500 ppm. No increase of structural malformations was observed. A NOAEL for maternal or embryo/fetotoxicity cannot be established in this study.

1 Kuna and Kapp (1981) exposed pregnant Sprague-Dawley rats (17-20 animals per group) to benzene
2 vapor at nominal concentrations of 0, 10, 50, or 500 ppm for 7h/day from GD 6 to 15. Mean analytical
3 concentrations were 9.75, 52.9, 513 ppm respectively. At GD 5 (before exposure) and GD 20, blood samples
4 were obtained from the dams and analyzed for RBC, WBC, and differential leukocyte counts. Dams were
5 sacrificed at GD 20 and the fetuses were investigated. No mortality or signs of toxicity were observed in the dams
6 at any dose level. Body weight gain of the dams was dose-relatedly and significantly decreased at 50 and 500
7 ppm. No effects were seen on any of the hematological parameters measured at GD 20 (5 days after the last
8 exposure). A clear dose-related effect was noted on mean body weight and the crown-rump length of the fetuses at
9 50 and 500 ppm. Delayed ossification was observed at 50 and 500 ppm especially in the rib cage and the
10 extremities. At the highest dose some skeletal variants (angulated ribs, ossification sequence out of order in
11 forefeet) as well as a decrease in the number of caudals were seen. The number of metacarpals, metatarsals, and
12 phalanges showed a dose-related decrease but did not reach statistical significance. One fetus at the 500 ppm level
13 showed exencephaly. The number of fetuses with slightly dilated brain ventricles were 0, 0, 5, and 4 for the
14 various treatment groups respectively while in the highest dose group also 3 animals showed clearly dilated
15 ventricles (anomaly). As a consequence the number of fetuses and litters with variants was statistically significant
16 increased at 50 and 500 ppm whereas at 500 ppm some indications for structural irreversible effects were
17 observed. The NOAEL for maternal and embryo/fetotoxicity in this study is 10 ppm.
18
19 Coate et al. (1984) exposed pregnant Sprague-Dawley rats (n=40 per group) to benzene vapor at nominal
20 concentrations of 0, 1, 10, 40, and 100 ppm for 6h/day from GD 6 to 15. Mean analytical concentrations were
21 within ±10% of the target level. The study included two separate control group to cope with spontaneous changes
22 in parameters within groups. Dams were killed at day 20 and pups were investigated. The experimental method
23 was essentially performed according OECD 414 although not all results from all endpoints were reported in the
24 publication. At 100 ppm, maternal body weight gain was marginally reduced between GD 6 and 15. The only
25 consistent finding was a statistically reduced mean fetal weight for both sexes at 100 ppm. Although not statically
26 significant, fetal body length was also slightly reduced at 100 ppm. No other toxicologically relevant findings
27 were reported. In this study, benzene inhalation induced only a weak general fetotoxicity and the NOAEL for
28 maternal toxicity is 100 ppm while the NOAEL for embryo-fetotoxicity is 40 ppm.
29
30 Kuna et al. (1992) performed a rat fertility study in which 26 female and 13 male Sprague-Dawley rats in
31 each group were exposed to benzene vapor at levels of 0, 1, 10, 30 or 300 ppm (analytical levels). Exposures were
32 done 6h/day, 5d/wk for a 10 week premating and mating period, GD 0 to 20, and from day 5-20 of lactation.
33 Mating was done with 2 females per male. No effect was observed on maternal body weight gain and no
34 toxicological relevant effects were noted for any of the reproductive parameters. Pup weight at 30 and 300 ppm
35 tended to be lower than controls (especially at postnatal day 14 and 21) but no statistical significance was
36 obtained. Absolute liver weight was decreased in female pups at day 21 while relative (but not absolute) kidney
37 weights were increased in female pups at 10, 30, and 300 ppm.
38
39 **3.3.3 Mice**
40 Keller and Snyder (1986) exposed pregnant Swiss Webster mice (5/group/dose) to analytical levels of
41 benzene vapor at 0, 5, 10, or 20 ppm from day 6 to 15 of gestation (6h/day). Fetuses were removed at day 16 of
42 gestation (2m/2f per dam), sacrificed at neonatal day 2 (2m/2f per litter), or sacrificed at an age of 6 weeks (1m/1f
43 per litter) after exposure in utero. Litter sizes, litter weights, numbers of dead resorbed or malformed fetuses were
44 all within control limits. Cell suspensions were made from liver (fetuses and 2-day neonates) or bone marrow and
45 spleen (6 weeks). BFU-E, CFU-E, and GM-CFU-C were determined. BFU-E was increased in 16-d fetuses at 5
46 ppm (male) and 10 ppm (female). At other time points, no significant effects were observed. For CFU-E,

significant increases were observed in 16 day fetuses at 5 and 10 ppm, but a significant decrease was observed at 20 ppm. In 2 day neonates, no effects were seen at 5 ppm but a significant increase at 20 ppm. CFU-E in 2 day neonates exposed to 10 ppm was decreased in one part of the animals and increased in the other part of the animals. At 6 wk of age no consistent effect was observed. GM-CFU-C was increased significantly in 20 ppm exposed 2 day neonates and decreased in 10 ppm exposed 2 day old males. At the other time points no effects were observed. Animals exposed in utero to benzene (10 ppm) showed an exaggerated response compared to air-exposed controls, in GM-CFU-C and CFU-E when exposed at 10 weeks of age to 10 ppm benzene for 2 weeks. Maternal toxicity and in particular maternal bone marrow toxicity was not investigated in this study.

In a further study, Keller and Snyder (1988) used the same experimental protocol (5/group/dose; 0, 5, 10, or 20 ppm from day 6 to 15 of gestation (6h/day)). Peripheral blood cell counts were investigated in all animals. In addition, differential cell counts were investigated in liver (fetuses and 2-day neonates) or bone marrow and spleen (6 weeks). No effects were observed on maternal toxicity, litter size, sex ratio, fetal/litter weights, dead pups, resorptions, or malformations (data were not included). Circulating levels of total red and white blood cells were not affected in any group. Differential blood cell counts in 2-day neonates showed a dose-related decrease in early nucleated red cells in all benzene exposed groups and a decreased number of late nucleated red cells at 20 ppm. In addition, an increased number of non-dividing granulocytes (80 % vs 68% in controls) in 2-day neonates. In 16-day fetuses or 6-week adults, these effects were absent. Differential cell counts of the hematopoietic organs showed no clear effects in 16-day fetuses but a significant increase in dividing (3.5 vs 1.38%) and non-dividing granulocytes (10.4 vs 5.4%), a decrease in late nucleated red cells and an increase in lymphocytes was observed in livers of 2-day neonates at 20 ppm. In 6-week adults (after exposure in utero), a decrease in early nucleated red cells was observed in bone marrow at 20 ppm, and increased numbers of dividing and non-dividing granulocytes in spleen at 20 ppm. Maternal toxicity and in particular maternal bone marrow toxicity is not investigated in this study.

These studies by Keller and Snyder (1986, 1988) indicate that exposure of mice in utero may induce some lasting changes in hematological progenitor cells. However, the effects are differential over time and over dosages from 5 to 20 ppm while maternal (bone marrow) toxicity was not investigated. Therefore it cannot be concluded whether these effects are due to direct actions of benzene on the developing fetal bone marrow are due to maternal toxicity. Moreover, it should be noted that mice, especially Swiss Webster mice (Neun et al., 1992), are quite susceptible to benzene-induced hematotoxicity. Therefore, the clinical relevance of these findings is not clear at this moment.

3.4 Genotoxicity

Reports on the genotoxicity of benzene in gene mutation assays are generally negative or inconclusive (BUA, 1988; EHC 1993; ATSDR, 1997). In addition, negative or inconclusive results have also been found when benzene per se is tested in vitro for chromosome abnormalities. However, in vivo studies generally show that benzene is genotoxic inducing chromosomal aberrations and Sister Chromatid Exchanges (SCE). These effects have been observed in various human studies as well as a range of animal studies. Most observations in humans involve chronic exposure to benzene (see review by Zhang et al., 2002). Clare et al. (1984) reported that SCE's but not chromosomal aberrations were increased in workers acutely exposed to an accidentally released fume of benzene. Neither exposure concentration or duration, however, were available (see section 2.2.2).

In the table below the most relevant animal studies are summarized which involved acute (single) inhalation exposure to benzene.

1

2 **Table 15 In vivo genotoxicity studies in animals with acute inhalation exposure.**

Animal Species & strain	Exposure concentration	Exposure duration	Observations	Reference
Mouse (M) B6C3F1, C57BI/6J	1000, 3500 ppm (analytical)	30 min (1000 ppm) 60 min (1000 + 3500 ppm)	Micronuclei (MN) measured in lung fibroblasts obtained 24h after exposure and cultured for 72h. Sign 3 to 4-fold increase of MN in all treatment groups (highest response in 60 min-1000 ppm group). The MN were due to both chromosome loss and chromosome breaks.	Ranaldi et al., 1998
Mouse (M/F) DBA/2	3130 ppm (analytical)	4h	No sign. increase in chromosomal aberrations in bone marrow cells. Significant increase of about a factor 2 in SCE's. Cell cycle in males disturbed but not in females. Prettreatment with phenobarbital enhanced the induction of SCE's primarily in females whereas the cell cycle was almost completely stopped in males (but not females). This treatment also resulted in significant increase of chromosomal aberrations. Percentage of aberrant cells in males increased from 8.8 (control) to 12.8 (benz) to 42.4% (benz+phenob) in males, and from 10.8 (control) to 12.0 (benz) to 25.6% (benz+phenob) in females.	Tice et al., 1980
Mouse (M) DBA/2	0, 10, 100, 1000 ppm (analytical)	6h	Dose-related sign. increase SCE frequency in circulating lymphocytes at ≥ 10 ppm. Dose-related sign. increase of MN in bone marrow PCE's at ≥ 10 ppm. Mitotic Index (MI) sign. decreased ≥ 10 ppm. The number of circulating leucocytes was not affected at any dose-level.	Erexson et al., 1986
Mouse (M) B6C3F1	600 ppm	6h/day 1 or 5 days	Single 6h exp induced a decrease in MI and a delay in cell cycle. SCE frequency increased from 5.2/cell in controls to 8.9/cell in exposed animals. Repeated 5 day exposure had no effect on MI, cell cycle or SCE frequency.	Brooks et al., 1987 (Abstract only)
Rat (M) Wistar	0, 1, 10, 100, 1000 ppm (analytical within 10%)	6h	At 1000 ppm, breathing frequency was increased. A dose related increase in percentage of aberrant cells was observed, being statistically sign. only at ≥ 100 ppm (0.25, 0.41, 0.91, 1.23, 4.49 for all groups respectively, without gaps). The most obvious type of aberrations were chromosome gaps and chromosome breaks or fragments.	Styles and Richardson, 1984
Rat (M) Sprague-Dawley	0, 10, 30 ppm and 0, 0.1, 0.3, 1, 3 ppm (analytical)	6h	Dose-related sign. increase SCE frequency in circulating lymphocytes at ≥ 3 ppm. Borderline sign increase (+10%) observed at 1 ppm. A dose related sign. increase in MN in bone marrow PCE's at ≥ 1 ppm. MI sign. decreased at 3 and 30 ppm, at 10 ppm decreased but not sign. The number of circulating leucocytes was not affected at any dose-level.	Erexson et al., 1986

3

4 In addition to these single inhalation experiments, various studies have been performed using repeated
5 inhalation exposure. Mice (CD-1, 10m/10f per group) exposed to 1, 10, 30 or 300 ppm for 6h/day, 5d/wk for 13
6 weeks, showed no increase in aberrations or percentage aberrant cells at levels up to 30 ppm. At a level of 300

ppm a significant increase in percentage aberrant cells and the mean number of aberrations per cell were observed in both sexes (mitotic index was changed only at the highest dose) (Cortina et al., 1984). Sprague Dawley rats (10m/10f per group) exposed to the same exposure regimen showed a (non-significant) increase in percentage aberrant cells at all dose levels but these effects showed no dose relationship. However, the largest increase was observed at the highest dose of 300 ppm (Cortina et la., 1984).

Au et al. (1991) reported increases in chromosome aberrations (breaks) in CD-1 mice after nose-only exposure to 0.04, 0.1, and 1.0 ppm for 22h/d, 7d,wk, for 6 weeks. The increase at 0.1 ppm was somewhat larger than at 1.0 ppm, possibly due to induction of detoxifying metabolism at the higher dose. Increase in aberrations was more substantial in females than in males.

Fujie et al. (1992) exposed rats (most likely Long Evans) to 0, 10, 20, or 60 ppm benzene (2h/d, 5d/wk) for 2 weeks. A dose related increase in the percentage of aberrant cells was observed 12h after the last inhalation experiment (about 8% at 10 ppm, 10% at 20 ppm, and 20% at 60 ppm). It should be noted that Long Evans rats seem more susceptible to the clastogenic effects of benzene than other strains. Experiments with oral and i.p, administration showed that the induced aberration were reversible (Fujie et al., 1992).

In a repeated inhalation experiment, Farris et al. (1996) exposed B6C3F1 mice to 1, 10, 100, or 200 ppm benzene vapor (6h/day, analytical concentrations) for 8 weeks. The results showed an increase in micronuclei in polychromatic erythrocytes from bone marrow and in normochromatic erythrocytes in blood at levels of 100 and 200 ppm but not at 1 and 10 ppm. Bone marrow cells with MN were already at a maximal level after 1 or 2 weeks while the number of blood cells with MN progressively increased over exposure time. In addition, a reduction in total RBC counts as well as PCE count was observed at 100 and 200 ppm.

In a dominant-lethal study from Bio/dynamics (Schroeder, 1980) CD rats (20 males/dose) were exposed to benzene vapor at 0, 1, 10, 30, or 300 ppm for 10 weeks (6h/day, 5d/wk; analytical concentrations). The males were caged for 7 days with 2 females/male prior to treatment, and after benzene treatment 2 x 7 days with 2 females each. No treatment related mortality occurred and body weight gain was unaffected. An increased number of animals showed lacrimation during the first 3 weeks of the study at 10 ppm and above. No effects were observed on fertility rates, pregnancy rates, the number of corpora lutea, the number of implantation sites, and implantation efficiency. In the high dose group an slight increase in dead implants and mutagenic ratio was observed although not statistically significant. This increase was attributed to females that were mated with one single male. Histopathology revealed 1/18 male with seminiferous tubule degeneration and 1/18 with focal syntitial cells formation. However, these males showed normal pregnancy rates. The male causing the increased mutagenic ratio at the high dose, showed normal testes and epididymides.'

Cirranni et al. (1991) exposed Swiss CD-1 mice to a single oral dose of 880 mg benzene/kg bw. In bone marrow cells the percentage of aberrant cells increased from 1.1% in controls to maximally 20.8% at 24h post-administration. Thereafter, the level decreased to about 5-6% at 48h. In spermatogonia of these mice, also an increase in percentage aberrant cells was seen from 1.2% in controls to maximally 6.3% at 24 post-administration. Thereafter, the level decreased to about 3% at 48h. Measuring the maximal response at 24h, dose response experiments showed that aberrations in bone marrow cells increased non-linear 1.1, 3.0, 4.8, and 20.8% aberrant cells at 0, 88, 440, and 880 mg/kg bw respectively) giving a much higher response at the highest dose level. In spermatogonia, a different pattern can be seen: 1.2, 3.0, 5.0, and 6.3% aberrant cells at 0, 220, 440, and 880 mg/kg bw respectively). This study indicates that benzene is able to induce clastogenic effects in sperm cells. For

comparison, these oral doses correspond roughly to 1000 – 4000 ppm inhalation exposure for 1h (mouse is 20g, 50% absorption at inhalation, minute volume is 45 ml/min).

3.5 Carcinogenicity

In an overview paper of Maltoni et al. (1989) the various experiments performed at the Bologna Institute of Oncology were summarized. Experiments were done with Sprague Dawley and Wistar rats and Swiss and RF/J mice. However, inhalation experiments were performed only with Sprague Dawley rats. Exposure of rats to 200 and 300 ppm (4 or 7h.d, 5d/wk, 7 to 85 weeks) showed increased incidence for zymbal gland carcinomas and oral cavity carcinomas. Weak evidence for induction of nasal cavity and mammary gland carcinomas and hepatomas was reported. It was claimed that the carcinogenic response was increased when exposure started early in embryonic life.

Cronkite et al. (1989) showed that exposure of CBA/Ca BNL mice to 300 ppm benzene (6h/day, 5d/wk) for 16 weeks resulted in increased mortality starting shortly after exposure. Benzene exposed animals showed an increased incidence in myelogenous neoplasms (especially males) and an increased incidence in various neoplasms (Zymbal, harderian, mammary adenocarcinomas, squamous cell carcinomas, papillary adenocarcinoma of lung, and benign tumors; 21.7% in control males, 52.6% in exposed males). Exposure of male mice to 100 ppm for 16 weeks resulted in a slight increase in mortality rates and an increase in the incidence of various neoplasms (20.0% in control males, 44.7% in exposed males).

