



Benzene Reference Exposure Levels

Technical Support Document for the Derivation
of Noncancer Reference Exposure Levels
Appendix D1

**Public Review Draft
June 2013**



**Air, Community, and Environmental Research Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency**

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(benzol; benzole; cyclohexatriene)

CAS: 71-43-2



1 Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360(b)(2)). In response to this statutory requirement OEHHA developed a Technical Support Document (TSD) that describes acute, 8-hour, and chronic RELs. The TSD was adopted in December 2008 (OEHHA, 2008) and presents methodology reflecting the latest scientific knowledge and techniques, and in particular explicitly includes consideration of possible differential effects on the health of infants, children, and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Chapter 731, Statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*). These guidelines have been used to develop the RELs for benzene presented in this document; this document will be added to Appendix D of the TSD.

Benzene is a solvent, and acute high inhalation exposure may lead to eye, nose, and throat irritation and central nervous system depression in humans. Prolonged or repeated exposures have been associated with both blood cell proliferation and reduction in blood cell numbers, including peripheral lymphocytopenia, pancytopenia, and aplastic anemia. The non-cancer adverse health effects of benzene result from the ability of its metabolites to adversely affect rapidly dividing cells, especially in the bone marrow where detoxifying enzymes for its toxic metabolites are present at low levels compared to the liver. Children may be more sensitive to benzene because so many of their tissues are undergoing rapid cell division and differentiation for growth and development to stimulate and maintain growth. This review includes relevant material published through January 2013 and is a technical review of those studies specifically applicable to developing non-cancer acute, 8-hour, and chronic inhalation RELs for benzene.

Although benzene is a known human carcinogen (IARC Group 1), this document does not discuss issues related to the cancer potency factor. That was derived previously and is available at www.oehha.ca.gov/air/hot_spots/index.html.

1.1 Benzene Acute REL

<i>Reference Exposure Level</i>	27 µg/m³ (0.008 ppm; 8 ppb) (for a 6 hour exposure)
<i>Critical effect(s)</i>	Developmental hematotoxicity in fetal and neonatal rats
<i>Hazard Index target(s)</i>	Developmental; Immune System; Hematologic System

1.2 Benzene 8-Hour REL

<i>Reference Exposure Level</i>	7 µg/m³ (0.002 ppm; 2 ppb)
<i>Critical effect(s)</i>	Decreased peripheral blood cells in Chinese workers
<i>Hazard Index target(s)</i>	Hematologic System

1.3 Benzene Chronic REL

<i>Reference Exposure Level</i>	7 µg/m³ (0.002 ppm; 2 ppb)
<i>Critical effect(s)</i>	Decreased peripheral blood cells in Chinese workers
<i>Hazard Index target(s)</i>	Hematologic System

2 Physical and Chemical Properties (HSDB, 2007)

<i>Description</i>	clear, colorless liquid
<i>Molecular formula</i>	C ₆ H ₆
<i>Molecular weight</i>	78.1 g/mol
<i>Density/Specific gravity</i>	0.8787 @ 15°C/4°C
<i>Boiling point</i>	80.1°C
<i>Melting point</i>	5.5°C
<i>Vapor pressure</i>	94.8 mm Hg @ 25°C (0.125 atm)
<i>Flashpoint</i>	-11°C
<i>Explosive limits</i>	upper = 8.0% by volume in air lower = 1.4% by volume in air
<i>Solubility</i>	miscible with ethanol, chloroform, ether, carbon disulfide, acetone, oils, and glacial acetic acid; slightly soluble in water (1,790 mg/L @ 25°C)
<i>Octanol/water partition coefficient</i>	log Kow = 2.13
<i>Odor threshold</i>	0.875 ppm (2.8 mg/m ³) (Haley, 1977) 4.68 ppm (HSDB, 2007)

<i>Odor description</i>	aromatic odor (sweet); gasoline-like odor
<i>Metabolites</i>	hydroquinone, benzoquinone, catechol, phenol
<i>Conversion factor</i>	1 ppm = 3.26 mg/m ³ = 42 µmol/m ³

3 Occurrences and Major Uses

Benzene was widely used in the past as a multipurpose organic solvent. This use was discouraged due to its high toxicity and, at least in the United States, its use as a solvent has decreased. Present uses include benzene as a raw material in the synthesis of styrene, phenol, cyclohexane, aniline, and alkyl benzenes and in the manufacture of various plastics, resins, and detergents. Synthesis of many pesticides and pharmaceuticals also involves benzene as a chemical intermediate. Benzene is emitted in large quantities from oil refineries and petroleum storage facilities. The tire industry and shoe factories use benzene extensively. Annual production in the U.S. was estimated to be 12.32 billion pounds (6.16 million tons) in 1993 (HSDB, 2007). In 2010 estimated U.S. production was 1.8 billion gallons (13.3 billion pounds) (Balboa, 2011).

Benzene exposure also arises from cigarette smoking (including passive smoking), home use of solvents or gasoline, and leaking underground storage tanks (Goldstein and Witz, 2009).

Estimates for benzene emissions from the Statewide 2008 California Toxics Inventory (CTI) were 1,284 tons from stationary sources, 117 tons from area-wide sources, 5,024 tons from on-road mobile sources, 4,393 tons from other mobile sources, and 46 tons from natural sources (CARB, 2008). The top 25 benzene emitters in the Air Toxics Hot Spots program in 2008 emitted between 4,000 and 49,000 pounds per year.

A survey of indoor levels of volatile organic chemicals reported a geometric mean of 0.69 µg/m³ (0.22 ppb) for benzene (range = 0.29 – 2.11 µg/m³) in 29 small- and medium-sized commercial buildings in California (Wu et al., 2011).