Farris et al. (1993) performed a inhalation carcinogenicity study with CBA/Ca mice using a 16 week exposure period. Male mice (125/group) were exposed to 0 or 300 ppm for 6h/d and 5d/wk and held for 18 months. Benzene exposure resulted in a shift of the mortality curve showing increased and early lethality after exposure. Exposed mice showed increased incidences of various tumors: malignant lymphoma (12% vs 2% in controls), preputial gland squamous cell carcinoma (60% vs 0%), lung adenoma (36% vs 14%), zymbal gland carcinoma (11% vs 1%), forestomach squamous cell carcinoma (7% vs 0%), and harderian gland adenoma (6% vs 5%). In particular, the first cases of malignant lymphoma were observed shortly after the exposure period (most of them between week 16 and 50) while lymphomas in controls were not observed before week 90. In addition, granulocytic hyperplasia was observed in bone marrow (36% vs 8%) and spleen (6% vs 0%). The absolute cell counts in peripheral blood showed no significant changes except for a slight increase in total leukocytes and neutrophils.

3.6 Summary animal data

Lethality

Only few animal studies provide adequate LC50 values. For rats, a 4h LC50 of 13,700 ppm and a 6h LC50 of 9536 were reported (Drew and Fouts, 1974; Bonnet et al., 1982). In addition, 16,000 ppm for 4h produced 66% mortality (Smyth et al., 1962). In mice, a 6h LC50 of 14,122 ppm and a 7h LC50 of 9980 were reported (Bonnet et al., 1982; Svirbely et al., 1943). From the mortality rates in Bonnet et al. (1982) and Svirbely et al. (1943) it can be concluded that the dose-response curve is very steep: within a concentration range with a factor of 3 the mortality rate increases from 0% to 100%. Furthermore, the data of Svirbely et al. (1943) show that delayed mortality after exposure is not a major factor with benzene exposure, most animals die during the exposure.

In addition to these studies, a range of single dose studies of variable quality provide information for the lack of mortality (see Table 20). In rabbits and cats, 7700 ppm for 6h produced no mortality (Engelhardt and Estler, 1935). In addition, no rabbits died during 3h exposure at 20,000 ppm (Kujime, 1990). In cats, deep narcosis was reached at about 52,000 ppm for about 60 min (Von Oettingen, 1940). In guinea pigs exposed to 15,000 ppm for 20 min (Von Oettingen, 1940), or to 1232 ppm (6h/day) for 2 weeks (Rotter, 1975) no mortality was reported. For rats, no mortality was observed after exposure to 7332-8224 ppm for 15 min (Magos et al., 1990), 10,000 ppm for 2h (Furnas and Hine, 1958), 5940 ppm for 4h (Molnar et al., 1986), or 2000 ppm for 6.5h/day for 5 days (Coate, 1983). No mortality was observed in mice exposed to 2000 – 8300 ppm for 30 min (Nielsen and Alarie, 1982), 5020 ppm for 8h (Uyeki et al., 1977), 1540 or 3080 ppm for 6h (Estler, 1935), or 3000 ppm for 6h (Dempster et al., 1984).

Irritation

Benzene is not a sensory irritant as shown by the absence of respiratory depression in the RD50 test (Nielsen and Alarie, 1982). In fact, an increase in respiratory rate was observed in this test. Little information is available concerning eye or airway irritation in animals. In rats exposed to about 12,000 ppm for 1h/day, grayish white turbidity of the cornea was apparent after 6-8 days but first signs may be noted after 25 min at the first day (Von Oettingen, 1940). In rats exposed to 40,000 ppm for 20 – 35 min, local irritation of the respiratory tract was reported (Furnas and Hine, 1958). In a dominant lethal study, an increased number of rats showed lacrimation during the first 3 weeks of exposure at levels ≥ 10 ppm (6h/day, 5d/wk) but it is not known whether this occurs directly after the first exposure (Schroeder, 1980). Repeated exposure to 388 ppm (6h/day, 5d/wk) showed ocular distress in mice (lacrimation, chromodacryorrhea, chemosis, corneal opacity) starting after 4 weeks of exposure but no effects were reported for rats, rabbits, dos, cat, or monkey (Thackara et al., 1979).

Neurotoxicity

Benzene induces, similar to many other volatile organic compounds (VOC), CNS depression. These CNS effects are dose-related in a continuum of slight effects (lightheadedness) to narcosis and eventually death, due to paralysis of the respiratory center. These effects are primarily related to the concentration of benzene incorporated in membrane lipids of the brain (De Jongh et al., 1998). Unfortunately, no adequate information is available with respect to brain concentrations of benzene associated with death, narcosis, or lightheadedness.

Animal data related to neurotoxicity involve two categories: information on narcotic effects and neurobehavioral changes. In rabbits and cats, light narcosis was reached after 60 min and 50 min respectively at 7700 ppm. Deep narcosis was not reached for 4h at this level (Engelhardt and Estler, 1935). At 15,400 ppm, these values were 29 and 18 min for light narcosis and 229 and 37 min for deep narcosis (rabbit and cat respectively). At 23,100 ppm, similar result were obtained for rabbits and cats (light narcosis reached in 30 min on average and deep narcosis in 40 min on average). At higher concentrations of 30,800 ppm, time to light narcosis was 22 min and time to deep narcosis was 30 min (Engelhardt and Estler, 1935). At a very high level of 35,000 – 45,000 ppm, slight anesthesia was reported in rabbits after ± 3 min and loss of reflexes after about 6-12 min (Carpenter et al., 1944). At 30,000 ppm, rats showed convulsive twitching in 10 min. At 25,000 ppm for 1h EEG changes were present but not at 10,000 ppm (Furnas and Hine, 1958). Exposure of rats to 5020 ppm during 8h, resulted in signs of CNS depression during the first hour but this resolved thereafter (Uyeki et al., 1977).

Neurobehavioral changes follow a specific pattern for this type of solvents: at subnarcotic levels hyperreactivity and increased locomotor behavior is observed (compare euphoria noted in humans) and at higher levels decreased activity and lethargy may result. The latter type of effects are primarily considered relevant for AEGL development. Decreased locomotor activity was observed in rats exposed to 5940 ppm for 4h, but not at

1 4000 ppm (Molnar et al., 1986). Exposure of mice to 300 or 900 ppm for 6h/d for 5 days results mostly in an
2 increased level of activity, although at 900 ppm intermediate results were found (approximation of narcotic
3 threshold ?) (Evans et al., 1981). Hindlimb grip strength was decreased by 10% after 6h exposure to 1000 or 3000
4 ppm, but not a 300 ppm (Dempster et al., 1984). Frantik et al. (1994) reports a 30% change in neurological
5 response, a relatively mild endpoint, of 929 ppm for rats (4h exposure) and 856 ppm for mice (2h exposure). In
6 contrast to these findings, Li et al. (1992) report behavioral changes at 12.52 ppm (2h/day for 30 days). The
7 presentation of the results does not indicate any temporal pattern or whether the significant effects are actually
8 present after a single 2h exposure. Furthermore, this study was inadequately reported with respect to methods,
9 measurements and results, and its validity should therefore be considered with great care.
10
11 The neurotoxic effects of benzene are reversible besides some changes in enzyme activities (Jonek et al.,
12 1965; Varona et al, 1998) there are no indications for structural damage to nervous tissues (Furnas and Hine,
13 1958).
14
15 *Cardiac sensitization*
16 The effects of benzene inhalation on cardiac sensitization (resulting in tachycardia, arrhythmias and
17 ventricular fibrillation) have been studied in monkeys, dogs, and rats (Nahum and Hoff, 1934; Wegria et al.,
18 1943; Chenoweth, 1946; Morvai et al., 1976; Tripathi and Thomas, 1986; Magos et al., 1990). However, most
19 studies lack appropriate exposure information while the experimental procedures used differ (mostly in trachea
20 cannulated animals under anesthesia sometimes using very short periods of benzene inhalation). Based on the
21 description of the experimental set-up, high exposure concentrations were probably used. In dogs very high
22 exposure concentrations were used (~ 50,000 ppm). Only Magos et al. (1990) provide some quantitative exposure
23 information although this study is not actually an adrenalin-type of sensitisation test. Exposure of rats to levels up
24 to 7332-8224 ppm for 15 min does not induce changes in heart rate or ECG by itself. However, coronary ligation
25 (i.e. ischemia) or injection of aconitine (a K-channel blocker) induced a more severe increase in ectopic beats in
26 benzene exposed animals (15 min at 7332-8224 ppm) than already observed in controls. No significant increase in
27 ectopic beats by these procedures was observed in animals exposed 2743-3119 ppm for 15 min (Magos et al.,
28 1990).
29
30 *Hematological effects*
31 From numerous repeated dose experiments with animals it is known that benzene exposure results in
32 bone marrow toxicity resulting in changes in hematological parameters. Effects on various types of endpoints can
33 be observed: changes in circulating cells (e.g. RBC and WBC), changes in unilineage progenitor cells (e.g. CFU-
34 E, CFU-GM), and changes in the multilineage or pluripotent stem cells (CFU-S) in bone marrow. The first two
35 types of effects are in principle reversible, but a loss of pluripotent stem cells is not. Therefore, it is important to
36 separate effects on circulating cells from effects on unilineage progenitor cells and specifically stem cells. For all
37 types of effects it is generally accepted that some form of repeated exposure is needed to induce the effects.
38 However, little is known about the actual occurrence of effects after a single exposure for 10 min to 8 hours.
39
40 In repeated dose experiments (5 days up to 16 weeks) dose related decreases in circulating WBC and
41 RBC are generally observed at 100 ppm and above (6h/day repeated exposures) in several species but mice are
42 the most susceptible (Johnston et al., 1979; Green et al., 1981a and 1981b; Coate, 1983; Rozen and Snyder, 1985;
43 Ward et al., 1985; Cronkite et al., 1989; Farris et al, 1997). Levels within the range of 10-30 ppm generally do not
44 induce changes in circulating WBC or RBC. Generally WBC changes are more severe and occur at lower
45 concentrations than RBC changes. In contrast, Rozen et al. (1984) showed decreased circulating lymphocytes in
46 mice after 6 days exposure at levels as low as 1 ppm. Dempster et al. (1984) showed that WBC were decreased

1 after a single 6h exposure to 1000 and 3000 ppm but not at 100 ppm. Two days exposure (2 x 6 hours) to 300
2 ppm also reduced WBC.
3 Effects on progenitor cells involve effects on the pluripotent stem cells (CFU-S) and unilineage
4 progenitor cells (CFU-GM, CFU-E). Changes in CFU-S are the most serious since damage to the pluripotent stem
5 cells may irreversibly compromise the formation of all cell types. The most prominent data are from Uyeki et al.
6 (1977) who showed that a single 8h exposure to 5020 ppm in mice resulted in a reversible decrease of colony
7 forming cells in femoral bone marrow. Three days of exposure (3 x 8h) resulted in a substantial decrease of CFU-
8 S. Total bone marrow and splenic cellularity were not affected after a single 8h exposure. Unfortunately, CFU-S
9 was not determined after a single exposure by Uyeki et al. (1977). Other studies investigated the effect on CFU-S
10 after repeated exposure. Green et al 1981b showed effects on CFU-S and GM-CFU-C at ≥ 103 ppm but not 9.9
11 ppm (6h/day, 5 days). After longer exposures up to 16 weeks effects on CFU-S and CFU-GM and CFU-E were
12 observed at 100 ppm and above (Cronkite et al., 1989; Farris et al., 1997). At 400 ppm (6h/day), changes in CFU-
13 S were observed after 5 days of exposure, but not after 1 or 4 days (Cronkite et al., 1989).
14
15 It is important to note that the bone marrow toxicity of benzene is mediated through several metabolites
16 instead of the parent compounds itself (see section 4).
17
18 *Developmental toxicity*
19 Although some slight differences exist between studies, the effecets of benzene on the developing fetus
20 are quite consistent. There are no indications that benzene induces structural irreversible effects in animals
21 (Schwetz, 1983). However, benzene consistently shows forms of developmental retardation in rats, i.e. decreased
22 fetal body weight and length, delayed ossification, and skeletal variants (Green et al., 1978; Kuna and Kapp,
23 1981; Coate et al., 1984; Kuna et al., 1992) sometimes already occurring before overt maternal toxicity appears.
24 NOAELs for embryo/fetotoxicity were 100 ppm or 300 ppm, 10 ppm, 40 ppm, and 10 ppm. The developmental
25 study of Tatrai et al. (1980) used 24h exposure per day from GD 7-14 and showed maternal and
26 embryo/fetotoxicity at and above a level of 150 ppm. Taken all information together, the developmental effects of
27 benzene are quite similar to those of toluene. The pattern of effects shares characteristics of the so-called “fetal
28 alcohol syndrome” which is induced primarily after repeated exposure.
29
30 In mice, two studies by Keller and Snyder (1986, 1988) investigated the effect of exposure in utero on
31 hematological parameters in fetuses, 2-day neonates, and 6 week young adults. In these studies no effects on litter
32 parameters, resorptions, or numbers of dead or malformed fetuses were found. These studies indicate that
33 exposure of mice in utero at levels of 5, 10 or 20 ppm (gestation day 6-15) may induce effects on the
34 hematopoietic system. The effects appear partly reversible and partly prolonged until 6 weeks of age. The effects
35 also show differential effects for the three age groups. It should be noted that maternal toxicity and especially
36 maternal bone marrow toxicity was not investigated in these studies. Because mice, especially Swiss Webster
37 mice (Neun et al., 1992), are very sensitive to the hematotoxic effects of benzene, the relevance of these
38 observations for humans remains unclear.
39
40 No adverse reproductive outcomes were evident from a dominant lethal study in rats (Schroeder, 1980).
41
42 *Genotoxicity*
43 Similar to humans, inhalation exposure of animals results in clastogenic effects in animals. Acute
44 inhalation experiments show the induction of micronuclei, sister chromatid exchanges, and chromosome
45 aberrations. MN were increased in mice at 1000 and 3500 ppm for 0.5-1h, and ≥ 10 ppm for 6h, and in rats at ≥ 1
46 ppm for 6h (Ranaldi et al., 1998; Erexson et al., 1986). SCE were increased in mice at 600 ppm for 6h and 3130

ppm for 4h, and in rats at ≥ 3 ppm for 6h (Tice et al., 1980; Erexson et al., 1986). Chromosome aberrations, the most meaningful endpoint for benzene toxicity (Zhang et al., 2002), were increased in rats at ≥ 100 ppm for 6h (not at 10 ppm) but no increase in CA was observed in mice exposed to 3130 ppm for 4h (Tice et al., 1980; Styles and Richardson, 1984). Repeated inhalation experiments showed generally a similar pattern with approximately the same effect concentrations. However, it was also shown in non-inhalation studies that part of the induced aberration were in fact reversible, showing a smaller percentage of aberrant cells after a few days (e.g. Ciranni et al., 1991; Fujie et al., 1992). No adverse effects were observed in a dominant lethal study with rats (Schroeder, 1980). After oral doses of 88, 440, or 880 mg/kg bw in mice, a dose related increase in aberrant cells was observed in spermatogonia (Cirranni et al., 1991).

Carcinogenicity

Although benzene has been identified as a human carcinogen inducing tumors of the hematopoietic system, long-term benzene exposure in animals induces malignant lymphoma but not acute myelogenous leukemia. Instead other types of tumors are observed: primarily zymbal gland tumors, preputial gland squamous cell carcinoma, lung adenoma, and nasal cavity tumors. This observations trigger two important questions: 1) whether animal models are adequate for studying the carcinogenic effects of benzene and 2) whether benzene is able to induce other types of tumors in humans except for hematopoietic tumors. Both questions remain to be resolved.

4 SPECIAL CONSIDERATIONS

4.1 Metabolism and Disposition

The toxicokinetics of benzene have been extensively studied and numerous reviews on this matter exist, also in monographs (e.g. Haley, 1977; Fishbein, 1984; Snyder, 1987; BUA 1988; Henderson et al., 1989; Travis et al., 1990; Marcus, 1990; Snyder et al., 1993; EHC 1993; ATSDR 1997; Krewski et al., 2000; Lovern et al., 2001; EU 2002). In this section only an overview will be given of the most important aspects of benzene toxicokinetics by the route of inhalation exposure, given the aim of this document i.e. deriving acute exposure limits. That implies that an elaborate discussion on the formation and action of the various metabolites with respect to chronic exposure and induction of leukemia is out of the scope of this chapter.

4.1.1 Absorption

During inhalation exposure, benzene is rapidly taken up from the air. In an historical study by Lehmann (1910), 3 humans inhaled 11 to 16 mg/l (± 3400 – 4900 ppm) for 5 to 15 minutes. Initial absorption was calculated to be about 80% (vapor generation and benzene measurement may be limited). In a human volunteer study using 23 healthy students and laboratory assistants, Srbova et al. (1950) showed that during exposure to 47 – 110 ppm for 2h, the initial uptake of benzene from the air was 60 – 80% within the first minutes of inhalation. After 15 min, the average retention of benzene was about 50% (range 30-60) which slightly decreased over the rest of the 2h period (range 20-50%). Respiratory uptake was 47% in a study with 6 volunteers exposed to 52-62 ppm for 4h (Nomiyama and Nomiyama, 1974a) and 48-52% in a study with 3 volunteers exposed to 1.6 or 9.4 ppm for 4h (Pekari et al., 1992).

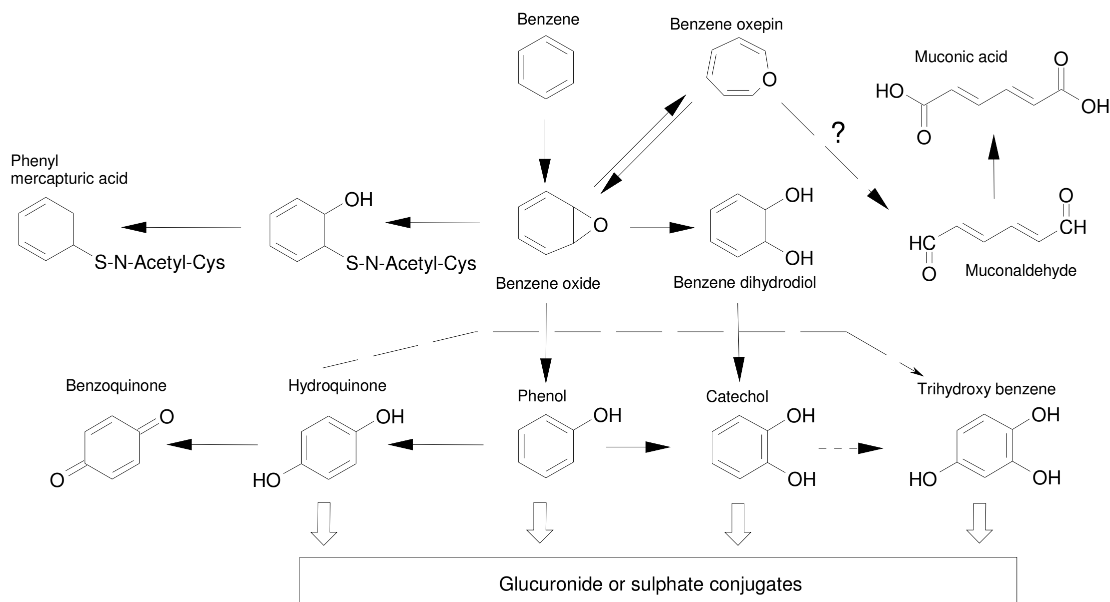
Animal studies on the absorption of benzene via inhalation indicate similar results. In a 6h inhalation experiment rats retained about 33% and 44% of the inhaled benzene at 33 and 75 ppm respectively while mice

1 retained 50% and 52% at 29 and 75 ppm. The fractional uptake decreased when exposure levels rose: only 15%
2 was retained in rats at 2260 ppm for 6h and only 9.7% in mice exposed to 2570 ppm for 6h (Sabourin et al.,
3 1987). Uptake in mice in terms of body load was about 1.5 to 2-fold higher than in rats, as can be expected based
4 on allometric scaling calculations. In an old and limited reported study by Lehmann (1910) using trachea
5 cannulated rabbits, it was reported that 37-54% of the inspired benzene was absorbed during the first 30 min of
6 exposure. Thereafter, absorption varied between 10 and 40% over 5 hours. Exposure levels ranged from 22 – 97
7 mg/l (6790 – 30000 ppm; Lehmann, 1910).
8
9 From the rapid uptake in blood several investigators have calculated or estimated the blood/air partition
10 coefficients. Travis et al. (1990) estimated values of 22.0 for mice, 15.0 for rats, and 7.4 for humans. Using Monte
11 Carlo techniques, Watanabe et al. (1994) estimated a range of 1.66 to 17.9 for humans. Robinson and Climenko
12 (1941) calculated a value of 7 to 8 based on nominal exposure concentrations. Schrenk (cited in Robinson and
13 Climenko, 1941) reported a value of 6.58 for dogs. Srbova et al. (1950) report a value of 7.78 for humans from an
14 in-vitro determination.
15
16 **4.1.2 Distribution**
17 Benzene taken up in the blood is rapidly transported throughout the whole body. Because benzene is
18 lipophilic, lipid-rich tissues contain the highest amounts of benzene (see also Table 2, which reports benzene
19 levels in victims of benzene intoxication). However, true saturation of fatty tissue may require up to 2 or 3 days
20 (Haley, 1977). Sato et al. (1975) and Hunter and Blair (1972) showed that uptake and excretion of benzene was
21 correlated with the body fat content in both rats and humans. In fatal cases, high amounts of benzene are found in
22 fat tissue and the brain (see Table 2). Well-perfused tissues (such as liver and kidney) contain substantial but
23 lesser amounts of benzene. Benzene has been reported to cross the human placenta and levels in cord blood may
24 be equal or higher than those in maternal blood (ATSDR, 1997). Blood levels in humans after benzene inhalation
25 are presented in Table 16.
26
27 **Table 16 Blood levels of benzene after inhalation exposure of humans**

Reference	Exposure level	Exposure duration	Study characteristics	Blood level (steady state, at the end of the exposure)
Srbova et al., 1950	Up to 110 ppm	2h	23 volunteers, results reported in a limited way	± 400-500 µg/l
Sato et al., 1975	25 ppm	2h	5 male volunteers 5 female volunteers (Japanese students)	± 200 µg/l ± 150 µg/l estimated from graphs
Pekari et al., 1992	9.4 ppm 1.7 ppm	4h 4h	3 male volunteers 3 male volunteers dynamic conditions	± 78 - 94 µg/l ± 16 - 23 µg/l estimated from graphs

28
29 No information is available about the time course of distribution in humans after inhalation exposure
30 (ATSDR, 1997, EU 2002).
31
32 In animal studies a similar distribution can be observed. The parent compound is preferentially stored in
33 fat (see also Table 9, benzene levels in rabbits), although this process depends on the relative perfusion of the
34 tissues. Rickert et al. (1979) performed experiments in rats showing the time course of benzene distribution and

1 its metabolites. During an 8h inhalation exposure to 500 ppm benzene, benzene concentrations reached steady
2 state within 4h in blood, 6h in fat tissue, and 2h in bone marrow. Steady state concentrations were 11.5 µg/ml in
3 blood, 37.0 µg/g in bone marrow, 164 µg/g in fat tissue, 9.9 µg/g in liver, 15.1 µg/g in lung, 25.3 µg/g in kidney,
4 4.9 µg/g in spleen, and 6.5 µg/g in brain. Half times to approach steady state in rats were 1.4h in blood, 2.0h in
5 fat, 0.9 - 1.9h in liver, lung, kidney and spleen, and 2.6h in brain. Distribution to bone marrow was too rapid, to
6 allow calculation of a half life in this experiment.
7
8 Inhalation exposure of pregnant mice (10 min to maximum 2000 ppm nominal, no further details
9 provided) to ^{14}C benzene shows that benzene (and/or its metabolites) cross the placenta. Fetal uptake of benzene
10 was calculated to be about 8% of the rate of maternal brain uptake. Fetal levels of benzene and its metabolites
11 were generally much lower than corresponding maternal tissues (Ghantous and Danielsson, 1986).
12
13 **4.1.3 Metabolism**
14 Metabolism is basically similar in humans and experimental animals. However, quantitative differences
15 between species have been demonstrated. Since benzene (hemato-)toxicity (at least during repeated exposure) is
16 highly determined by its metabolites, differences in benzene metabolism ultimately result in differences in toxic
17 responses.
18
19 The metabolism scheme below is taken from ATSDR (1997), with some adaptation.
20



21
22
23 The liver is the predominant site for benzene metabolism but bone marrow has also been indicated as a
24 site for benzene metabolism. Benzene is converted to a number of metabolites by the cytochrome P-450
25 dependent mixed-function oxidase system. The P-450 enzyme CYP2E1 has the greatest affinity for benzene. CYP
26 2B1 may be involved in benzene metabolism only at higher concentrations (Snyder, 2002).
27
28 The first step in benzene metabolism is the formation of benzene oxide via cytochrome P-450 mixed-
29 function oxidase (Marcus,1990). From this metabolite, two toxification and two detoxification pathways progress.

The first toxification pathway occurs after the non-enzymatic rearrangement of benzene oxide to phenol. Phenol conjugates are the predominant metabolites of benzene metabolism (see below). Phenol can be metabolized to the toxic metabolites hydroquinone and benzoquinone. Phenol can also be metabolized to catechol and trihydroxy benzene. Another toxification route proceeds probably through the formation of benzene oxepin from benzene oxide. Ring opening of this structure results in muconaldehyde and muconic acid. The specific route and intermediates involved in ring-opening are still subject of research. Although this is a relative minor route of benzene metabolism, these metabolites are known hematotoxins and may be therefore important with respect to benzene toxicity.

Detoxification occurs through the formation of phenyl mecapturic acid which can be eliminated through the bile. Another detoxification occurs through the formation of glucuronide or sulfate conjugates of phenol, hydroquinone, catechol, and trihydroxy benzene, which can be eliminated in the urine. Phenol and its conjugates form the major part of benzene metabolites (30-70% of total metabolites) in all species. In humans, phenol-sulfate is the major metabolite excreted in urine and a relation between urinary (phenol)sulfate levels and benzene exposure has been established (Inoue et al., 1986; Inoue et al., 1988; Hunter and Blair, 1972).

With increasing exposure levels in the air, metabolism becomes saturated (see also below) resulting in a shift of production of toxic metabolites to detoxification routes. This indicates that at relatively high inhalation exposure (300 ppm or higher in experimental animals), the extend of production of toxic metabolites may be underestimated when extrapolated to low exposures.

With respect to the hematotoxic effects of benzene it is now known that this requires hepatic metabolism of benzene. Benzene metabolites formed in the liver (where the majority of CYP 2E1 is located) are transported to the bone marrow. The pivotal role of hepatic metabolism in benzene toxicity is illustrated by the fact that partial hepatotectomy reduces benzene toxicity (Sammett, et al., 1979). In mice lacking functional CYP 2E1 expression, benzene induced micronuclei induction was prevented (Valentine et al., 1996).

The hematotoxic effects result ultimately from an interaction of several benzene metabolites (Snyder, 2000a,b). The metabolites phenol, hydroquinone, p-benzoquinone, catechol, trihydroxybenzene, and muconaldehyde all have been associated with various forms of bone marrow toxicity which includes decreases in bone marrow cellularity, inhibition of erythropoiesis, inhibition of spindle formation, inhibition of DNA polymerase, inhibition of topoisomerase II, changes in cytokine production, and DNA adduct formation (Snyder, 2000a,b; Krewski et al., 2000). With respect to inhibition of erythropoiesis, it should be mentioned that the most effective combination was found to be exposure to both hydroquinone and muconaldehyde.

The mechanism(s) by which benzene metabolites induce DNA damage is still not fully clear and several lines of theories exist. DNA adduct formation, oxidation of DNA by reactive oxygen species, release of iron and subsequent chelation by hydroquinone or trihydroxybenzene have been proposed

Benzene is able to induce its own metabolism but compounds like ethanol and various others can also induce CYP 2E1 activity (EU 2002). On the other, hand benzene metabolism is inhibited by simultaneous exposure to toluene which is metabolized by the same enzyme (Snyder, 2000a,b). Through competitive inhibition, simultaneous toluene exposure results in a decreased formation of benzene metabolites as well as a decrease in hematotoxicity (Inoue et al., 1988).

1 Considerable species variation exists with respect to benzene metabolism. In a sum-up of various
2 metabolism studies Henderson et al. (1989) showed that mice metabolize benzene faster than rats and convert
3 more of the benzene to toxic metabolites than do rats, primarily at exposure levels up to about 200 ppm. This is
4 partly due to increased inhalation per unit body weight but also due to a higher activity of hepatic metabolism.
5 Metabolic saturation occurs at levels between 200 ppm (rats) and 780 ppm (mice) (Sabourin et al., 1989; Lovern
6 et al., 2001). A 6h exposure to 50 ppm resulted in only trace amounts of hydroquinone (and its conjugates) in rat
7 lungs, blood, and liver but in 100-fold higher amounts in mice. Muconic acid was found at 2-fold (blood) to 20-
8 fold (liver) higher quantities in mice compared to rats (Henderson et al., 1989). In cynomologous monkeys (ip
9 treatment) and chimpanzees (iv treatment), phenol sulfate was the major metabolite (51% in chimps, 62% in
10 monkeys). Monkeys produced about 27% of hydroquinone conjugates while chimps produced 8% of such
11 products. However, these primate species produced less muconic acid metabolites than mice or rats (Lovern et al.,
12 2001).
13
14 With respect to bone marrow metabolism, the cellular GSH content and quinone reductase specific
15 activity were 2- and 28-fold higher in rats than in mice which again indicates that the mouse is systemically
16 exposed to higher amounts of toxic metabolites (EU 2002).
17
18 From in vitro experiments with hepatocytes or liver microsomes it was shown that the range of metabolite
19 production from human tissue more or less spans the range between mice and rats. However, no definitive
20 conclusions can be drawn from this (Medinsky et al., 1996; Snyder, 2002).
21
22 **4.1.4 Elimination**
23 A major portion of benzene in humans and animals is eliminated unchanged in exhaled air. In human
24 volunteers, about 16 to 42% of the absorbed benzene was excreted in air within 5 to 7h after an exposure of 47-
25 110 ppm for 2h (Srbova et al., 1950). In men and women exposed to 52-62 ppm for 4h about 16% was eliminated
26 unchanged in exhaled air. This has been described by a 3-compartment model with half lives of 2.6, 28, and 90h
27 (Nomiyama and Nomiyama, 1974b, ATSDR, 1997). The excretion rate of the slow compartment (probably body
28 fat) was lower in women compared to men (Nomiyama and Nomiyama, 1974b; Sato et al., 1975) indicating that
29 benzene remains longer in the body of females than males. After 4h exposure to 1.6 or 9.4 ppm, about 8-14% was
30 excreted unchanged in the exhaled air (Pekari et al., 1992). After exposure to 100 ppm, 12% (not clear if authors
31 mean percentage of total dose or absorbed dose) was eliminated via the lungs and 0.1-0.2% in the urine. Of the
32 metabolized benzene, 29% was eliminated as urinary phenol, 2.9% as pyrocatechol, and 1% as hydroquinone
33 (cited in Haley 1977). Based on measured urinary phenol levels, Pekari et al. (1992) estimated that in 3 volunteers
34 11-23 % of the pulmonary uptake of benzene was excreted in urine as phenol. In male volunteers exposed to
35 levels of 19 – 125 ppm for a few hours (not specified, maximal 6h), it was determined that 50 – 87% of the total
36 dose was excreted as phenol (and conjugates) (Hunter and Blair, 1972).
37
38 In rats after a 6h exposure to 500 ppm, benzene was eliminated through the lungs in a biphasic process
39 with half lives of 0.7 and 13.1h respectively (Rickert et al., 1979) In humans, a triphasic process was observed
40 (see above) but apparently the third phase (halflife of 90h in humans) was not covered by Rickert et al. (1979).
41 Sabourin et al. (1987) investigated the elimination of ^{14}C-benzene equivalents during and after 6h exposures of
42 about 11 to 990 ppm in rats and mice. In
43 Table 17, the results of this study are shown expressed as % of the total amount excreted.

1
2

3 **Table 17 Excretion in mice and rats during and 56 hours after exposure (Sabourin et al., 1987)**

Exposure concentration	Expired air (ethanol + KOH traps) [a]	Feces	Urine	Pelt + Carcass [b]	Total
		MOUSE			
13	3.8	2.6	56	37.9	121
29	3.5	3.1	57	36.8	113
130	5.2	2.0	51	41.0	101
260	11.7	2.1	62	24.5	112
870	48.3	3.4	41	7.0	106
		RAT			
11	3.5	7.5	81	7.8	116
29	1.8	8.6	82	7.5	117
130	2.4	3.2	87	8.1	107
990	14.9	4.7	74	6.5	119

4 a) only determined after exposure.
5 b) At low concentrations, the pelt contained 23-36% in mice and about 6% in rats.
6
7 This study clearly shows that the elimination of unchanged benzene increases at exposure levels above
8 which metabolism becomes saturated. Fecal excretion was rather stable over the whole range but about 2-fold
9 higher in rats than in mice. Urinary excretion was the main elimination route, but its contribution declines at the
10 highest exposure levels (Sabourin et al., 1987).
11
12 **4.1.5 Dermal absorption**
13 Dermal absorption of benzene can be considered in two ways. First, dermal contact may occur as a
14 consequence of direct contact of the skin with liquid benzene. Second, dermal contact may occur also as a
15 consequence of contact with benzene vapor.
16 Franz (1984) determined dermal absorption in man, monkey, and mini-pig using ^{14}C-benzene. In the
17 monkey (3/sex) and mini-pig (one of each sex), 0.5 ml was applied to the shaven back skin and allowed to flow
18 (labeled vehicle experiments showed the treated surface to be 55-75 cm^2), equal to 0.006 – 0.009 ml/cm^2. In
19 human male volunteers 0.4 ml was applied to 80 cm^2 on the ventral forearm, equal to 0.005 ml/cm^2. No occlusion
20 was used and benzene was allowed to evaporate from the application site under a ventilation hood to prevent
21 inhalation exposure. Urine, but not expired air, was collected over 2-4 days. In humans, more than 80% of the
22 total excretion in urine occurred within the first 8h. However, total excretion after direct dermal absorption was
23 0.023 ± 0.022 % of the applied dose. In monkeys and mini-pig a similar excretion pattern was observed with total
24 excretion of 0.065 ± 0.037% of the applied dose for monkeys and 0.042 ± 0.017 % for min-pig. The number for
25 monkeys was statistically different from the human value. After subcutaneous injection, it was found that 45.3%
26 of the dose was excreted in the urine. This value was used to correct the values for dermal absorption by a factor
27 of 2.2 (in order to account for excretion via other routes (e.g. bile and expired air). The dermal absorption (% of
28 applied dose) values become 0.14 ± 0.08 in monkey, 0.09 ± 0.04 in mini-pig, and 0.05 ± 0.05 in human. In vitro
29 studies performed by the same author showed values of respectively 0.19, 0.23, and 0.10% were given for
30 monkeys, min-pig, and humans respectively. However, the in vitro studies did not contain a validity check.
31 Nevertheless, it is important to note that absorption through skin is very rapid for benzene (maximal rate after 20-

40 minutes) and both the maximal penetration rate as well as the fraction absorbed increases with increasing dose because at lower dosages benzene rapidly evaporates from the skin surface.