Indoor benzene levels were measured prior to and after the Ireland Public Health Tobacco Act of 2002 ban on smoking in pubs (McNabola et al., 2006). The average ambient concentration of benzene measured inside two Dublin pubs in March 2004 prior to the ban was 4.83 µg/m³ (1.5 ppb). The average ambient level outside the pubs was 0.84 µg/m³ (0.26 ppb). In August 2004 after the ban the average indoor level was 0.54 µg/m³ (0.2 ppb). The average ambient outside level was 0.13 µg/m³ (0.04 ppb).

The TEACH (Toxic Exposure Assessment, Columbia/Harvard) study characterized personal exposures to urban air toxics among 41 high school students living in Los Angeles in 2000 (Sax et al., 2006). Exposure was analyzed using 48-hr personal monitoring, outdoor ambient monitoring, and in-home ambient monitoring. The students were mainly Hispanic (93%), and were required to be non-smokers from non-smoking families. The mean outdoor concentration of benzene was 3.32 µg/m³ (1 ppb), while the

mean in-home concentration was $3.87 \mu\text{g}/\text{m}^3$ (1.2 ppb). The mean personal concentration was $4.64 \mu\text{g}/\text{m}^3$ (1.4 ppb).

Nazaroff and Singer studied hazardous air pollutants including benzene within US residences. Data analyses indicated that some 16 million US juveniles (2 months to 16 years old) were exposed to benzene from Environmental Tobacco Smoke (ETS) in the home (Nazaroff and Singer, 2004). Assuming that from 14 to 20 cigarettes are smoked per day in each residence, with an average of 430 μg benzene per cigarette, the resulting indoor air level was calculated to be 1.1-2.5 $\mu\text{g}/\text{m}^3$ (0.3-0.8 ppb) and the daily intake of benzene for juveniles was 14 – 31 μg .

In 2002 the estimated statewide ambient concentration of benzene was approximately 0.6 ppb ($\sim 2 \mu\text{g}/\text{m}^3$) (CARB, 2004). Statewide the annual average benzene concentration has decreased from ~ 2.5 ppb in 1990 to ~ 0.5 ppb in 2007 (CARB, 2009). Table 3.1 shows benzene levels in ambient air at various sampling stations in the Bay Area Air Quality Management District in 2008. Benzene levels were determined by a modification of USEPA method TO-15, which uses gas chromatography/mass spectrometry. The minimal detectable level for benzene is 0.014 ppb ($0.046 \mu\text{g}/\text{m}^3$).

Table 3.1. Benzene Levels (ppb) at Monitoring Stations in the Bay Area in 2008

Station	Average	Maximum	Minimum	Samples
Benicia – VIP	0.106	0.350	0.030	60
Berkeley	0.269	1.00	0.050	122
Bethel Island	0.135	0.510	0.040	62
Concord - Treat Blvd.	0.167	0.450	0.050	62
Crockett - Kendall Ave	0.116	0.250	0.040	62
Fremont-Chapel Way 1	0.195	0.630	0.070	40
Fremont-Chapel Way 2	0.230	0.590	0.100	31
Fort Cronkhite	0.0681	0.200	0	62
Livermore - Rincon Ave.	0.197	0.540	0.060	62
Martinez - Jones St	0.172	0.610	0.030	60
Napa - Jefferson St	0.321	1.05	0.080	62
Oakland	0.234	0.520	0.060	62
Oakland - Filbert St.	0.191	0.610	0.040	60
Redwood City	0.244	0.710	0.090	62
Richmond - 7th St	0.165	0.320	0.020	62
San Francisco - Arkansas 1	0.176	0.410	0	62
San Francisco - Arkansas 2	0.182	0.470	0.060	31
San Jose - Jackson St. 1	0.316	1.11	0.050	120
San Jose - Jackson St. 2	0.296	1.00	0.110	31
San Pablo - Rumrill	0.232	0.440	0.100	62
San Rafael	0.190	0.370	0.050	62
Santa Rosa - 5th St	0.210	0.800	0.030	62
Sunnyvale - Ticonderoga	0.153	0.430	0.050	56
Vallejo - Tuolumne St	0.196	0.660	0.060	62

Source: BAAQMD. Toxic Air Contaminant Air Monitoring Data for 2008

Table 3.2 shows benzene levels in ambient air at ten fixed sampling locations in the South Coast Air Quality Management District between 2004 and 2006 taken as part of the Multiple Air Toxics Exposure Study (MATES) III study. Year 1 was April 2004 through March 2005. Year 2 was April 2005 through March 2006. The analytical method used generally followed the EPA Method TO-15; determination of volatile organic compounds collected in specially prepared canisters and analyzed by gas chromatography/mass spectrometry.

Table 3.2. Benzene Levels at 10 Fixed Sites in South Coast in 2004 through 2006

Location	Year 1 (4/2004 – 3/2005)			Year 2 (4/2005 – 3/2006)		
	Mean ppb	SD	Samples	Mean ppb	SD	Samples
Anaheim	0.44	0.28	118	0.42	0.33	115
Burbank	0.73	0.42	118	0.69	0.44	122
Central LA	0.59	0.30	117	0.57	0.31	121
Compton	0.82	0.70	118	0.78	0.67	118
Inland Valley	0.49	0.24	115	0.49	0.24	116
Huntington Park	0.76	0.46	98	-	-	-
North Long Beach	0.56	0.35	119	0.48	0.34	118
Pico Rivera	0.57	0.32	121	-	-	-
Rubidoux	0.45	0.25	114	0.43	0.26	120
West Long Beach	0.57	0.44	114	0.50	0.38	120

Source: <http://www.aqmd.gov/prdas/matesIII/Final/Appendices/f-MATESIIIAppendixVIFinal92008.pdf>

Note that all the levels in Table 3.2 are higher than the highest mean value (0.321 ppb) in the Bay Area (Table 3.1). Table 3.3 shows benzene levels from ten sites in a subproject of MATES III in which mobile monitoring stations (microscale) were intentionally put near a known emission source and the results compared to a fixed site nearby over a period of three to ten months.

Table 3.3. Benzene Levels (ppb) at Monitoring Stations in South Coast 2004-2006

Site	Monitor	Mean (ppb)	SD	Samples	Time interval
Commerce	Microscale*	0.69	0.33	62	11/2004 – 5/2005
Huntington Park	Fixed	0.93	0.52	46	“
Indio	Microscale	0.21	0.1	26	3/2005 – 5/2005
Rubidoux	Fixed	0.39	0.23	26	“
San Bernardino	Microscale	0.73	0.38	45	10/2004 – 2/2005
Inland Valley(SB)	Fixed	0.51	0.28	46	“
Sun Valley	Microscale	0.52	0.23	91	6/2005 – 3/2006
Burbank	Fixed	0.75	0.46	101	“
Santa Ana	Microscale	1.04	0.6	47	9/2005 – 1/2006
Anaheim	Fixed	0.61	0.4	46	“

*A mobile monitoring device was intentionally located near a known emission source.

Source: <http://www.aqmd.gov/prdas/matesIII/Final/Document/e-MATESIIIChapter5Final92008.pdf>

Benzene exists mostly in the vapor phase. It reacts with photochemically produced hydroxyl radicals with a calculated half-life of 13.4 days. In atmospheres polluted with NO_x or SO₂ the half-life can be as short as 4-6 hours (<http://www.epa.gov/ogwdw/pdfs/factsheets/voc/tech/benzene.pdf>).

4 Metabolism

Inhalation of benzene is the principal route of concern for the general public; approximately half the benzene inhaled by humans is absorbed (Nomiyama and Nomiyama, 1974; Pekari et al., 1992). In men and women exposed to 52-62 ppm benzene for 4 hours, 46.9 percent of the inhaled dose was absorbed. Of this absorbed fraction, 30.1 percent was retained and 16.8 percent was excreted unchanged in the expired air (Nomiyama and Nomiyama, 1974).

After absorption, benzene targets the liver, the kidney, the lung, the brain, and the blood-forming bone marrow. The parent compound likely causes the acute neurotoxic effects. Benzene metabolism is depicted in Figure 4.1.

(1) Benzene is metabolized in the liver to benzene epoxide by the cytochrome P450 system, primarily CYP2E1 (but also to varying extents by CYP1A1, CYP2B1, CYP2F1, and CYP2F2). Benzene epoxide has a half-life of approximately 8 minutes in rat blood (Lindstrom et al., 1997) and thus could travel from the liver to other organs including bone marrow.

(2) Benzene epoxide rearranges nonenzymatically to phenol, initially the major metabolite of benzene. Phenol is oxidized, also by CYP2E1, to hydroquinone. Hydroquinone can be oxidized to the toxic metabolite benzoquinone non-enzymatically by O₂ or enzymatically by myeloperoxidase (MPO) in bone marrow. Benzoquinone can be converted back to hydroquinone by NAD(P)H:quinone oxidoreductase (NQO1), a detoxifying reaction.

(3) Benzene epoxide is enzymatically transformed by microsomal epoxide hydrolase to benzene dihydrodiol, which is then dehydrogenated to catechol by dihydrodiol dehydrogenase.

Most of the catechol and phenol metabolites are excreted within 24 hours in the urine, while hydroquinone requires 48 hours (Teisinger et al., 1952).

(4) Benzene epoxide is also metabolized to a ring-opened product, trans, trans-muconaldehyde. t,t-Muconaldehyde can be metabolized to t,t-muconic acid (oxidation) and to 6-hydroxy-t,t-2,4-hexadienal (reduction)(not shown in Figure 4.1).

(5) Benzene epoxide can be conjugated with glutathione by glutathione-S-transferases to ultimately form S-phenylmercapturic acid (SPMA).

(6) Benzene epoxide also equilibrates with its oxepin, a seven-atom monocyclic structure, which may be an intermediate in one or more of the previous pathways (Figure 4.1).

(7) The benzene metabolites with hydroxyl groups (phenol, catechol, hydroquinone, and 1,2,4-benzenetriol) can form sulfates and glucuronidates (Nebert et al., 2002), and

Table 4.1. Percentage of water soluble metabolites in 24 hour urines (Sabourin et al., 1989)

	5 ppm (3 mice)	600 ppm (3 mice)	5 ppm (2 rats)	600 ppm (3 rats)
Phenyl glucuronide	1	15	1.4	1
Catechol glucuronide	Non-detect	Non-detect	Non-detect	Non-detect
Phenyl sulfate	36	52	56	73
Hydroquinone monoglucuronide	26	9	8.5	0.5
Hydroquinone sulfate	6.6	2.3	2.9	1.5
Pre-phenylmercapturic acid	6	15	9.5	17
Phenylmercapturic acid	1	Non-detect	1.3	1
Muconic acid	23	5	18.5	4
Unknown	Non-detect	Non-detect	1.4	Non-detect
Total	99.6	98.3	99.5	98

Benzene causes hematotoxicity through its phenolic metabolites, which lead to DNA strand breaks, chromosomal damage, sister chromatid exchange (SCE), inhibition of topoisomerase II, and damage to the mitotic spindle. The myelotoxic (and carcinogenic) effects of benzene are associated with free radical formation either as benzene metabolites, particularly benzoquinone, or as lipid peroxidation products (Smith et al., 1989; USEPA, 2002; Rana and Verma, 2005). The effects of intraperitoneal injection of benzene and various of its metabolites on erythropoiesis were studied in mice in vivo (Snyder et al., 1989). The most potent inhibitor of red blood cell (RBC) production was a mixture of hydroquinone (50 mg/kg) and t,t-muconaldehyde (1 mg/kg). Several other metabolites also inhibited red cell formation.