In another set of in-vitro experiments, Blank and McAuliffe (1985) used 3 different exposure conditions in a closed system: 1) benzene was applied as liquid to the skin, 2) benzene was applied as (saturated) vapor to the skin, or 3) benzene was applied as a saturated aqueous solution. It was shown that dermal absorption from pure solution was highest followed by benzene vapor. The steady state fluxes were 2.11 ±1.08 (pure liquid benzene), 1.04 ± 0.37 (vapor), and 0.22 ± 0.05 µl x cm^{-2} x h^{-1} (aqueous solution).

Naked human volunteers were exposed to benzene vapor at 1.0 mg/L (308 ppm) in an exposure chamber while breathing clean air through a face mask. Exposure lasted 1.5 or 7h. Urinary phenol excretion was measured over 24h. Based on these measurements the authors concluded that less than 1% of benzene is taken up by the skin (Hanke et al., 1961). However, this may be an underestimation because other elimination routes than urine were not covered.

Generally, the rate of dermal absorption from liquid benzene or benzene vapor through the skin is a rapid process. Compared to absorption the inhalation route (e.g. during exposure to vapor), dermal absorption provides only a small contribution (less then 1% of the uptake through inhalation).

4.2 Biomarkers of exposure and effects of benzene

Several potential biomarkers have been proposed for exposure to benzene and its effects (Snyder and Hong, in press; ATSDR 1997). In this paragraph a brief overview is given.

Biomarkers for exposure

Exposure to benzene has been linked to various metabolites appearing in urine. One of the earliest used biomarkers was the organic:inorganic sulfate ratio in urine. Urinary inorganic sulfate levels accounting for >80% of total sulfates are considered to be normal background, 70-80% indicate some exposure, 60-70% indicate a dangerous level of exposure, while levels < 60% indicate an extremely hazardous exposure (ATSDR 1997). Urinary sulfate levels are, however, not specific for benzene and are quite variable.

Urinary phenol measurements have been used for monitoring benzene exposure and a quantitative relation with inhaled benzene concentrations has been shown (Inoue et al., 1986, 1988). However, phenol levels are influenced by diet, exposure to other aromatic compounds, and smoking. In addition, conversion of benzene to phenol may be influenced by alcohol consumption and exposure to toluene (ATSDR, 1997).

Other metabolites used as biomarker are muconic acid and phenylmercapturic acid (Snyder and Hong, 2002). But also these metabolites are influenced by smoking, dietary status and food additives, while a quantitative relation between urinary levels of these metabolites and levels of benzene exposure is not yet clear (Snyder and Hong, 2002).

Other types of biomarkers for exposure are covalent binding of reactive metabolites to proteins (e.g. hemoglobin or albumin adducts) and benzene in urine and in expired air (Snyder and Hong, 2002).

In summary, most biomarkers for exposure lack specifity for benzene and are influenced by exposure to various other chemicals from different sources.

Biomarkers for benzene effects

The most important and occupationally employed method for the biomonitoring of adverse benzene effects is the peripheral blood count. In this respect, decreases in WBC and RBC are taken as indications for benzene toxicity (ATSDR 1997). When toxicity progresses, anemia and phenomena related to bone marrow depression can be observed (Snyder and Hong, 2002).

Other biomarkers of effect include genotoxic endpoints such as chromosome aberrations, sister chromatid exchanges, and micronuclei (Zhang et al., 2002, ATSDR, 1997). In this respect, chromosome aberrations are the most relevant endpoints as discussed in section 2.4.These effects may be observed already at relatively low levels (see section 2.4).

4.3 Mechanism of Toxicity

The mechanism of benzene toxicity should be viewed separately for acute CNS effects and bone marrow toxicity. With respect to its CNS depressing effects, benzene shares many characteristics with halothane. As with many volatile organic compounds (VOCs), the concentration of benzene in the brain is probably the pivotal factor for CNS effects. According to De Jongh et al. (1998), primarily the calculated concentration in the lipid phase of the brain shows a good correlation with acute mortality data for several VOCs. Taking into account kinetics, it was shown that LC50 values for 15 compounds correlated to a concentration of 70 ± 31 mM of these compounds in brain lipids (De Jongh et al.,1998). So, most likely the concentration of benzene in the brain lipid phase determines its CNS depressing effects and – eventually – death by paralysis of the respiratory center. In fact, measurements of benzene in rabbit brain after inhalation to 20,000 ppm showed that primarily the spinal cord and the medulla oblongata (containing the respiratory center), and the pons showed relatively high tissue levels of benzene.

With respect to the hematotoxic effects of benzene a much more complicated process is responsible for the toxic effects on bone marrow cells. Various reviews have addressed this issue and the mechanism of benzene-induced hematotoxicity is still a subject of extensive research (ACGIH, 1997; ATSDR, 1997; Snyder, 2000a, 2002; Snyder and Kalf, 1994). For a detailed discussion, the reader is directed to one of these reviews. Below only a few points of attention are formulated.

The hematotoxic effects of benzene are mediated by a range of metabolites of which most are formed in the liver (primarily by CYP2E1) and transported to the bone marrow. Additionally, some metabolites are also formed within the bone marrow. The balance of myeloperoxidase and NQ01 activities determines the individual suceptibility to damage by hydroxylated metabolites of benzene.

The toxic metabolites of benzene exert its toxic effects primarily on active dividing cells. In principal, a first effect will thus be exerted on the unilineage progenitor cells (CFU-GM, CFU-E) and consequently changes in peripheral blood cells. In a later phase, effects may be observed on the pluripotent stem cells (CFU-S) (Snyder and Kalf, 1994). Therefore, the effects of benzene hematotoxicity most often observed are reversible but when benzene toxicity progresses, the effects (e.g. on the pluripotent stem cells) may become irreversible. Various pathways and mechanisms have been proposed by which benzene metabolites exert their toxic action including: decreases in bone marrow cellularity, inhibition of erythropoiesis, inhibition of spindle formation, inhibition of DNA polymerase, inhibition of topoisomerase II, changes in cytokine production, and DNA adduct formation

(Snyder and Hedli, 1996; Snyder, 2000a,b; Krewski et al., 2000).
For this series of effects there is an ill defined dose x time relationship for establishing disease. For acute exposure within 8 hours, information on the dose x time relationship for hematotoxic effects is lacking (Snyder, 2001a). However, from the available information it can be concluded that the dose x time relationship is complex, the total AUC not being a good dose metric for predicting bone marrow toxicity (Cox, 1996; ACGIH, 1997). On a short term basis, it has been shown that the same total dose spread over a few days induces a larger effect than the same amount given in a shorter time (Cronkite et al., 1989). On the other hand, an important role for the level of exposure (concentration) has been indicated. For example, exposing male CD-1 mice via inhalation to 10 ppm of benzene for 6 hr/day, 5 days/week for 10 weeks (3000 ppm-hr of exposure) has no detectable impact on marrow cellularity or on colony-forming unit, granulocyte-macrophage (CFU-GM) stem cells in bone marrow, but 100 ppm for 6 hr/day for 5 days (also 3000 ppm-hr of exposure) significantly depresses marrow colony-forming unit, spleen (CFU-S) and CFU-GM cells (Green et al., 1981). NMRI mice continuously exposed to 21 ppm of benzene via inhalation for about a week show very significantly depressed marrow cellularity (cells/tibia) and CFU-GM content per tibia, while mice exposed to up to 14 ppm for up to 8 weeks--a much larger AUC dose--show no significant changes in bone marrow cellularity or CFU-GM content (Cox, 1996). Other AUC dose violations and anomalies, such as the fact that exposure for 3 days per week may have a larger impact on erythropoiesis than exposure for 5 days per week have been documented for benzene metabolism, cytotoxicity, and genotoxicity (Cox, 1996). Also, from the occupational studies on benzene induced leukemia, indications were found for a role of temporary high (peak) exposures (see section 0). However, there is now growing evidence that the induction of leukemia by benzene does not follow the 'one-hit' theory applied for genotoxic substances. In fact, it is proposed that the clastogenic effects of benzene are not due to direct interaction with DNA but are more likely to be the consequence of protein interactions. Induction of leukemia by benzene therefore requires a cascade of reactions and therefore some form of repeated dosing (Snyder, 2002).

In conclusion, the C^n x T relationship for hematotoxicity of benzene is not adequately known, and certainly no information is available within the time periods (10 min to 8h) of the AEGL framework.

4.4 Structure-Activity Relationships

No quantitative structure activity relationships have been found for benzene. However, benzene shares many characteristics with other alkylbenzenes including toluene and xylene with respect to their CNS depressing action (see e.g. Tegeris and Balster, 1994). This effect is related to the amount of parent substance in the brain. Compared to other aromatic substances, benzene is equally or less potent then toluene, ethylbenzene, propylbenzene, or xylenes to induce behavioral changes in rats and mice (Molnar et al., 1986; 'Tegeris and Balster, 1994; Frantik et al., 1994) and substantially less potent then toluene, styrene, xylenes, monochlorobenzene, or dichlorobenzene to induce mortality in rats and mice (Bonnet et al., 1982). However, benzene is the only susbtance of this series to show hematotoxicity and bone marrow effects because of the difference in the metabolites that are produced.

4.5 Other Relevant Information

4.5.1 Interspecies Variability

With respect to interspecies variability for benzene toxicity, little information is available. For CNS depression effects, the most prominent factor is the amount of benzene reaching the brain (De Jongh et al., 1998). During inhalation, benzene is taken up rapidly into the blood and diffuses into the brain (see section 4.1.2). Therefore, the concentration in the brain is directly related to the concentration in the blood. For this type of

substances, it can be expected that CNS effects follow the rules of allometric scaling over a range of species. From this it can be concluded that small animals are more susceptible to the CNS-effects of benzene than larger animals. In the technical support document of toluene, much more data on blood levels and body loads in various species (inlcuding humans) are available showing that internal uptake of toluene is higher in small animals compared to dogs and humans. The data from Sabourin et al. (1987) on the uptake of benzene in mice and rats shows that the body load of mice is about 1.5-2 fold higher than rats up to about 900 ppm. In addition, inhibition of locomotor activity in mice seems to occur at lower external concentrations compared to rats. (see section 3.2 and 3.6). However, an important aspect for benzene toxicity is also the amount of benzene that is metabolized. Mice do metabolize benzene faster and to a greater extend than rats (see section 4.1.3). The more benzene is metabolised, the less will be present in the brain. With respect to hematotoxicity, mice are considered to be the most sensitive species (Henderson, 1996). Although a study of limited design, Thackara et al. (1979) investigated the response of circulating cells in a range of species and showed a clear response in mice (and to a lesser extend in female rats) but no clear effects in (male) rats, guinea pigs, rabbits, dogs, cats, and monkeys. This pattern of species differences is supported by Svirbely et al. (1944) on rats and dogs, White et al. (1983) and Ward et al. (1985) on mice and rats. These differences are related to differences in toxicokinetics and metabolism but do not necessarily mean that there are differences in susceptibility within the bone marrow itself.

Whether humans are more susceptible to benzene than mice, is presently not known because of the lack of appropriate human data. From metabolism studies with monkeys, it appears that these species produce less polyhydroxylated rings or muconic acid in their urine than mice which might indicate reduced sensitivity of primates (Snyder and Kalf, 1994). A tentative conclusion from Snyder and Kalf (1994) was that those species displaying the highest levels of polyhydroxylated phenolic metabolites in urine appear the more sensitive to benzene. On the other hand, the effect levels and no-effect-levels (as far as can be deduced from human occupational studies) are within the same order of magnitude for rodents and humans (see section 2.6 and 3.6).

4.5.2 Intraspecies Variability

With respect to CNS depression it is not expected that much difference exists within the human population. However, case reports on acute lethality indicate considerable variation (see for example benzene levels in Table 2). It should be noted that the differences in benzene levels found in fatal cases are influenced by concentration and duration of exposure, the level of activity during exposure, but also to factors like the time elapsed between the exposure and the measurements, and the usage of artificial respiration after the incident. Nevertheless, it has been proposed that sudden cardiac arrest may be a factor of importance explaining the large variation in exposure and mortality (Bass, 1970; Litovitz, 1988). However, no quantitative information is available on any range of variation for this endpoint.

In general it has been stated that the uptake and systemic exposure benzene and its metabolites is related to the body fat content. In this respect, women are thought to be more susceptible because they have a higher body fat content in general (Nomiyama and Nomiyama, 1974b; Sato et al., 1975). However, from metabolism studies, it cannot be concluded that this difference between man and women is substantially larger than what could be expected based on the overall individual variability.

With regard to the hematotoxic action of benzene, a wide variation in response has been observed in the human population. Sensitivity to benzene may result from a series of polymorphisms in enzymes which modulate the production of toxic metabolitesm (Snyder, 2000). From the review of Snyder (2000), the following table is taken.

1
2 **Table 18. Factors that determine susceptibility to benzene (hemato)toxicity in humans**

Factors leading to sensitivity	Factors leading to resistance
High CYP2E1 activity (increased production of toxic metabolites)	Low CYP2E1 (decreased production of toxic metabolites)
Low GSH transferase	High GSH transferase
Low marrow NQ01	High NQ01
High marrow myeloperoxidase	Low marrow myeloperoxidase

3
4 There are no data that provides a clear picture of the occurence of these susceptibility factors and their
5 combinations in the human population. Nevertheless it should be noted that these factors play a role primarily in
6 chronic toxicity and their relevance for setting AEGLs is probably less relevant.
7
8 **4.5.3 Skin Irritation and Sensitization**
9 Benzene has been identified as a slight to moderate skin irritant in experimental animals, primarily due to
10 its defattening properties (BUA 1988, EU 2002). Undiluted benzene applied 10 to 20 times to the ears or shaven
11 abdomen of rabbits (under (semi)occlusion) in a 2-4 week period (no further details provided) induced slight to
12 moderate erythema, edema, and superficial necrosis. The latter was characterized by exfoliation of skin patches
13 (Wolf et al., 1956). Administration of 2 drops of undiluted benzene into the rabbit eye, resulted in moderate
14 conjunctival irritation (inflammation and swelling of eyelids) and slight transient corneal injury (Wolf et al.,
15 1956).
16
17 High concentrations of benzene vapors (not defined) are irritating to the mucous membranes of the eyes,
18 nose, and respiratory tract in humans (Gerarde, 1960). In a case report on fatal exposure to benzene fumes, Avis
19 and Hutton (1993) described second degree skin burns on the face, trunk and limbs of 3 victims. Autopsy
20 revealed hemorrhagic airless lungs with alveolar hemorrhage and pulmonary edema. No data on exposure levels
21 were provided but exposure was a few minutes.
22
23 Reports on skin sensitization were not available. Based on a long history of benzene usage and
24 occupational exposure, there are no indications that benzene would be a skin or respiratory sensitiser (BUA, 1988;
25 EHC 1993, EU 2002).
26
27 **4.6 Ambient Concentrations and other sources of exposure**
28
29 The use of benzene is widespread and it is ubiquitous in the environment. In this section, a brief overview
30 of ambient concentrations of benzene is provided as well as some exposure due to specific activities.
31
32 Benzene is found throughout the world as a consequence of natural sources (vegetation, wood-fires,
33 vulcanic activities). However, most benzene in the environment originates from human activities. As a
34 consequence, levels in urbanized areas are higher than those in rural areas. In various cities throughout the world
35 the following levels have been reported over the last two decades: Canadian cities 6 – 98 ppb, U.S. cities 0.4 –
36 112 ppb, German cities 2 – 206 ppb, Dutch cities 1 – 57 ppb, London 48 ppb on average with a maximal level of
37 185 ppb (Brief et al., 1980; Slooff et al., 1987; EHC, 1993; ATSDR 1997). In rural and remote areas, average
38 levels are about 4 – 10 times lower.
39

Specific sites or activities associated with relatively high background exposures to benzene are smoking, travelling by' car, handling gasoline, or living near industrial or waste sites. Smoking is probably the most important source of exposure for benzene for smokers. Cigarette smoke may contain 46 – 77 ppb benzene (Slooff et al., 1987) while one cigarette may deliver 10 – 480 µg benzene (average about 55 µg) (Slooff et al., 1987; ATSDR 1997). Homes of smokers contain 3.3 ppb on average compared to 2.2 ppb in homes of non-smokers (ATSDR, 1997). In a smoke-filled bar levels of 8-11 ppb have been determined (ATSDR, 1997).

Because benzene is a constituent of gasoline and car exhaust, driving a car may result in increased benzene exposure. ATSDR reports 12.5 ppb in a moving car. RIVM has reported a mean level of 20 ppb inside cars with a maximum of 832 ppb. Measurements on highways give about 8 ppb while car driving in German cities was stated to result in levels of 16 – 172 ppb (Slooff et al., 1987). In garages, levels up to 60 ppb have been measured (ATSDR, 1997)

During handling of gasolines and related products, transient exposure to peaks of benzene may occur. At gasoline stations levels up to 3 ppm may occur (Brief et al., 1980, Slooff et al., 1987; ATSDR, 1997). "Open" loading or transfer of benzene-containing products may be associated with peaks levels of about 52 ppm. Levels for personnel working on tank-lorries may range from 0.01 to 30 ppm, for people working on tank-ships 0.7 – 52 ppm (Slooff et al., 1987). Around waste sites or near chemical facilities levels up to 250 ppb have been reported (Brief et al., 1980).

It should be noted that environmental benzene levels in the US and Europe show a gradual decrease over the last decade due to various policy regulations (e.g. decreased emissions, decreased content in gasolines, use of catalytic converters on automobile exhaust). In California, a statewide decline of about 49% was observed over the years 1990 to 1995. On average levels decreased from 2.7 to 1.3 ppb, with maximum values of 5.4 ppb in 1990 and 2.6 in 1995 (in Burbank) (Hammond, undated). In the Netherlands, a similar trend was observed. Benzene levels near streets (measurements providing the highest levels) decreased gradually from 1.5 ppb in 1990 to about 0.6 ppb in 2000. A similar downward trend was found for rural areas and industrial areas (Hammingh, 2000).

5 RATIONALE AND PROPOSED AEGL-1

5.1 Human Data Relevant to AEGL-1

Two types of effects induced by benzene in humans are relevant to AEGL-1: 1) discomfort as a consequence of slight CNS depression and 2) skin, eye, and airway irritation. The primary human data concerning CNS effects is based primarily on historic literature and estimations. CNS effects are claimed to occur above 250 ppm (Fishbein, 1984). Gerarde (1960) estimates that 8h exposure to 25 ppm does not induce any effects while 5h at 50-150 ppm induces headache, lassitude and weariness (without reference to primary data).

Srbova et al. (1950) reports no subjective symptoms in volunteers exposed to 110 ppm for 2h (inhalation by facemask or mouthpiece, no exposure of skin and eyes). The number of individuals exposed at this level was not reported but in total 23 volunteers were involved in the study.

Occupational (repeated) exposure shows changes in EEG patterns at personal exposure levels of 45-154 ppm (with peaks to 308 ppm, 1-2h averages) (Kellerova, 1985) or dizziness and headache at levels of 1- 40 ppm

(Yin et al., 1987, peak levels unknown). CNS symptoms and irritation were also reported in sewage workers. During the occurrence of unusual odors limited area sampling showed benzene levels between 30-300 ppm (Kraut et al., 1988). However, in these occupational studies, signs and symptoms were not coupled directly to individual exposure levels, co-exposure to other substances cannot be excluded, whereas the influence of peak exposures cannot be ruled out.

With respect to skin and eye irritation limited human information is available. Acute exposure to very high levels can induce skin burns (Avis and Hutton, 1993). Airway irritation was reported in 3 individuals exposed to 3400-4900 ppm for 5-15 minutes (Lehmann, 1910). Degassing operators exposed to benzene for 1 day to 3 weeks (exposure > 60 ppm; limited GC measurement indicates that initial levels could have been about 653 ppm as indicated by measurement in a similar tank) showed eye, skin, nose and throat irritation (Midzenski et al., 1992). Occupational repeated exposure of seawage workers (with peaks of benzene exposure between 30 and 300 ppm) was associated with a distinct odor and eye irritation in 3 out of 19 workers (Kraut et al., 1988). Signs of upper airway irritation were also observed in Chinese workers exposed to an average level of 1-40 ppm (Yin et al., 1987, peak levels unknown). In a recent spill of 'benzene heartcut' (40-60% benzene, 40-60% naphta) in the UK, workman noticed a 'benzene-like' odor and reported a burning sensation in the nose and skin irritation. Limited measurements indicated 50 ppm benzene in open air and 100 ppm indoors (A Keddie, UK/HSE, personal comm., no details provided).

5.2 Animal Data Relevant to AEGL-1

Benzene is, similar to toluene, not a sensory irritant in the RD50 test. In fact, an increase in respiration rate was observed up to levels of 8300 ppm for 30 min (Nielsen and Alarie, 1982). However, local airway irritation was observed in rats exposed to 40,000 ppm for 25-35 min (Furnas and Hine, 1958). In addition, Von Oettingen (1940) reported irritation of mucous membranes in rats at exposures of 1000-2440 ppm (duration unknown) and eye irritation in rabbits exposed to 12,000 ppm for 1h. Other animal studies related to behavioral changes or narcotic effects are not considered relevant for AEGL-1 development.

5.3 Derivation of AEGL-1

The available animal data are not considered to be adequate for AEGL-1 development because they do not provide a treshold level. The most controlled human study representing exposure within the AEGL time frames is Srbova et al. (1950) reporting no subjective symptoms during exposure to 110 ppm for 2h. However, no skin and eye exposure was involved in this study and clinical symptoms were not systematically investigated. Repeated occupational exposure was associated with irritation and CNS symptoms at time weighted average concentration of 1-40 ppm and above (Yin et al., 1987, Kraut et al., 1988) and nose and skin irritation were reported during a benzene-hearcut spill in the UK at 50 (open air) or 100 ppm (indoors) (only two limited measurements). However, in all these cases co-exposure to other substances cannot be excluded whereas the contribution of peak level is unknown. Airway irritation during benzene exposure was reported after 5-15 minutes at 3400-4900 ppm in 3 humans.

Taken all together, it is expected that mild CNS effects will be the first noticeable effects of benzene exposure and that irritation occurs only at higher exposures or are due to co-exposure to other substances. Therefore, the AEGL-1 values should be based on mild CNS effects. Also for toluene, mild CNS-effects were selected as the basis for the AEGL-1 values.

1 Although CNS effects of benzene are already known for over 100 years, very little is known about the
2 time-concentration-effect relationship of slight CNS effects due to benzene exposure in humans, and no
3 quantitative point of departure is available for a solid PBPK modeling. Although the study by Srbova et al. (1950)
4 has some weaknesses (no details on all individual exposures and time durations, the lack of symptoms was
5 reported by a single remark, no active investigation of health effects), the 110 ppm level for 2h is taken as a
6 NOEL for CNS effects. This is the starting point for AEGL-1 development. This NOEL appears to be supported
7 by the fact that a substantial number of volunteers was exposed in a controlled or occupational setting to levels of
8 1 – 76 ppm for 7h TWA (Inoue et al., 1986; 64 men, 88 women, occupational), 32 ppm ± 25 ppm for 8h TWA
9 (Inoue et al., 1988; 65 workers, occupational) or 19-125 ppm for 6-8h (Hunter and Blair, 1972; male laboratory
10 staff, controlled exp.) although none of these studies actually included statements on health effects. Because CNS
11 effects are the consequence of systemic benzene exposure, time extrapolation should be applied. Based on the
12 experiments described by Von Oettingen (1940) with cats, a n-value of 1 was identified for light and deep
13 narcosis in cats (see section 6.2). Although these data from cats are from an old experiment and details about the
14 experiment are lacking, the extensive number of concentrations and time points reported provides sufficient
15 confidence in the results indicating that using N=3 for extrapolation to short time periods is too conservative.
16 Therefore, time extrapolation is performed using N=1 and N=2 for longer and shorter time periods respectively.
17 Because human data are used, the interspecies uncertainty factor is 1. The intraspecies uncertainty factor is 3
18 because it has been found from experience with anesthetic gases that CNS depression does no vary by more than a
19 factor 2-3 between groups in the population. This results in the following AEGL-1 values.
20

21 **Table 19 AEGL-1 values for benzene**

AEGL-1 VALUES FOR BENZENE in ppm (mg/m^3)					
AEGL Level	**10 minutes**	**30 minutes**	**1 hour**	**4 hours**	**8 hours**
AEGL-1	130 (420)	73 (240)	52 (170)	18 (58)	9.0 (29)

22
23

24 # 6 RATIONALE AND PROPOSED AEGL-2

25

26 ## 6.1 Human Data Relevant to AEGL-2

27

28 For AEGL-2 development, five types of toxicity endpoints may be relevant: 1) CNS effects, 2)
29 hematotoxicity, 3) carcinogenicity, 4) developmental toxicity, 5) genotoxicity. For all these types of endpoints,
30 relevant data are discussed.
31

32 *CNS effects*
33 CNS depression is generally viewed as the most obvious effect of acute benzene exposure. It should be
34 noted that CNS effects of benzene – similar to various other VOC's – first starts with excitement or euphoria and
35 when concentrations rise or exposure prolongs, signs of CNS depression and narcosis develop. The latter type of
36 effect is primarily considered relevant for AEGL-2 development. Fishbein (1984) states in his review that signs of
37 CNS depression occur at levels above 250 ppm. Gerarde (1960) estimates that 50-150 ppm for 5h results in

headache, lassitude, and weariness, 500 ppm for 1h produces symptoms of illness while 1500 ppm for 1h results in serious symptoms. However, no references to primary data were given.

Lehmann (1910) reported excitement and dizziness in 3 volunteers at 3400-4900 ppm for 5-15 minutes. Degassing operators working in confined tanks for 1 day to 3 weeks (2.5-8h/day) showed signs of CNS effects (dizziness, nausea, headache, drowsiness). Exposure levels measured after several days showed levels > 60 ppm (limited GC measurements indicate that the initial levels could have been about 653 ppm maximum as determined in a similar tank). Because exposure was measured only after several days while work continued it must be assumed that these workers had no actual impairment of escape (Midzenski et al., 1992). In a case study by Drozd and Bockowski (1967), 4 workers were exposed intermittently (periods of 2-3h) to benzene levels in a confined space. One of these workers developed CNS symptoms after 2 days. Levels measured with the door closed in a simulation experiment showed values of 600-1500 ppm. This condition is at least representative for the first two hours of exposure because these men performed work with the door closed. During this period no impairment of escape is anticipated. See section 2.2.2. for details on these studies.

In general, area concentrations in workplaces in the past were mostly within the range of 10 to about 500 ppm on a routine basis. However, also area levels of 1000 – 1500 ppm were observed. The massive amount of data that fit the general picture (including the recent data summarised by Wong (2002)) provides sufficient confidence in the exposure ranges. Unfortunately, acute occuring health effects were almost never reported and at least not coupled to exposure data on the individual level. Most studies focussed on hematological changes and carcinogenicity when studying health effects. As described by Wong (2002), benzene intoxication is substantial after prolonged exposure in these conditions (intoxication rates of 25-38% of the workers have been reported). However, one must assume from the large extent of people involved in these occupational exposures, and the fact that these exposure levels occurred on a regular basis that those condition were not severely adverse on an acute scale and will certainly not have resulted in an impaired ability to escape, although the occurrence of some health effects such as headache, dizziness, and irritation cannot be excluded at these levels.

Occupational repeated exposures showed CNS effects at levels over a wide range of 30 ppm to 1800 ppm (Greenburg, 1926b; Yin et al., 1987; Kraut et al., 1988). Signs quickly recovered when workers were removed from the exposure areas. However, from the description of these studies no indications are present to indicate acute impairment of work performance or any inability to escape.

Hematotoxicity

Hematotoxicity is the most obvious effect of repeated benzene exposure, often related to occupational settings. There are no specific human data providing an effect or no-effect level for hematotoxic effects due to acute benzene exposure. Workers exposed to benzene vapors in fuel tanks for 1 day up to 3 weeks (exposure > 60 ppm; max. probably 653 ppm as indicated by measurement in a similar tank), showed no consistent hematological abnormalities (Midzenski et al., 1992). No further data for acute exposures are available.

Carcinogenicity

Benzene is an established human carcinogen and there is sufficient evidence to assume a causal relationship between benzene exposure and (acute) non-lymphatic leukemia (ANLL) or acute myeloplastic leukemia (AML). Carcinogenicity will however not be used as basis for developing AEGL-2 values.

First, the Standing Operating Procedures for AEGL development states that at present AEGL values based on carcinogenicity are not developed because of reasons explained in the SOP (NRC, 2001).

Second, there are scientific reasons for not developing AEGL-values for benzene based on carcinogenicity. Various publications have shown that the dose-response-relationship for benzene and leukemia is non-linear. This holds especially for the low dose range for which it has been claimed that the leukemia risk follows a sub-linear relation (see section 2.5). This agrees with the fact that growing evidence might indicate that benzene-induced leukemia does not follow the rules of the 'one-hit' theory but rather requires some cascade of events that are not characterised by direct DNA interaction (Snyder and Hong, 2002). The latter may require some form of repeated exposure rather than a single peak exposure. Some observations from epidemiological studies reporting that 'peak' exposure or 'high level' exposure may render a relatively higher risk than low dose chronic exposure should be placed in this perspective. In these studies 'peak exposure' is rather defined relative to general long-term exposure levels of < 10 ppm and refers to (incidental) additional peak exposures of about 30 to 100 ppm. The results of Green et al. (1981) support this view since exposure of mice to 10 ppm (6h/day, 10 weeks) induced no major hematotoxicity whereas 100 ppm (6h/day) for only 5 days (cumulative dose is equal) did.

However, single peak exposures in context of the AEGL framework may actually refer to levels above 500 or even above 1000 ppm. For such exposure other aspects are important. A major factor is saturation of the metabolising capacity for benzene since metabolites of benzene are responsible for the induction of leukemia. In rodents, metabolism becomes saturated at exposure levels above a few hundreds of ppm. This implicates that with exposure to higher levels of benzene vapor the generation of toxic metabolites increases less than proportionally (see section 4.1.3). This is supported by observations of Cronkite et al. (1989) showing that a 2 day exposure to 3000 ppm in mice induced less hematotoxicity then a 19 day exposure to 316 ppm (cumulative dose is equal). In fact, model calculations for leukemia risk based on the exposure concentration as dose metric (presented in the most recent TLV update document (ACGIH, 1997)) indicates that the risk increases substantial between 20 ppm and about 120 ppm but does not increase much further up to levels of 260 ppm. Taken all information together, dose-response extrapolation from the epidemiological data of e.g. the Pliofilm cohort to either low or substantially high exposure does not follow linear relationships. Therefore validity of the standard linear calculation of the cancer risk for exposures of less then 24 hours – over dose and time – for benzene is very uncertain. The values generated in Appendix D should be viewed with great care.

Developmental toxicity

Benzene exposure has been suggested to be associated with menstrual disorders and decreased female fertility. However, these studies all suffered from major shortcomings. No association was found between spontaneous abortions and parental benzene exposure (ATSDR, 1997, Stücker et al., 1994). Another series of studies has investigated a possible relation between parental exposure and childhood leukemia. Although these studies may provide some indications for a possible role of parental benzene exposure, the pattern that emerges from these studies is inconsistent and does define any associated exposure levels. These studies do not allow any definitive conclusions on the effects of parental exposure to benzene and possible childhood leukemia. Nevertheless, it remains questionable whether the exposures involved are really relevant for setting AEGL values since some form of repeated exposure might be necessary.

Genotoxicity

Benzene is a clastogenic agent that induces CA's, SCE's, and MN's (Zhang et al., 2002). Only CA's are considered to be a relevant cytogenetic endpoint for predicting future leukemia risks. However, benzene does not induce a specific pattern of CA's but may result in genomic instability by causing recombination, double strand breaks and mitotic spindle disruption (Zhang et al., 2002). Only one study investigates the occurrence of CA and SCE in workers exposed to a single peak of benzene vapor (brief exposure due to spillage, concentration and duration not defined). CA were not increased. In occupational studies involving repeated low dose exposure, CA

occur already at low exposure levels at around 2 ppm (Sarto et al., 1984; Picciano 1979; Zhang et al., 1996). It should be noted that part of the benzene-induced aberrations are transient (see animal data). Furthermore, there is no quantitative relation available between the induction of aberrations and future leukemia risks. CA should be considered as markers for future risks only. Therefore, genotoxicity is not a valid endpoint for setting AEGL-2 values for benzene.

6.2 Animal Data Relevant to AEGL-2

For AEGL-2 development, four types of toxicity endpoints may be relevant: 1) CNS effects, 2) hematotoxicity, 3) developmental toxicity, 4) genotoxicity. For all these types of endpoints, relevant data are discussed. Carcinogenicity data in animals will be taken into account since rodents do not develop leukemia like humans.

CNS effects

CNS effects with a relation to 'impairment of escape' are difficult to establish from animal experiments because of the interpretation and extrapolation of the endpoints studied in animals to human abilities. Exposure of rabbits and cats to 7700 ppm induced light narcosis after 50 min, deep narcosis was not reached within 4h (Estler, 1935). Von Oettingen (1940) showed time-concentration-response curves for light and deep narcosis in cats. Because time frames of 3 min – 7 hours were used, these curves are relevant to AEGL setting. The curves show that CNS depression can be described using n=1 in the formula C^n x T = constant.