Transgenic mice, in which the gene for CYP2E1 has been knocked out (*cyp2e1^{-/-}*), do not show toxicity when exposed to 200 ppm benzene for 6 hr (Valentine et al., 1996). CYP2E1 protein and its associated enzymatic activity were not detected in early fetal human liver samples (Vieira et al., 1996).

Transgenic male mice, in which the gene for microsomal epoxide hydrolase was knocked out, did not show benzene toxicity (e.g., decreased white blood cell (WBC) counts) when exposed to 50 ppm benzene for two weeks, while control male mice did (Bauer et al., 2003). In humans, susceptibility to chronic benzene poisoning has been related to the epoxide hydrolase genotype (Sun et al., 2008). Specifically the risk of benzene poisoning increased in the subjects with microsomal epoxide hydrolase (EPHX1) GGAC/GAGT diplotype (P = 0.00057) or AGAC/GAGT diplotype (P =

0.00086). Surprisingly, neither diplotype altered the level of microsomal epoxide hydrolase enzyme activity.

Yoon and co-workers investigated the involvement of the aryl hydrocarbon receptor (AhR) in benzene hematotoxicity using wild-type (AhR^{+/+}), heterozygous (AhR^{+/-}), and homozygous null (AhR^{-/-}) male mice (Yoon et al., 2002). No hematotoxicity and no changes in peripheral blood and bone marrow cells were induced in AhR^{-/-} mice by a 2-week inhalation exposure to 300 ppm (978 mg/m³) benzene. The lack of hematotoxicity was associated with the lack of p21 over-expression, regularly seen in wild-type mice following benzene inhalation. (p21, also known as Cdkn1a in mice and CDKN1A in humans, is a cyclin-dependent kinase inhibitor important in the braking of the cell division cycle.) Combined treatment of AhR^{-/-} mice with two benzene metabolites (phenol and hydroquinone) induced hematopoietic toxicity. The aryl hydrocarbon receptor may have a role in the regulation of hematopoiesis and in benzene-induced hematotoxicity (Gasiewicz et al., 2010).

In Wistar rats of both sexes with a large body fat content, benzene was eliminated more slowly and remained in the body for a longer time than in rats with a small body fat content. Accordingly, the decrease in WBC during chronic benzene exposure was seen only in rats with large volumes of fat tissue. In humans, the elimination of benzene was slower in women than in men. The slower elimination in women is due primarily to the bulky distribution of body fat tissue. The authors concluded that women may be inherently more susceptible to benzene, which has a high affinity for fat tissue (Sato et al., 1975).

The pharmacokinetics of benzene follows a two-compartment model in the rat. The rapid phase has an elimination half-life ($t_{1/2}$) of 0.7 hours, and the $t_{1/2}$ for the longer phase is 13.1 hours (Rickert et al., 1979). The long elimination half-life for benzene is due to the formation of catechol, quinone, and hydroquinone in the bone marrow. These reactive metabolites are not readily excreted, and are cytotoxic to the stem cells in the bone marrow (Greenlee et al., 1981).

A three-compartment model was fit to human data on benzene disposition and bone-marrow metabolism (Watanabe et al., 1994). The general relationship between cumulative quantity of metabolites produced and inhalation concentration, was S-shaped, inflecting upward at low concentrations, and saturating at high concentrations.

Kim, Rappaport and associates studied the relationships between levels of five benzene metabolites and benzene exposure among 250 exposed and 136 control Chinese workers (Kim et al., 2006a; Kim et al., 2006b). Benzene metabolism was nonlinear with increasing benzene levels above 0.03 ppm. They then statistically tested whether human metabolism of benzene is better fit by a kinetic model having two pathways rather than only one (i.e., CYP2E1) (Rappaport et al., 2009). Michaelis-Menten-like models were fit to urinary benzene metabolites and the corresponding air concentrations of benzene (range: < 0.001 ppm - 299 ppm (0 - 975 mg/m³)) for 263 nonsmoking Chinese females. The different values of Akaike's information criterion

(AIC) obtained with the two models gave strong statistical evidence favoring two metabolic pathways. The low-affinity pathway (likely due to CYP2E1) had an affinity (“ K_m ”) of 301 ppm (981 mg/m³) for benzene in air; the value for the high-affinity pathway (unknown but possibly due to CYP2F1 or CYP2A13) was 0.594 ppm, a 500-fold difference. The exposure-specific metabolite level predicted by the two-pathway model at non-saturating benzene concentrations was 184 μ M/ppm of benzene, which is close to an independent estimate of 194 μ M/ppm for a nonsmoking Chinese female (Weisel et al., 2003). Rappaport estimated that a nonsmoking woman would metabolize about three times more benzene from the ambient environment under the two-pathway model (184 μ M/ppm) than under the one-pathway model (68.6 μ M/ppm). A follow-up study examined the individual urinary metabolites of benzene in each woman (Rappaport et al., 2010). The data indicated that the predicted high-affinity enzyme is predominant at less than 1 ppm and favors the ring-opening pathway to t,t-muconaldehyde (see Figure 4.1). The concept of increased efficiency in benzene metabolism at levels below 3 ppm has important implications for low level environmental exposures. The concept has been challenged (Price et al., 2012; 2013) and defended by the original authors (Rappaport et al., 2013).

4.1 Physiologically-based Pharmacokinetic Modeling

Based partly on an early PBPK model for styrene (Ramsey and Andersen, 1984), Medinsky and coworkers developed a model to describe the uptake and metabolism of benzene in F344/N rats and B6C3F1 mice and to determine if the observed differences in toxic effects between mice and rats could be explained by differences in metabolic pathways or by differences in uptake (Medinsky et al., 1989a). For inhalation concentrations up to 1,000 ppm (3,260 mg/m³) for six hours, mice metabolized at least two to three times as much total benzene (per kg body weight) as rats. In regard to metabolites, rats primarily formed phenyl sulfate, a detoxification product (see Table 4). In addition to phenyl sulfate, mice formed hydroquinone glucuronide and muconic acid which are part of toxicity pathways. The formation of hydroquinone showed the greatest difference between mice and rats. Metabolic rate parameters (V_{max} and K_m) were very different for hydroquinone conjugation and muconic acid formation compared to formation of phenyl conjugates and phenyl mercapturic acids. Assumed toxication pathways had high affinity, low capacity kinetics; detoxification pathways were low affinity, high capacity. Model simulations suggested that hydroquinone and muconic acid comprised a larger fraction of the total benzene metabolized at lower benzene levels for both rats and mice than at higher levels (where detoxification metabolites predominated). (See also Table 4.1)

The animal model was extended to predict benzene metabolism in people exposed near occupational exposure limits in effect at the time of the paper’s publication (Medinsky et al., 1989b). For 8 hr inhalation exposures less than 10 ppm (32.6 mg/m³), metabolites of hydroquinone, the precursor of the toxic benzoquinone, were predicted to predominate in people. Lower levels of muconic acid, a metabolite of muconaldehyde, were predicted below 10 ppm. Above 10 ppm, detoxification metabolites, including phenyl conjugates, predominated (Medinsky et al., 1989a).

A PBPK model, based on that of Medinsky above, was developed to describe the disposition of benzene in 3 and 18 month-old C57BL/6N mice and to examine the key parameters affecting changes in benzene disposition with age (McMahon et al., 1994). The model included a rate constant for urinary elimination of metabolites as an age-related increase in K_m for production of hydroquinone conjugates from benzene. The study indicated that age-related changes in physiology, including decreased elimination of hydroquinone conjugates at 18 months, were responsible for altered disposition of benzene in aged mice.

Travis and colleagues developed three PBPK models to describe the pharmacokinetics of benzene in mice, rats, and humans, respectively, using five anatomical compartments: liver, fat, bone marrow, muscle (poorly perfused), and other richly perfused organs (e.g., brain, heart, kidney, and viscera), all interconnected by the arterial and venous blood flow (Travis et al., 1990). Benzene metabolism showed Michaelis-Menten kinetics and occurred primarily in the liver (human V_{max} = 29.04 mg/h) and secondarily in the bone marrow (human V_{max} = 1.16 mg/h). Graphical comparison of model results with empirical data in the three species from previously published reports was quite favorable for exposure by inhalation and gavage, and from intraperitoneal and subcutaneous injection.

Bois and colleagues modeled the distribution and metabolism of benzene in humans using population pharmacokinetics, Bayesian statistical inference, and PBPK modeling (Bois et al., 1996). Using existing data, they derived distributions for model parameters. The model adequately fit both prior physiological information and recent experimental data (Pekari et al., 1992). An estimate of the relationship between benzene exposure up to 10 ppm (32.6 mg/m³) and the median population fraction metabolized in the bone marrow is 52 percent (90% CI = 47-67%), and was linear in the three subjects studied by Pekari et al. (1992). At 1 ppm continuous exposure, the occupational inhalation exposure threshold in the U.S., the estimated quantity metabolized in the bone marrow ranged from 2 to 40 mg/day, a 20-fold variation.

Cole and coworkers developed a PBPK model in mice to relate inhaled benzene levels to tissue doses of benzene, benzene oxide, phenol, and hydroquinone (Cole et al., 2001). Parameter values in the literature were used. Additional parameters, estimated by fitting the model to published data, were first-order rate constants (k_i) for pathways lacking in vitro data and the concentrations of microsomal and cytosolic protein. The model was constrained by using the in vitro metabolic parameters (V_{max} , first-order rate constants (k_i), and saturation parameters), rather than in vivo values. Even though data from multiple laboratories and experiments were used, model simulations matched the data reasonably well in most cases. No extrapolation to humans was attempted.

Yokley and associates developed a human PBPK model that quantified tissue levels of benzene, benzene oxide, phenol, and hydroquinone after inhalation and oral benzene exposures (Yokley et al., 2006). The model was integrated into a statistical framework that acknowledges sources of variation due to inherent intra- and inter-individual

variation, measurement error, and other data collection issues. They estimated the population distributions of key PBPK model parameters. They hypothesized that observed interindividual variability in the dosimetry of benzene and its metabolites resulted primarily from known or estimated variability in key metabolic parameters and that a statistical PBPK model that explicitly included variability in only those metabolic parameters would sufficiently describe the observed variability. They identified parameter distributions for the PBPK model to characterize observed variability through the use of Markov chain Monte Carlo analysis applied to two data sets. The identified parameter distributions described most of the observed variability, but variability in physiological parameters such as organ weights may also be helpful to predict the observed human-population variability in benzene dosimetry.

Various benzene exposure scenarios were simulated for adult men and women using PBPK modeling (Brown et al., 1998). Women had a higher blood/air partition coefficient and maximum velocity of metabolism for benzene than men. Women generally had a higher body fat percentage than men. Physicochemical gender differences resulted in women metabolizing 23-26 percent more benzene than men in the same scenario, although benzene blood levels are generally higher in men. The authors stated that women may be at higher risk for certain effects of benzene exposure.