Molnar et al. (1986) showed an increase in activity in rats at low concentrations and a decrease in activity at higher levels. At 1500 ppm for 4h or at 2000 ppm for 2h, no activity changes were observed. However, a decrease in locomotor activity was only seen at the highest dose 5940 ppm for 4h with an increase in activity (with some incoordination and tremor) at 4000 ppm for 4h (Molnar et al., 1986). Frantik et al. (1994) calculated a 30% response levels for neurological endpoints that would not impair normal locomotion. The 30%-response level was 929 ppm in rats (4h exposure) and 856 ppm in mice (2h exposure). Exposure of mice to 5020 ppm for 8h does induce reversible prostration with muscle twitching (Uyeki et al., 1977). Loss of equilibrium and hindlimb ataxia was observed in mice during a 6h exposure at 10,000 ppm but at 5000 ppm only hyperactivity was observed (Estler, 1935). Evans et al. (1981) showed hyperactivity at 300 and 900 ppm exposure for 6h/day (5 days) but also some signs of light narcosis at 900 ppm. After a single exposure of 6h to 1000 or 3000 ppm, hindlimb grip strength was decreased by 10% in mice, no effect was observed at 300 ppm (Dempster et al., 1984). Tegeris and Balster (1994) showed a significant decrease in locomotor activity in mice exposed to 4000 and 8000 ppm for 20 min, but not at 2000 ppm. In contrast, Li et al (1992) showed behavioral changes in grip strength and avoidance responses of mice at 12.52 ppm (2h/day for 30 days). However, this study has major shortcomings.

Hematotoxicity

As explained in previous sections of this document. With respect to hematotoxicity, a clear separation should made between effects on circulating cells (e.g. WBC, RBC), effects on unilineage progenitor cells (e.g. CFU-E, CFU-GM), and effects on mulitlineage stem cells (CFU-S). The first two effects are in principle reversible but the latter is not. In repeated dose animal experiments, changes in circulating cells are generally observed at 100 ppm and above (6h/day exposures) in various species but primarily in mice. Levels that do not induce any changes in circulating cells are 10 – 25 ppm in mice (Green et al., 1981a, 1981b; Cronkite et al., 1989; Farris et al., 1997), 30 ppm in rats (Ward et al., 1985), or 20 ppm in pigs (Johnston et al., 1979). Dempster et al. (1984), showed effects in circulating cells in mice after a single 6h exposure at 1000 or 3000 ppm but not at 100 ppm. A single 8h exposure at 5020 ppm reduced the number of colony forming cells in the femur of mice. After 3

x 8h exposure, a significant effect was shown on CFU-S, the pluripotent stem cell (Uyeki et al., 1977). After 5 day exposure, changes in CFU-S in mice were observed at 103 ppm or 400 ppm but not at 9.9 ppm (Green et al., 1981a, 1981b; Cronkite et al., 1989). Changes in unilineage progenitor cells such as CFU-GM and CFU-E were observed at 100 ppm in repeated exposures but not at 400 ppm for 1 or 4 days (Cronkite et al., 1989; Farris et al., 1997). There is some evidence from dosimetric studies that exposure to benzene over a number of days produces more severe hematotoxic effects than the same dose applied during one or two days (Dempster et al., 1984; Cronkite et al., 1989) indicating that hematotoxicity and bone marrow effects are less likely to occur after a single exposure. In this respect, it should be noted that the hematotoxic effects of benzene are induced by various benzene metabolites rather than the parent compound itself and that metabolism becomes saturated at exposure above a few hundred ppm.

Developmental effects

Although some variation exist between studies (6 or 7h exposures per day), benzene is not considered to induce structural irreversible effects. However, benzene consistently shows forms of developmental retardation in rats, i.e. decreased fetal body weight and length, delayed ossification, and skeletal variants (Green et al., 1978; Kuna and Kapp, 1981; Coate et al., 1984; Kuna et al., 1992). NOAELs for embryo/fetotoxicity were < 100 ppm or 300 ppm, 10 ppm, 40 ppm, and 10 ppm. The developmental study of Tatrai et al. (1980) does not comply with this general pattern showing maternal mortality, total resorptions and dead fetuses at levels of 150 ppm and above (24h exposure/day). Taken all information together, the developmental effects of benzene are quite similar to those of toluene. The pattern of effects shares characteristics of the so-called "fetal alcohol syndrome" which is induced primarily after chronic exposure (see also the technical support document of toluene).

In mice, two studies by Keller and Snyder investigated the effect of exposure in utero on hematological parameters in fetuses, 2-day neonates, and 6 week young adults. In these studies no effects on litter parameters, resorptions, or numbers of dead or malformed fetuses were found. The indicate that exposure of mice in utero at levels of 5, 10 or 20 ppm (gestation day 6-15) may induce effects on the hematopoietic system. The effects appear partly reversible and partly prolonged until 6 weeks of age. The effects also show differential effects for the three age groups. However, maternal (bone marrow) toxicity was not investigated so that no relation between the fetal effects and maternal toxicity could be evaluated. Because mice – and especially Swiss Webster mice used in this studies (Neun et al., 1992) – are very sensitive to the hematotoxic effects of benzene, the relevance of these observations for humans remains unclear.

Genotoxicity

Acute inhalation experiments show the induction of micronuclei, sister chromatid exchanges, and chromosome aberrations. MN were increased in mice at 1000 and 3500 ppm for 0.5-1h, and ≥ 10 ppm for 6h, and in rats at ≥ 1 ppm for 6h (Ranaldi et al., 1998; Erexson et al., 1986). SCE were increased in mice at 600 ppm for 6h and 3130 ppm for 4h, and in rats at ≥ 3 ppm for 6h (Tice et al., 1980; Erexson et al., 1986). Chromosome aberrations, the most meaningful endpoint for benzene toxicity (Zhang et al., 2002), were increased in rats at ≥ 100 ppm for 6h (not at 10 ppm) but no increase in CA was observed in mice exposed to 3130 ppm for 4h (Tice et al., 1980; Styles and Richardson, 1984). Repeated inhalation experiments showed generally a similar patterns with approximately the same effect concentrations. However, it was also shown in non-inhalation studies that part of the induced aberration were in fact reversible, showing a smaller percentage of aberrant cells after a few days (e.g. Ciranni et al., 1991; Fujie et al., 1992). For reasons provided above, CA should be considered as markers for future risks only. Therefore, genotoxicity is not a valid endpoint for setting AEGL-2 values for benzene.

1 **6.3 Derivation of AEGL-2**
2
3 The AEGL-2 values will not be based on the risk of leukemia as explained in section 6.1. In addition,
4 hematotoxicity will not be used for setting the AEGL-2 values. With respect to hematotoxicity, the first effects
5 noted will be reduced numbers of circulating cells and possibly unilineage progenitor cells. However, these effects
6 are in principle reversible although recovery may require some time. As such, these effects are not the prefered
7 AEGL-2 endpoints. Effects on the pluripotent stem cell, however, are serious and irreversible. Unfortunately, no
8 data are available showing effects on the pluripotent stem cell after a single dose. The most important data are
9 from Uyeki et al. (1974) showing reductions in pluripotent stem cells after 3 x 8 hour exposures at 5020 ppm over
10 three days. In general, it can be observed that major hematotoxicity develops after a couple of days. In addition,
11 Cronkite et al. (1989) showed that a two day exposure to 3000 ppm (6h/day) showed less hematotoxicity than a
12 19 day exposure at 316 ppm (6h/day) while recevory was faster in the 3000 ppm group. This indicates that some
13 form a repeated exposure is an important determinant. It is expected that knocking out a substantial number of
14 pluripotent stem cells requires a couple of thousand ppm in a single dose (Uyeki et al., 1974). In this respect, CNS
15 effects are expected to occur before major effects on the pluripotent stem cell will be induced.
16
17 The most prominent effect of acute benzene, therefore, is exposure is CNS depression. Because this is a
18 continuum from very slight dizziness to narcosis, the level that impairs escape should be identified for AEGL-2
19 derivation. There are no adequate dose-response studies available for humans, only estimations and indications.
20 Therefore, animal data will be used as the point of departure. Increased activity is not considered an AEGL-2
21 endpoint but clear decreases in neurobehavioral function are an AEGL-2 endpoint. In mice, 8h exposure at 5020
22 ppm induced reversible prostration. Molnar et al. (1986) showed increased locomotor activity in rats at 4000 ppm
23 for 4h and decreased activity at 5940 ppm. A single exposure of 6h of 1000 ppm induced a 10% decrease in grip
24 strength, no effect was observed at 300 ppm. Frantik et al. (1994) calculated a 30% neurological response level at
25 929 ppm in rats (4h exp) and 856 ppm in mice (2h exp). The effects observed by Frantik et al (1994) in rats and
26 mice and Dempster et al. (1984) in mice are considered to be below an AEGL-2 endpoint. Therefore, the highest
27 level showing no AEGL-2 effect, is 4000 ppm for 4h in rats (Molnar et al., 1986).
28
29 Based on the experiments described by Von Oettingen, time extrapolation is performed using n=1 and
30 N=2 for longer and shorter time periods respectively (see explanation in AEGL-1 section). An interspecies factor
31 of 3 is used because CNS depression due to benzene exposure does not appear to vary much between species. In
32 addition, the use of a larger interspecies factor would provide AEGL-2 values that do not comply with the
33 available human information (see section 6.1). Moreover, with respect to CNS depression in animals, benzene is
34 less or equipotent to other alkylbenzenes and toluene in particular (Molnar et al., 1986; Tegeris and Balster, 1994,
35 Frantik et al., 1994) which means that the AEGL-2 values for benzene should end up within the same order of
36 magnitude than the CNS-based AEGL-2 values for toluene. The interim AEGL-2 values for toluene are 990, 570,
37 510, 510, and 510 ppm for the 10 min, 30 min, 1 hour, 4 hour, and 8 hour period respectively. Proposed AEGL-2
38 levels for xylenes are 990, 480, 430, 430, and 430 ppm for the 10 min, 30 min, 1 hour, 4 hour, and 8 hour period
39 respectively. In contrast, to toluene and xylene which reach steady status in the blood within 2 or 4 hours, benzene
40 does not reach a steady state in blood in tissues before 4 hour. Therefore, for benzene time extrapolation should
41 continue over the whole AEGL time frame.
42
43 An uncertainty factor of 3 is used for intraspecies variation because it has been found from experience
44 with anesthetic gases that CNS depression does no vary by more than a factor 2-3 between groups in the
45 population. The total uncertainty factor is therefore 10. This provides the following AEGL-2 values.
46

TABLE 8: AEGL-2 VALUES FOR BENZENE in ppm (mg/m^3)					
AEGL Level	**10 minutes**	**30 minutes**	**1 hour**	**4 hours**	**8 hours**
AEGL-2	2000* (6500)	1100 (3600)	800 (2600)	400 (1300)	200 (650)

* The AEGL-2 value is higher than 10% of the lower explosive limit of propane in air (LEL = 1.4 % (14,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

7 RATIONALE AND PROPOSED AEGL-3

7.1 Human Data Relevant to AEGL-3

Death due to benzene exposure is probably caused either by severe CNS depression resulting in paralysis of the respiratory center. Some reports have suggested sudden cardiac arrest due to cardiac sensitization as a cause of lethality. However, there are no human studies providing quantitative concentration-time-mortality relationships or providing quantitative exposure information on cardiac sensitization.

From historic literature some indications are present for lethal or near-lethal levels. These indications and estimations are probably based on the combination of findings in old animal experiments and early experiences with human exposures. Flury (1928) states that 20,000 ppm for 5-10 min would be lethal and 7500 ppm for 30-60 min is dangerous. The same values are reported by Gerarde (1960) but no reference to primary data was given.

In addition, some limited studies provide evidence for non-lethal exposure levels in humans. Lehman (1910) exposed 3 volunteers to levels of 3400-4900 ppm for 5-15 min reporting only excitement and dizziness. In addition, the studies of Drozd and Bockowski (1967) and Midzenski et al. (1992) provide some indications for non-lethal exposure levels. These studies are described thoroughly in section 2.2.2 along with their caveats and methodological considerations and are not described here again because they are not quantitatively used in the derivation of AEGL-3 values. In occupational settings, benzene levels have been reported up to about 500 ppm (both personal and area sampling). Area levels between 1000-1500 ppm have been reported in some instances also. In historic studies by Greenbrug (1926b, 1939) *mean* area levels of 70 up to 1800 ppm were determined in workplaces by 20 minutes stationary air sampling. Maximal area levels measured ranged from 110 to as high as 4140 ppm without any report on acute lethality (Greenburg 1926b, Greenburg 1939). These data are supported by recent exposure levels from factories in China (Wong, 2002). See section 2.2.3 for detailed discussion.

7.2 Animal Data Relevant to AEGL-3

Only few adequate animal studies on acute lethality are available. For rats, a 4h LC50 of 13700 ppm and a 6h LC50 of 9536 ppm were reported (Drew and Fouts, 1974; Bonnet et al., 1982). In addition, 16000 ppm for 4h produced 66% (4/6) mortality (Smyth et al., 1962). In mice, a 6h LC50 of 14122 ppm and a 7h LC50 of 9980 ppm were reported (Bonnet et al., 1982; Svirbely et al., 1943). From the mortality rates in Bonnet et al. (1982) and Svirbely et al. (1943) it can be concluded that the dose-response curve is very steep: within a concentration range of about 3-fold, the mortality rate increases from 0% to 100%. Svirbely et al. (1943) using 18 animals per

1 dose, found no mortality at 4890 ppm for 7h. In addition, the study by Svirbely et al. (1943) showed that delayed
2 mortality after exposure is not an important factor for benzene induced mortality. Therefore, also studies with a
3 limited or absent observation period after the exposure provide relevant data on (the lack of) mortality.
4
5 Other studies reporting exposure levels without mortality are summarized below.
6
7 **Table 20 Summary of benzene exposure without mortality in animals**

Duration	Exposure level without mortality (ppm)	Species	Ref
15 min	7332-8224	rat	Magos et al. 1990
30 min	8300	mice	Nielsen and Alarie, 1982
2 h	10,000	rat	Furnas and Hine, 1958
3h	20,000	rabbit	Kujime, 1990 (abstract)
4 h	5940	rat	Molnar et al., 1986
6 h	3000	mice	Dempster et al., 1984
6 h	7700	Rabbit + cat	Estler, 1935
6.5 h (17 days)	2000	Rat + mice	Coate, 1983
7 h	4890	mice	Svirbely et al., 1943
8 h	5020	mice	Uyeki et al., 1977

8
9 The highest reliable NOEL for mortality is 5940 ppm for 4h in rats (Molnar et al., 1986) which is
10 supported by Uyeki et al. (1977) and Svirbely et al. (1943) for mice.
11
12 Animal studies that study benzene-induced cardiac irregularities had no clear descriptions of the exposure
13 levels but from the description of the experimental set-ups one may assume very high vapor levels (Nahum and
14 Hoff, 1934; Morvai et al., 1976; Tripathi and Thomas 1986). In dogs, a 5% vapor (50,000 ppm) was used to study
15 benzene induced cardiac irregularities. In the study of Magos et al. (1990), benzene exposure at 7332-8224 ppm
16 for 15 min did not induce changes in cardiac function itself but an exacerbation of effects was seen when
17 experimental ischemia or a potassium channel blocker were introduced.
18
19 **7.3 Derivation of AEGL-3**
20
21 There are no reliable quantitative human data available for setting an AEGL-3. The limited human data,
22 however, can be used as supporting background information. Only few adequate LC50 studies are available in
23 animals and the available studies do not allow the derivation of a substance specific value for the factor of n.
24
25 The observed NOEL for mortality of 5940 ppm for a 4h exposure rats (Molnar et al., 1986) is taken as
26 starting point AEGL-3 development. Because the mortality of benzene is caused by severe CNS depression
27 (paralysis of the respiratory center), this effect is correlated to the benzene level in the brain lipid fraction (De
28 Jongh et al., 1998). This concentration will be related directly to a build-up of benzene in the tissue, which is
29 directly related to the inhalation rate (see e.g. the data from Sabouring et al., 1987). Therefore, it is expected that
30 humans require higher external concentrations compared to rodents, to obtain a similar level of benzene in the
31 blood or brain as is observed also for other VOC's (trichloroethylene, toluene). In the technical support document
32 for toluene, kinetic information that supports this view is available for various species (including humans). For
33 this reason, an interspecies uncertainty factor of 1 is used. The use of a higher uncertainty factor for interspecies

variation, would actually result in conservative AEGL-3 values which are too low compared to the – although limited – human experiences. In addition, values would not match with those of toluene since benzene is less or about equipotent with regard with CNS depression and mortality (Svirbely et al., 1943; Tegeris and Balster, 1994; Bonnet et al., 1982). Furthermore, various animal studies with various duration provide information about relatively high non-lethal concentrations for benzene (see Table 20).

It has been found from experience with anesthetic gases that CNS depression does no vary by more than a factor 2-3 between groups in the population. It should be noted that especially for toluene, a range of human studies is available. An uncertainty factor of 3 is used for intraspecies variation because it has been found from experience with anesthetic gases that CNS depression does no vary by more than a factor 2-3 between groups in the population. This is supported by the small range in concentrations found between 0 and 100% mortality (Svirbely et al., 1943; Bonnet et al., 1982). Based on the experiments described by Von Oettingen, time extrapolation is performed using n=1 and n=2 for longer and shorter time periods respectively (see AEGL-1 section for explanation).

This approach results in the following AEGL-3 levels. The values are extrapolated from a 4h endpoint to 10 min because the resulting values are supported by other animal data within a exposure time of 15 min to 2h (Von Oettingen, 1940; Furnas and Hine, 1958, Nielsen and Alarie, 1982; Magos et al, 1990).

Table 21 AEGL-3 values for benzene

AEGL-3 VALUES FOR BENZENE in ppm (mg/m^3)					
AEGL Level	**10 minutes**	**30 minutes**	**1 hour**	**4 hours**	**8 hours**
AEGL-3 (based on 5940 ppm 4h)	See below ¶	5600* (18,000)	4000* (13,000)	2000* (6500)	990* (3200)

* The AEGL-3 value is higher than 10% of the lower explosive limit of propane in air (LEL = 1.4 % (14,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

¶ The 10-min AEGL-3 value is higher than 50% of the lower explosive limit of propane in air (LEL = 1.4 % (14,000 ppm)). Therefore, extreme safety considerations against hazard of explosion must be taken into account. The calculated 10-min AEGL-3 value is 9700 ppm (31,000 mg/m^3).

The resulting AEGL-3 values are supported by the data of Svirbely et al. (1943) which would provide basically similar values if the NOEL for mortality from this study would be used as a starting point. The AEGL-3 values also appear to be compliant with the limited quantitative human experiences from occupational and accidental exposures. In addition, these values are considered to be protective also for sudden cardiac arrest due to cardiac sensitization. Most cases of human mortality in which cardiac sensitization is thought to play a major role are related to vapor exposure in confined spaces which are considered to result from high exposure levels (sometimes possibly close to saturated levels). In dogs, this effect is studied using a 5% vapor (about 50,000 ppm, Chenoweth, 1946). The only study with quantitative exposure information was performed by Magos et al. (1990) in rats. However, this is not an adrenalin-type of sensitisation test but studies the effect of experimental ischemia (coronary ligation) and a potassium channel blocker on cardiac irregularities. Benzene exposure itself had no effect on heart rate or ECG. However, exposure to benzene at 7332-8224 ppm for 15 min exacerbates the effects of coronary ligation and injection of a potassium channel blocker compared to controls.

1 # 8 SUMMARY OF PROPOSED AEGLS
2
3 ## 8.1 AEGL Values and Toxicity Endpoints
4
5 ### 8.1.1 Odor threshold and Level of Distinct Odor Awareness (LOA)
6
7 A Level of distinct Odor Awareness (LOA) of 7.5 ppm was derived as explained in Appendix D.
8

TABLE 10: SUMMARY/RELATIONSHIP OF PROPOSED AEGL VALUES [a]

Classification	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour
LOA	7.5				
AEGL-1 (Nondisabling)	130 (420)	73 (240)	52 (170)	18 (58)	9.0 (29)
AEGL-2 (Disabling)	2000* (6500)	1100 (3600)	800 (2600)	400 (1300)	200 (650)
AEGL-3 (Lethal)	See below ¶	5600* (18,000)	4000* (13,000)	2000* (6500)	990* (3200)

9 * The AEGL-2 or AEGL-3 value is higher than 10% of the lower explosive limit of propane in air (LEL = 1.4 % (14,000 ppm)).
10 Therefore, safety considerations against hazard of explosion must be taken into account.
11 ¶ The 10-min AEGL-3 value is higher than 50% of the lower explosive limit of propane in air (LEL = 1.4 % (14,000 ppm)).
12 Therefore, extreme safety considerations against hazard of explosion must be taken into account.
13 The calculated 10-min AEGL-3 value is 9700 ppm (31,000 mg/m^3).
14
15
16 ## 8.2 Comparison with Other Standards and Criteria
17

EXTANT STANDARDS AND GUIDELINES FOR BENZENE in ppm

Guideline	Exposure Duration				
	10 minutes	30 minutes	1 hour	4 hours	8 hours
AEGL-1	130	73	52	18	9.0
AEGL-2	2000*	1100	800	400	200
AEGL-3	See below¶	5600*	4000*	2000*	990*

ERPG-1 (AIHA)[a]			50 ppm		
ERPG-2 (AIHA)			150 ppm		
ERPG-3 (AIHA)			1000 ppm		
PEL-TWA (OSHA)[b]					1 ppm
IDLH (NIOSH)[c]					500 ppm
REL-TWA (NIOSH)[d]					0.1 ppm
TLV-TWA (ACGIH)[e]					10 ppm
MAK (Germany)[f]					
MAC (The Netherlands)[h]					1 ppm

* The AEGL-2 or AEGL-3 value is higher than 10% of the lower explosive limit of propane in air (LEL = 1.4 % (14,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

¶ The 10-min AEGL-3 value is higher than 50% of the lower explosive limit of propane in air (LEL = 1.4 % (14,000 ppm)). Therefore, extreme safety considerations against hazard of explosion must be taken into account. The calculated 10-min AEGL-3 value is 9700 ppm (31,000 mg/m^3).

[a] **ERPG (Emergency Response Planning Guidelines, American Industrial Hygiene Association)** (AIHA, 1991)

[b] **OSHA PEL-TWA (Occupational Health and Safety Administration, Permissible Exposure Limits - Time Weighted Average)** (OSHA, 1989)
is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[c] **IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)** (NIOSH, 1996), is based on acute inhalation toxicity ……

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

[e] **ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average)** (ACGIH, 1996)
The time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

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

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

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1 **APPENDIX A**
2
3 **Time Scaling Calculations for AEGLs**

1 **AEGL-1**
2
3 Key study: Srbova et al. (1950)
4
5 Toxicity endpoint: no subjective symptoms in human volunteers exposed up to 110 ppm for 2 hours.
6 Considered to be a NOEL for mild CNS effects.
7
8 Scaling: Based on data presented in Von Oettingen (1940) on light and deep narcosis in cats an n-
9 value of 1 was observed. Because these are secundary data, this n-value is not used
10 directly. The data do show, however, that default value of n=3 for extrapolation to shorter
11 durations is too conservative. Therefore, a n-value of 2 is used for extrapolation to shorter
12 durations and an n-value of 1 is used for extrapolation to longer durations.
13
14 Uncertainty factors: Interspecies factor = 1 because human data are used.
15 Intraspecies factor = 3 since experience with anethestic gases have shown that the
16 variability between groups in the population does not vary by more than a factor 2-3.
17 Calculations: To shorter durations: C^2 x t = k
18 $(110\ ppm)^2$ x 120 min = k
19 k = 1,452,000 ppm^2 x min
20 To longer durations: C x t = k
21 (110 ppm) x 120 min = k
22 k = 13,200 ppm*min
23
24 10-minute AEGL-1 $(1,452,000\ ppm^2 \times min/10\ minutes)^{1/2}$ / 3 = 130 ppm
25
26 30-minute AEGL-1 $(1,452,000\ ppm^2 \times min/30\ minutes)^{1/2}$ / 3 = 73 ppm
27
28 1-hour AEGL-1 $(1,452,000\ ppm^2 \times min/10\ minutes)^{1/2}$ / 3 = 52 ppm
29
30 4-hour AEGL-1 (13,200 ppm x min/240 minutes) / 3 = 18 ppm
31
32 8-hour AEGL-1 (13,200 ppm x min/480 minutes) / 3 = 9 ppm

1 **AEGL-2**
2
3 Key study: Molnar et al. (1986)
4
5 Toxicity endpoint: Highest reliable level without AEGL-2 endpoint observed in rats (4000 ppm for 4h). At
6 5940 ppm for 4h a reduction in locomotor activity was observed.
7
8 Scaling: Based on data presented in Von Oettingen (1940) on light and deep narcosis in cats an n-
9 value of 1 was observed. Because these are secundary data, this n-value is not used
10 directly. The data do show, however, that default value of n=3 for extrapolation to shorter
11 durations is too conservative. Therefore, a n-value of 2 is used for extrapolation to shorter
12 durations and an n-value of 1 is used for extrapolation to longer durations.
13
14 Uncertainty factors: Interspecies factor = 3 because CNS-dependent effects of benzene (and VOC's in
15 general) do not appear to vary mcuh between species. In addition, the use of a larger
16 interspecies factor would provide values that do not match the human experience.
17 Furthermore, the AEGL-values for benzene should be in prespective relative to the values
18 for toluene and xylenes.
19 Intraspecies factor = 3 since experience with anethestic gases have shown that the
20 variability between groups in the population does not vary by more than a factor 2-3.
21 Total uncertainty factor = 10
22
23 Calculations: To shorter durations: C^2 x t = k
24 $(4000\ ppm)^2$ x 240 min = k
25 k = 3.84 x 10^9 ppm^2 x min
26 To longer durations: C x t = k
27 (4000 ppm) x 240 min = k
28 k = 960,000 ppm*min
29
30 10-minute AEGL-1 $(3.84 \times 10^9\ ppm^2 \times min/10\ minutes)^{1/2} / 10 = 2000\ ppm$
31
32 30-minute AEGL-1 $(3.84 \times 10^9\ ppm^2 \times min/30\ minutes)^{1/2} / 10 = 1100\ ppm$
33
34 1-hour AEGL-1 $(3.84 \times 10^9\ ppm^2 \times min/10\ minutes)^{1/2} / 10 = 800\ ppm$
35
36 4-hour AEGL-1 $(3.84 \times 10^9\ ppm \times min/240\ minutes)/^{1/2} / 10 = 400\ ppm$
37
38 8-hour AEGL-1 (960,000 ppm x min/480 minutes) / 10 = 200 ppm

1 **AEGL-3**
2
3 Key study: Molnar et al. (1986)
4
5 Toxicity endpoint: Highest reliable level without mortality observed in rats (5940 for 4h).
6
7 Scaling: Based on data presented in Von Oettingen (1940) on light and deep narcosis in cats an n-
8 value of 1 was observed. Because these are secundary data, this n-value is not used
9 directly. The data do show, however, that default value of n=3 for extrapolation to shorter
10 durations is too conservative. Therefore, a n-value of 2 is used for extrapolation to shorter
11 durations and an n-value of 1 is used for extrapolation to longer durations.
12
13 Uncertainty factors: Interspecies factor = 1 because CNS-dependent effects of benzene (and VOC's in
14 general) do not appear to vary much between species. In addition, the use of a larger
15 interspecies factor would provide values that do not match the human experience.
16 Futhermore, the AEGL-values for benzene should be in perspective relative to the values
17 for toluene and xylenes
18 Intraspecies factor = 3 since experience with anethestic gases have shown that the
19 variability between groups in the population does not vary by more than a factor 2-3.
20 Total uncertainty factor = 3
21
22 Calculations: To shorter durations: C^2 x t = k
23 $(5940\ ppm)^2$ x 240 min = k
24 k = $8.468064 \times 10^{10}\ ppm^2$ x min
25 To longer durations: C x t = k
26 (4000 ppm) x 240 min = k
27 k = 1,425,600 ppm*min
28
29 10-minute AEGL-1 $(8.468064 \times 10^{10}\ ppm^2 \times min/10\ minutes)^{1/2}$ / 3 = 9700 ppm
30
31 30-minute AEGL-1 $(8.468064 \times 10^{10}\ ppm^2 \times min/30\ minutes)^{1/2}$ / 3 = 5600 ppm
32
33 1-hour AEGL-1 $(8.468064 \times 10^{10}\ ppm^2 \times min/10\ minutes)^{1/2}$ / 3 = 4000 ppm
34
35 4-hour AEGL-1 $(8.468064 \times 10^{10}\ ppm \times min/240\ minutes)/^{1/2}$ / 3 = 2000 ppm
36
37 8-hour AEGL-1 (1,425,600 ppm x min/480 minutes) / 3 = 990 ppm

1 **APPENDIX B**
2
3 **Derivation Summary for Benzene AEGLs**

1 # ACUTE EXPOSURE GUIDELINES FOR BENZENE
2 # (CAS NO. 71-43-2)

AEGL-1 VALUES

10 minutes	30 minutes	1 hour	4 hours	8 hours
130 ppm	73 ppm	52 ppm	18 ppm	9.0 ppm

Srbova et al. (1950)

Test Species/Strain/Number: Human volunteers

Exposure Route/Concentrations/Durations: Inhalation, 110 ppm, 2h

Effects: No subjective symptoms

Endpoint/Concentration/Rationale: 110 ppm for 2h is considered a NOEL for mild CNS effects.

Uncertainty Factors/Rationale: Interspecies factor = 1 because human data are used. Intraspecies factor = 3 since experience with anethestic gases have shown that the variability between groups in the population does not vary by more than a factor 2-3.
Total uncertainty factor: 3

Modifying Factor: none

Animal to Human Dosimetric Adjustment: -

Time Scaling: n =2 to shorter durations, n = 1 to longer durations

Data Adequacy: Study is described in limited way. However, level of exposusre is indirectly supported by metabolism studies with humans in which substantial numbers of volunteers or workers were exposed. In addition, levels are supported by historic knowledge on occupational exposure.

3

ACUTE EXPOSURE GUIDELINES FOR BENZENE (CAS NO. 71-43-2)

AEGL-2 VALUES

10 minutes	30 minutes	1 hour	4 hours	8 hours
2000 ppm*	1100 ppm	800 ppm	400 ppm	200 ppm

Reference: Molnar et al. (1986)

Test Species/Strain/Sex/Number: male CFY rats (8 per dose),

Exposure Route/Concentrations/Durations: Inhalation: 0, 250, 500, 800, 1500, 2000, 4000, 5940 ppm for 1,2 ,3 or 4 hours.

Effects: Increase in locomotor activity at 2000 and 4000 ppm for 4h with some incoordination and tremor, decrease in locomotor activity at 5940 ppm for 4h.

Endpoint/Concentration/Rationale: 4000 ppm for 4h is highest (reliable) level without AEGL-2 endpoint.

Uncertainty Factors/Rationale:
Interspecies factor = 3 because CNS-dependent effects of benzene (and VOC's in general) do not appear to vary mcuh between species. In addition, the use of a larger interspecies factor would provide values that do not match the human experience. Furthermore, the AEGL-values for benzene should be in prespective relative to the values for toluene and xylenes.
Intraspecies factor = 3 since experience with anethestic gases have shown that the variability between groups in the population does not vary by more than a factor 2-3.
Total uncertainty factor = 10

Modifying Factor: Not applicable

Animal to Human Dosimetric Adjustment: -

Time Scaling: n = 2 to shorter durations, n = 1 to longer durations

Data Adequacy: Study is supported by other neurobehavioral studies in rats. Mice appear to be more susceptible but have a higher body load.
AEGL-2 values are in agreement with historic knowledge on occupational exposure and impairment of the ability to escape or functioning properly.

* The AEGL-2 or AEGL-3 value is higher than 10% of the lower explosive limit of propane in air (LEL = 1.4 % (14,000 ppm)). Therefore, safety considerations against hazard of explosion must be taken into account.

1 # ACUTE EXPOSURE GUIDELINES FOR BENZENE
2 # (CAS NO. 71-43-2)

AEGL-3 VALUES

10 minutes	30 minutes	1 hour	4 hours	8 hours
See below¶	5600 ppm*	4000 ppm*	2000 ppm*	990 ppm*

Reference: Molnar et al. (1986)

Test Species/Strain/Sex/Number: male CFY rats (8 per dose),

Exposure Route/Concentrations/Durations: Inhalation: 0, 250, 500, 800, 1500, 2000, 4000, 5940 ppm for 1,2 ,3 or 4 hours.

Effects: No mortality

Endpoint/Concentration/Rationale: 5940 ppm for 4h is the highest (reliable) level without mortality within the AEGL time frame.

Uncertainty Factors/Rationale:
Interspecies factor = 1 because CNS-dependent effects of benzene (and VOC's in general) do not appear to vary much between species. In addition, the use of a larger interspecies factor would provide values that do not match the human experience. Futhermore, the AEGL-values for benzene should be in perspective relative to the values for toluene and xylenes
Intraspecies factor = 3 since experience with anethestic gases have shown that the variability between groups in the population does not vary by more than a factor 2-3.
Total uncertainty factor = 3

Modifying Factor: none

Animal to Human Dosimetric Adjustment: -

Time Scaling: n = 2 to shorter durations, n =1 to longer durations.

Data Adequacy: The 5940 ppm level for 4h is supported by various other animal studies. In addition, there are animal data with short term exposure (10-30 minutes) showing that extrapolation back to 10 minutes is allowed. Furthermore, the levels are supported by historic knowledge on occupational exposure without acute mortality.

3 * The AEGL-3 value is higher than 10% of the lower explosive limit of propane in air (LEL = 1.4 % (14,000 ppm)). Therefore,
4 safety considerations against hazard of explosion must be taken into account.
5 ¶ The 10-min AEGL-3 value is higher than 50% of the lower explosive limit of propane in air (LEL = 1.4 % (14,000 ppm)).
6 Therefore, extreme safety considerations against hazard of explosion must be taken into account. The calculated 10-min AEGL-3 value is
7 9700 ppm (31,000 mg/m^3).

1 # APPENDIX C: SUMMARY OF STUDIES ON CHILDHOOD LEUKEMIA AND PARENTAL BENZENE
2 # EXPOSURE

3 **Table 22 Studies on childhood leukemia and parental benzene exposure**

Reference	Study description (all case-control, except one)	Exposure characterization	Relevant findings	Remarks
Maternal exposure				
Shu et al. 1988	309 leukemia cases, 618 controls (56% ALL, 30% ANLL(most AML))	Personal interview on occupation and occupational exp.	Increased leukemia risk for chemical industry (OR 3.3). High risk for physicians (OR 5.7) or pharmacists (OR 19.7). Occupational benzene exposure had OR of 2.3: differentiated for ALL (OR 1.3) and ANLL (OR 4.0)	Shanghai population with naturally increased percentage of ANLL cases. Benzene exposure was self reported over a 12 yr period: serious risk of positive recall bias. Highest risk groups not further investigated
Buckley et al., 1989	178 case-control pairs, only ANLL cases.	Life time occup. History, inquiry about 52 chemicals, exposure self reported and occup. Classification. Exp. duration was taken into account	Association between maternal occup. Exposure to paints/pigments (OR 2.2, mainly pre-conception). Although benzene exposure was specifically included in the questionaires, no specific association between risk and benzene exposure was reported.	Controlling for household pesticide and petroluem exposure, and marijuana use reduced the association. Specific chromosome damage observed in adults with ANLL (chrom. 5 + 7) was not observed in children. Serious risk of positive recall bias.
McKinney et al., 1991	109 leukemia or non-Hodgkin cases, 2 controls per case. Brittish populations	Complete history of employment and exposure to substances. Self reported and occup. classification	Benzene exp. of the mother (2 cases, 1 control) had an OR of 4.0 (95% CI 0.30-117).	An occupation of the mother in literary, artistic or sports had an OR of 4.37 (0.45 – 118) as large as the risk of benzene. High possibility of chance since number of cases were too low. Leukemia and non-Hodgekin tumors were not separated. Some parents already involved in previous study (bias ?)