5 Acute Toxicity of Benzene

5.1 Acute Toxicity to Adult Humans

Deaths from acute exposure to benzene are often related to physical exertion and release of epinephrine with subsequent cardiac failure. Frequently, the person trying to rescue a collapsed victim will die during the effort of lifting the unconscious person (HSDB, 2007). Anesthesia may develop at concentrations above 3,000 ppm (9,600 mg/m³). At exposures greater than 1,000 ppm (3,200 mg/m³) (duration unspecified), CNS symptoms include giddiness, euphoria, nausea, and headaches; heightened cardiac sensitivity to epinephrine-induced arrhythmias may develop (Snyder, 1987). These effects may be accompanied by symptoms of mild irritation to the eyes and mucous membranes. Acute hemorrhagic pneumonitis is highly likely if benzene is aspirated into the lung (HSDB, 2007). Respiratory tract inflammation, pulmonary hemorrhages, renal congestion, and cerebral edema have been observed at autopsy in persons with acute benzene poisoning. In these cases, blood levels of 2 mg percent (2 mg/100 ml) benzene were not associated with hematological changes (Winek and Collom, 1971).

A case report described three deaths due to acute benzene poisoning from a shipboard accident (Avis and Hutton, 1993). Exposure levels were not estimated. Autopsies showed skin, respiratory, and cerebral injury. Benzene levels in body fluids and tissues were consistent with the lipophilicity of benzene. In a single fatal acute case of benzene intoxication aboard a chemical cargo ship (Barbera et al., 1998), the authors found blood clots inside the heart and main vessels, multi-organ congestion, and pulmonary

edema. They also measured benzene (rounded to whole numbers) in several organs: liver (379 µg/g tissue), heart (183 µg/g tissue), brain (179 µg/g tissue), kidneys (75 µg/g tissue), lungs (22 µg/g tissue), blood (32 µg/mL), and urine (2 µg/mL).

Systemic poisoning by benzene can occasionally result in neuroretinal edema and in retinal and conjunctival hemorrhage (Grant, 1986). Additionally, petechial hemorrhages of the brain, pleura, pericardium, urinary tract, mucous membranes, and skin may occur in cases of fatal, acute benzene poisoning (Haley, 1977).

Major concerns of systemic benzene toxicity include pancytopenia and acute myelogenous leukemia (IARC, 1982). Both are typically seen in chronic and subchronic exposures, but may be of concern following acute exposures as well. Cells of the myeloid pathway, erythroid in particular, are specific targets of benzene toxicity.

Fifteen degassers were acutely exposed over several days to > 60 ppm benzene during removal of residual fuel from fuel tanks on ships (Midzenski et al., 1992). The maximal level was approximately 653 ppm (2,129 mg/m³). Volatilization of benzene from the residual fuel was the suspected source of benzene. Twelve workers reported mucous membrane irritation. Eleven reported neurotoxic symptoms. Workers with more than 2 days (8 hours/day) of acute exposure were significantly more likely to report dizziness and nausea than those with 2 or fewer days. Blood cell analyses over a 4-month period after exposure found at least one hematologic abnormality consistent with benzene exposure in 9 degassers. For example, white blood cell counts were below normal in 4 workers. At one year, 6 workers had persistent abnormalities; an additional worker, with normal hematologic parameters initially, later developed an abnormality consistent with benzene exposure. Many large granular lymphocytes were found in the peripheral blood smears of six of the workers. There were no significant associations between the presence of hematologic abnormalities and either the number of hours of acute benzene exposure or the duration of employment as a degasser in this study.

Two studies may indicate an acute NOAEL for adult humans. Japanese students between the age of 18 and 25 breathed one of seven organic solvents for 2.7 to 4 hours in a 60 m³ chamber. No adverse effects were reported in three males and three females exposed to 52-62 ppm benzene for the full 4 hours (Nomiyama and Nomiyama, 1974). In a study of the absorption and elimination of inhaled benzene (Srbova et al., 1950), the authors mentioned that no adverse effects were seen in 23 adult volunteers exposed to 47 to 110 ppm benzene for 2 to 3 hours. Thus 110 ppm (359 mg/m³) is a possible 3-hour NOAEL for acute effects of benzene in humans (National Academy of Sciences, 2009).

5.2 Acute Toxicity to Infants and Children

No studies of the effects of acute or short-term inhalation exposure to benzene in infants and children were found in the published literature.

5.3 Acute Toxicity to Experimental Animals

The oral LD₅₀ in rats was calculated to be 3.4 g/kg in young rats and 4.9 g/kg in older rats (Kimura et al., 1971). Death was observed in 2 out of 10 rats exposed to 33,000 mg/m³ (10,300 ppm) for 12.5-30 minutes daily for either 1 or 12 days ((IARC, 1982)). A 4-hour LC₅₀ of 13,700 ppm (43,800 mg/m³) was reported in female rats (Drew and Fouts, 1974; IARC, 1982). An LC_{Lo} of 45,000 ppm (144,000 mg/m³) is reported in rabbits (NIOSH, 1994). In mice, an LC₅₀ of 9,800 ppm (31,400 mg/m³) is reported (NIOSH, 1994). Leukopenia has been demonstrated to occur in rabbits exposed to 240 ppm (767 mg/m³) for 10 hours/day for 2 weeks (IARC, 1982).

Brief inhalation of air saturated with benzene vapor (concentration unknown) resulted in ventricular extrasystole in cats and primates, with periods of ventricular tachycardia that occasionally terminated in ventricular fibrillation (Sandmeyer, 1981a).

The RD₅₀ is a chemical concentration that depresses the respiratory rate in mice by 50 percent due to sensory irritation of the upper respiratory tract. An attempt to determine the inhalation RD₅₀ for benzene was not successful (Nielsen and Alarie, 1982). The investigators showed that inhalation of 5,800 ppm (18,800 mg/m³) benzene in mice caused an increase in respiratory rate beginning at 5 minutes following onset of exposure. They speculated that the stimulation of respiratory rate resulted from the action of benzene on the central nervous system. In this study, the authors reported that benzene was not irritating to the upper airways of the animals up to 8,500 ppm (27,710 mg/m³).

Repeated subcutaneous dosing of mice with benzene for 6 to 20 days resulted in a dose-related decrease in red blood cell production as measured by the incorporation of ⁵⁹Fe into developing erythrocytes. The DBA mouse strain was more sensitive than the CD-1 and C57B6 strains. Research using multiple species indicated that mice are more sensitive to adverse effects on erythropoiesis from benzene than are rabbits which are more sensitive than rats (Longacre et al., 1980; Longacre et al., 1981a; IARC, 1982).

Acute exposure to benzene may disrupt erythropoiesis and result in genotoxicity. Subcutaneous injection of 5, 13, 33, and 80 mmol/kg (390, 915, 2,577, and 6,248 mg/kg) benzene in 8- to 10-week-old, male, Swiss-Webster mice inhibited erythropoiesis in a dose-dependent manner, as measured by uptake of radiolabeled iron in the bone-marrow 48 hours after benzene injection (Bolcsak and Nerland, 1983). Three metabolites of benzene (phenol, hydroquinone, and catechol) also inhibited iron uptake.

Results from subacute exposures further illustrate the hematotoxic effects of benzene and the potential for immunotoxicity. Inhalation of 103 ppm (334 mg/m³) benzene for 6 hours/day for 7 days by mice caused decreased spleen and marrow cellularity and decreased spleen weights (Green et al., 1981). Benzene inhalation at 0, 10, 30, 100, and 300 ppm (0, 32.6, 97.3, 326, and 973 mg/m³) for 6 hours/day for 5 days resulted in a decreased host-resistance to bacterial infection by *Listeria monocytogenes*

(Rosenthal and Snyder, 1985). The numbers of *L. monocytogenes* isolated from the spleen were increased in a dose-dependent manner on day 4 of infection. The total numbers of T- and B-lymphocytes in the spleen and the proliferative ability of the splenic lymphocytes were decreased in a dose-dependent manner by benzene exposures of 30 ppm (97.3 mg/m³) or greater. No decrement in host resistance or immune response was observed at 10 ppm (32.6 mg/m³) benzene. Later studies in mice have also shown that exposure to 10 ppm for a subacute duration does not significantly alter hematological parameters in blood, spleen, thymus, or bone marrow.

Inhalation of 0, 10, 31, 100, or 301 ppm (0, 32.6, 100.4, 326, or 978 mg/m³) benzene for 6 hours/day for 6 days resulted in a dose-dependent reduction in peripheral lymphocytes, and a reduced proliferative response of B- and T-lymphocytes to mitogenic agents in mice (Rozen et al., 1984). Total peripheral lymphocyte numbers and B-lymphocyte proliferation in response to lipopolysaccharide (LPS) were significantly reduced at 10 ppm (32.6 mg/m³). The proliferation of T-lymphocytes was significantly reduced at 31 ppm (100.4 mg/m³).

Farris et al. (1997) reported the hematological consequences of benzene inhalation in 12-week-old male B6C3F1/CrIBR mice exposed to 1, 5, 10, 100, and 200 ppm (3.26, 16.3, 32.6, 326, and 652 mg/m³) benzene for 6 hr/day, 5 days/week for 1, 2, 4, or 8 weeks. The study also evaluated hematology in small recovery subset groups at each concentration (4 weeks exposure to benzene, then up to 25 days in air). There were no significant effects on hematopoietic parameters from exposure to 10 ppm benzene or less. Thus 10 ppm (32.6 mg/m³) was a NOAEL for up to 8 weeks of exposure in this study. Exposure to 100 and 200 ppm benzene reduced the number of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, and most blood parameters. Replication of primitive progenitor cells in the bone marrow was increased during the exposure period as a compensation for the cytotoxicity. At 200 ppm, the primitive progenitor cells maintained an increased percentage of cells in S-phase through 25 days of recovery compared with controls.

Evans and coworkers used CD1 and C57BL/6J mice in a time-sampling protocol to quantify seven categories of behavior (stereotypic, sleeping, resting, eating, grooming, locomotion, and fighting). Animals were exposed 6 hours per day for 5 days to 0, 300, or 900 ppm (0, 960 or 2,930 mg/m³) benzene followed by two weeks of no benzene exposure. The authors designed the inhalation exposures to "reflect" occupational exposure at that time. An increase in active behavior in the form of eating and grooming was observed in both strains of mice following exposure to 300 ppm and 900 ppm benzene for 6 hours/day for 5 days (Evans et al., 1981).

Exposure of BALB/c male mice to 50 ppm (162 mg/m³) benzene on 14 consecutive days resulted in a significantly reduced blood leukocyte count (Aoyama, 1986).

6 Chronic Toxicity of Benzene

6.1 Chronic Toxicity to Adult Humans

The primary toxicological effects of chronic benzene exposure are on the hematopoietic system. Neurological and reproductive/developmental toxic effects are also of concern at slightly higher concentrations. Impairment of immune function and/or various types of anemia may result from the hematotoxicity. The hematologic lesions in the bone marrow can lead to peripheral lymphocytopenia and/or pancytopenia following chronic exposure. Severe benzene exposures can also lead to life-threatening aplastic anemia. These lesions may lead to the development of leukemia years later, after apparent recovery from the hematologic damage (Degowin, 1963).