Reference	Study description (all case-control, except one)	Exposure characterization	Relevant findings	Remarks
Kaatsch et al., 1998	2358 cases, 2588 controls (39% non-specified malignancies, 10% non-Hodgekin, 50% leukemias (of which 87% ALL, 12% ANLL))	Self administered questionaires and telephone interviews.	No associations were found between childhood cancers and exposure to plastic and resin fumes or benzene.	No details about subtypes of leukemia provided.
Shu et al., 1999	1842 cases (all ALL), 1986 controls	Detailed self reported occupational exposure questionnaire	No association of ALL with maternal benzene exposure	Large study, but only ALL investigated
Paternal exposure				
Shaw et al., 1984	255 leukemia cases, 2 controls per case (83% ALL, 10% AML) California population	"potentially exposed versus not exposed" based on occup. classification	No association between benzene exp. and leukemia	No leukemia subtypes studied. Exposure not quantified. No recall bias on benzene exposure
Shu et al., 1988	See above	See above	No association with paternal occupation / benzene exposure and leukemia	See above
Buckley et al., 1989	See above	See above	An increased OR was found for paternal solvent exposure (2.6 for 1-1000 days exp; 2.0 for > 1000 days exp) and petroleum products (OR 2.4). No specification for benzene.	Controlling for household pesticide and petroleum product exposure, and marijuana use, reduced the association to non-significant levels. See above also.
McKinney et al., 1991	See above	See above	No association of leukemia with chemical industry. Self reported benzene exposure was associated with increased risk: pre-conceptional OR 5.81, peri-conceptional OR 2.98, postnatal OR 1.39. Significant only for pre-conceptional.	See above.
Kaatsch et al., 1998	See above	See above	No associations were found between childhood cancers and exposure to plastic and resin fumes or benzene.	See above

Reference	Study description (all case-control, except one)	Exposure characterization	Relevant findings	Remarks
Shu et al., 1999	See above	See above	No association between ALL and paternal benzene exposure	See above
Feychting et al., 2001	***Cohort study***. Swedish population, 235635 children, followed for 12-15 years. 522 cases of childhood cancer (161 leukemias)	Father's exposure estimated from 2-26 months before birth through occup. classification. Three scale level: no exp, possible exp., probable exp.	Paternal exp to solvents had an OR of 1.25, to benzene had an OR of 1.23 (only 3 cases). Based on 1 case, paternal occup in filling station had an increased risk, however the same risk was observed for governmental administrative work (OR 2.72)	Exposure estimates were obtained before occurrence of cancer, no recall bias. Number of case is too low to provide reliable associations. No subtypes of leukemia studied.

1

APPENDIX D: CALCULATIONS FOR CARCINOGENIC RISKS OF A SINGLE EXPOSURE TO BENZENE

Benzene is an acknowledged human leukomogen. As explained in section 6.1 of this document linear extrapolation over dose and time are probably not correct for benzene. However, the SOP prescribes calculations for carcinogenicity to be given in this technical support document. The following calculations are based on the most recent carcinogenicity update of U.S. EPA from 1997. Using linear assumptions, EPA estimated that the leukemia risk associated with a lifetime exposure to 1 ppm ranged from 7.1 x 10^{-3} to 2.5 x 10^{-2}. These values (ranges) correspond to the following exposure at risk levels of 10^{-4}, 10^{-5} and 10^{-6}.

Risk level	**Range of exposure level for lifetime exposure**
10^{-4}	4 – 14 ppb
10^{-5}	0,4 – 1,4 ppb
10^{-6}	0,04 - 0,14 ppb

For (linear) extrapolation to a single 8h exposure value, the following adjustments are made:
(Exposure level at lifetime risk * 25600) / (24/8) = Exposure level lifetime risk * 8533

This yields the following numbers:

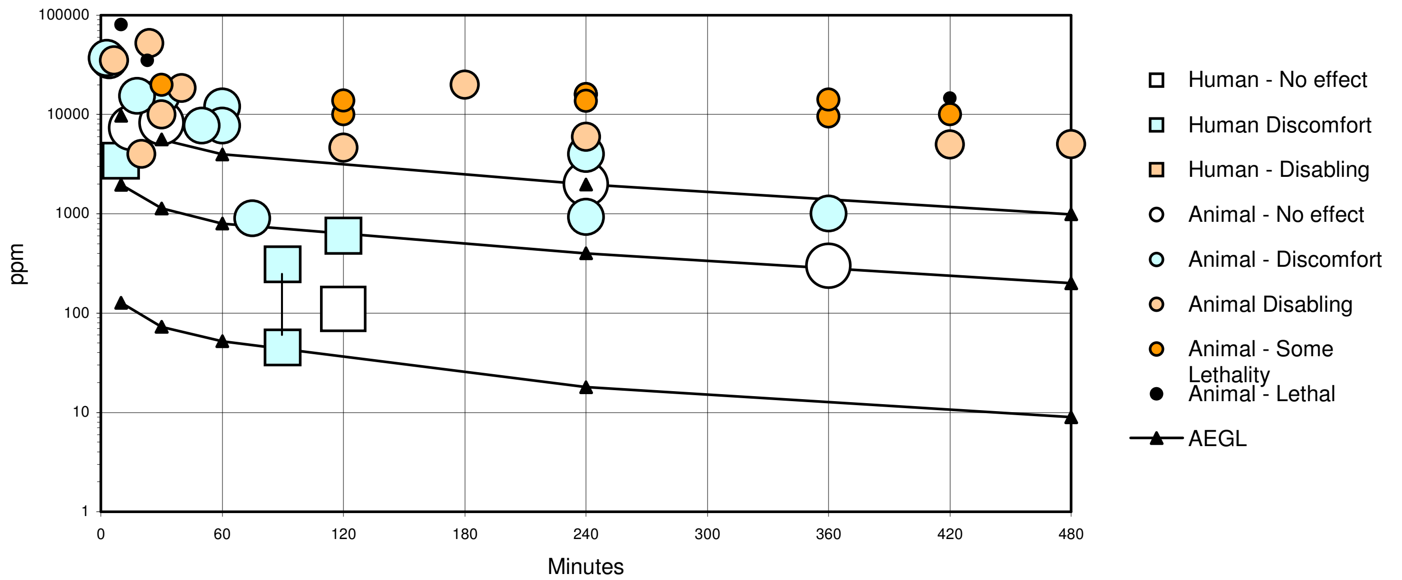
Risk level	**Range of exposure level for a single 8h exposure (without DRCF)**
10^{-4}	34 – 119 ppm
10^{-5}	3,4 – 11,9 ppm
10^{-6}	0,34 - 1,2 ppm

To account for the uncertainty regarding the variability in the stage of the cancer process at which benzene or its metabolites may act, a multistage factor of 6 is applied according to the procedure described in the AEGL Standing Operating Procedures. This yields the following (rounded) numbers.

Risk level	**Range of exposure level for a single 8h exposure (with DRCF)**
10^{-4}	6 – 20 ppm
10^{-5}	0,6 – 2,0 ppm
10^{-6}	0,06 - 0,2 ppm

For comparison, the EEGL values derived by NRC in 1986 were 38 ppm for 24h and 912 ppm for 1h. (for a risk level of 10^{-4}). The SMAC value based on carcinogenicity (risk 10^{-4}) was 12 ppm for 24h (NRC 1996).

1 APPENDIX E: CATEGORY PLOTS FOR BENZENE
2
3
4
5



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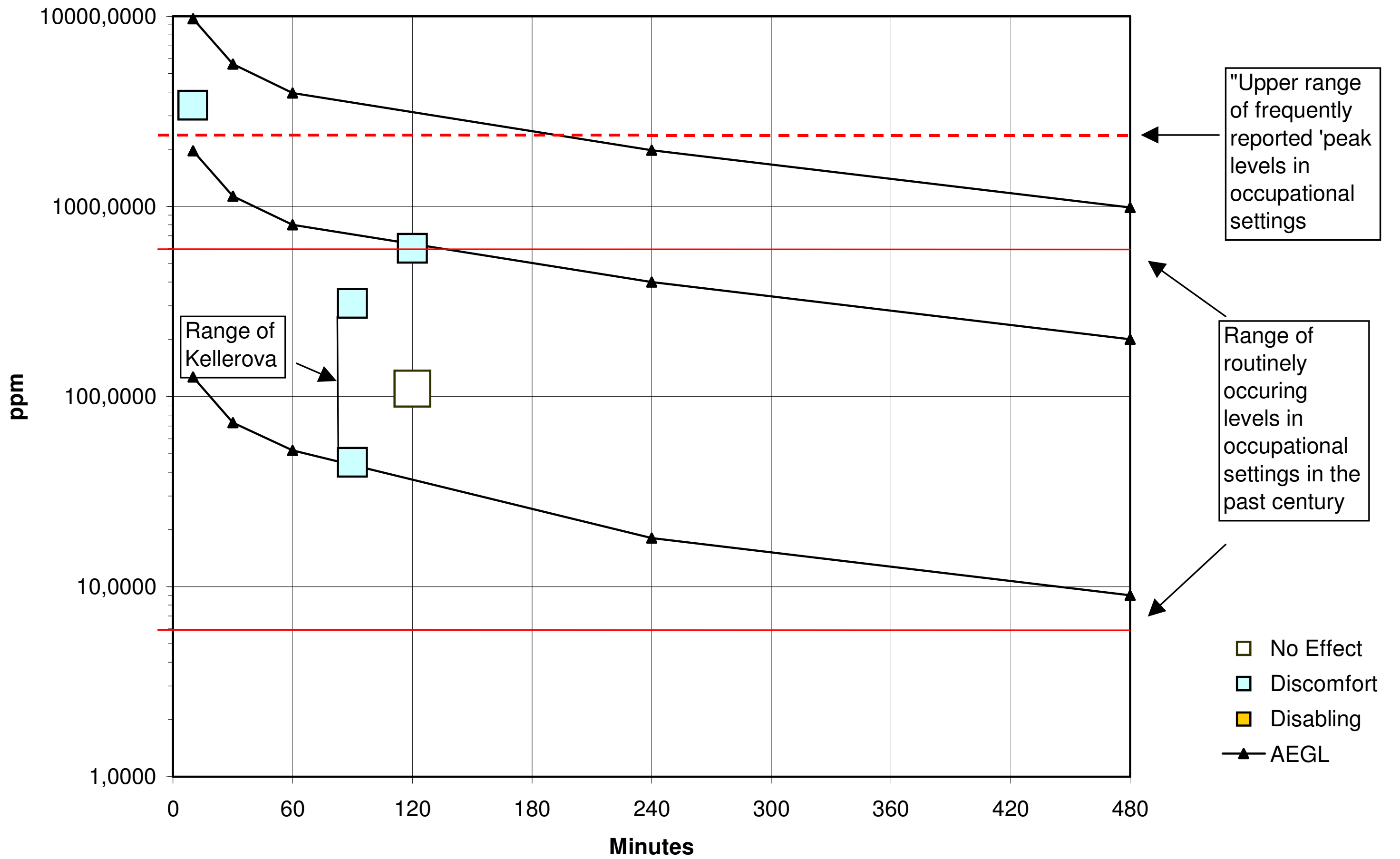

2
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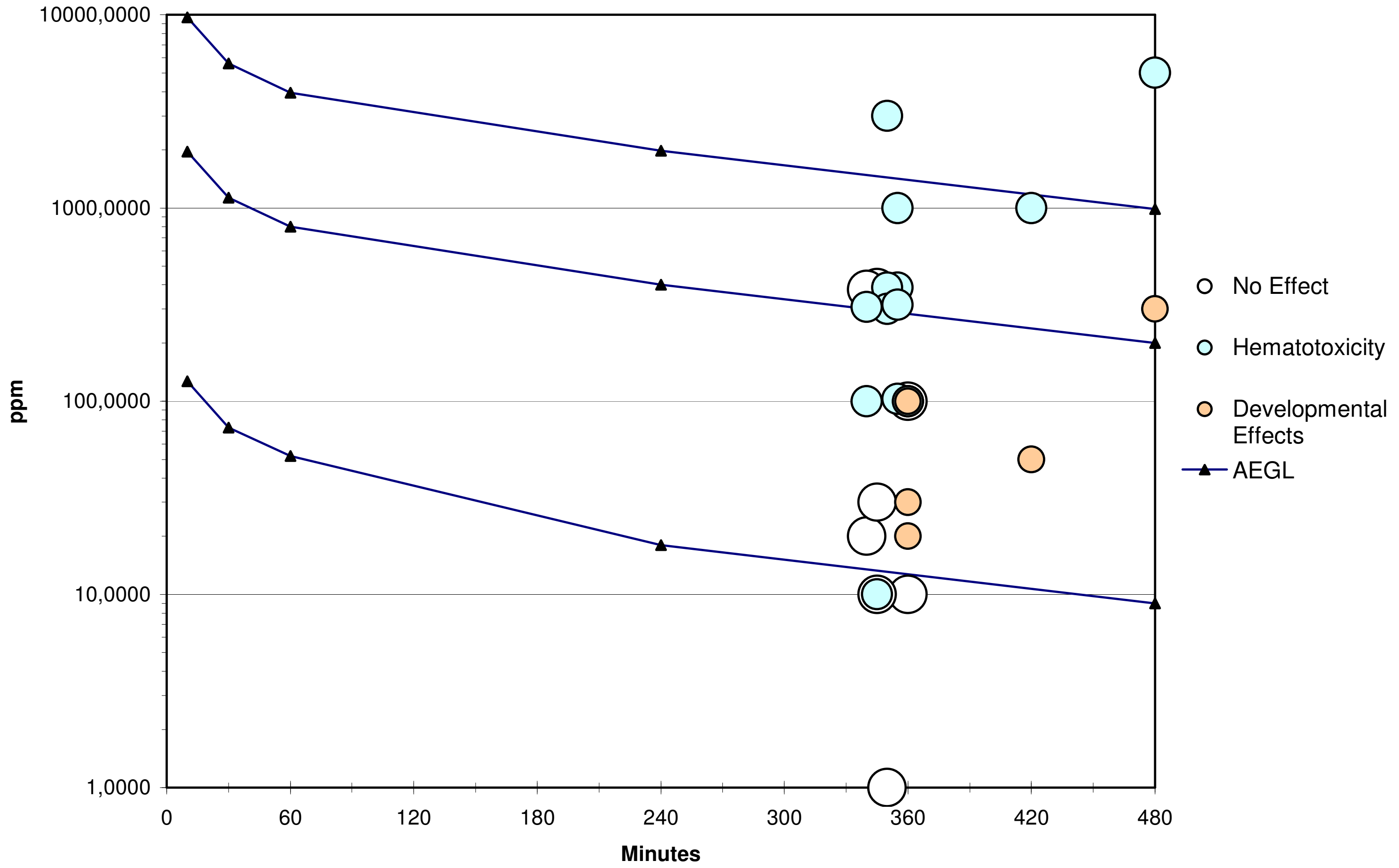

1
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APPENDIX F: DERIVTION OF THE LEVEL OF DISTINCT ODOR AWARENESS

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 by Van Doorn et al. (2002).

Reported odor thesholds for benzene have been reported to be 56 ppm (May, 1966) and 34 ppm (Punter, 1980). Because in these studies the thresholds for n-butanol were 11 ppm and 1.2 respectively, the level 2 corrected odor thesholds were 0.20 and 1.1 ppm for May (1966) and Punter (1980) respectively.

Using a default value for k_w and a mean value OT50 of 0.47 ppm as odor threshold, a LOA of 7.5 ppm was derived.

APPENDIX G: ABBREVIATIONS USED IN THIS DOCUMENT

ALL	Acute Lymphocytic Leukemia
AML	Acute myelogenous Leukemia
ANLL	Acute Non-Lymphocytic Leukemia
BFU-E	Burst Forming Units - Erythroid
CA	Chromosome aberrations
CFC	Colony Forming cells
CFU – E	COlony Forming Units – Erythroid
CFU – S	Colonyu Forming Units – spleen (stem cells)
CFU	Colony Forming Units
CFU-GM	Colony Forming Units – Granulocytes / Macrophages
CFU-HHP	Similar to CFU-S
CML	Chronic myeologenous Leukemia
GD	Gestation Day
MN	Micronucleus
OR	Odds Ratio
PMN	Polymorphonuclear Neutrophils
RBC	Red Blood Cells
SCE	Sister Chromatid Exchanges
VOC	Volatile Organic Compound
WBC	White Blood Cells