Investigators observed 28 cases of pancytopenia among 32 patients in Turkey, who were chronically exposed to benzene vapors from adhesives ranging from 150 to 650 ppm (489 to 2,119 mg/m³) for 4 months to 15 years (Aksoy et al., 1972). Bone marrow punctures revealed variable hematopoietic lesions, ranging from acellularity to hypercellularity. Central nervous system (CNS) abnormalities were reported in four of six individuals with pancytopenia following chronic occupational exposure to unknown concentrations of benzene for an average of 6 years (Baslo and Aksoy, 1982). The abnormalities included reduced sensory and motor nerve conduction velocities.

A retrospective longitudinal study correlated average benzene exposure with total white blood cell counts in a cohort of 459 Pliofilm rubber workers in Ohio (Kipen et al., 1988). The authors found a significant ($p < 0.016$) negative correlation between average workplace benzene concentrations and white blood cell counts for the years 1940-1948. A reanalysis of these data (Cody et al., 1993) showed significant decreases in red (RBC) and white (WBC) blood cell counts among a group of 161 workers during the 1946-1949 period compared with their pre-exposure blood cell counts. The decline in blood counts was measured over the course of 12 months following start of exposure. During the course of employment, workers with low monthly blood cell counts were transferred to other areas with lower benzene exposures; this potentially created a bias towards non-significance by removing sensitive subjects from the study population. Since there was a reported 75 percent rate of job change within the first year of employment, this bias could be highly significant. In addition, there was some indication that blood transfusions were used to treat some "anemic" workers, which would cause serious problems in interpreting the RBC data, since RBCs have a long lifespan (120 days) in the bloodstream. Two of Cody's co-authors performed the exposure analysis and determined a range of monthly median exposures of 30-54 ppm throughout the 12-month segment examined (Crump and Allen, 1984). Despite the above-mentioned potential biases, workers exposed above the median concentrations displayed significantly decreased WBC and RBC counts compared with workers exposed to the lower concentrations using a repeated measures analysis of variance (ANOVA).

The mortality from all cancers and leukemia, in addition to hematologic parameters, was investigated in male workers exposed to benzene for 1-21 years between 1952 and

1978 at a Gulf Oil refinery in Texas (Tsai et al., 1983). The cohort of 454 included maintenance workers and utility men and laborers assigned to benzene units on a “regular basis”. Exposures to benzene were determined using personal monitors. The median air concentration was 0.53 ppm (1.7 mg/m³) in the work areas of greatest exposure to benzene and the average length of employment was 7.4 years. The analysis of overall mortality revealed no significant excesses. Mortality from all causes (SMR = 0.58, $p \leq 0.01$) and from diseases of the circulatory system (SMR = 0.54, $p \leq 0.05$) was significantly below expected values based on comparable groups of U.S. males. The authors concluded that a healthy worker effect was present. An internal comparison group of 823 people, including 10 percent of the workers who were employed in the same plant in operations not related to benzene, showed relative risks (RR) of 0.90 and 1.31 for all causes and cancer at all sites, respectively ($p < 0.28$ and 0.23). A subset of 303 workers was followed for medical surveillance. Up to four hematological tests per year were conducted. Total and differential WBC counts, hemoglobin, hematocrit, RBC, platelets, and clotting times were found to be within normal (between 5% and 95% percentile) limits in this group. (This study was the basis of OEHHA’s previous chronic REL of 60 µg/m³.)

Complete blood count (CBC) data from employees who had ever participated in the Shell Benzene Medical Surveillance Program were compared to employees who had not participated (Tsai et al., 2004). The study included 1,200 employees in the surveillance program (mean eight hour TWA benzene exposure of 0.60 ± 5.60 ppm (median = 0.1 ppm) (2 ± 18 mg/m³) from 1977 to 1988 and of 0.14 ± 0.82 ppm (median = 0.1 ppm) (0.5 ± 2.7 mg/m³) since 1988) and 3,227 comparison employees. The study evaluated abnormality of six blood count parameters (WBC, lymphocytes, RBC, hemoglobin, mean corpuscular volume (MCV), and platelets) and the adjusted mean values of these parameters in the exposed group. No increased abnormality of the six parameters was found among exposed employees, however a significant decrease ($p = 0.02$) in the MCV was seen in the exposed workers. The mean values of the exposed employees, adjusted for age, gender, race, amount of time between first and last exam, and current smoking, were similar to those in the comparison group. No adverse hematological effects were found. However, the “exposed” group had a very wide range of benzene exposure levels as evidenced by the reported mean, standard deviation, and median values. The coefficient of variation (SD/mean) was greater than 100 percent. The median exposure value of 0.1 ppm (0.326 mg/m³) means that half the workers were exposed below that level.

Routine data collected from 1980 to 1993 for Monsanto’s medical/industrial hygiene system were used to study 387 workers with daily 8-hour time-weighted average exposures (TWA) of 0.55 ppm benzene (1.8 mg/m³) (range = 0.01 – 87.69 ppm; based on 4213 personal monitoring samples; less than 5 percent exceeded 2 ppm) (Collins et al., 1997). Controls were 553 unexposed workers. There was no increase in the prevalence of lymphopenia, an early, sensitive indicator of benzene toxicity, among exposed workers (odds ratio = 0.6; 95% confidence interval (CI) = 0.2 to 1.8), taking into account smoking, age, and sex. There also was no increase in risk among 266 workers exposed for 5 or more years (odds ratio = 0.6; 95% CI = 0.2 to 1.9). There were no

differences between exposed and unexposed workers for other measures of hematotoxicity, including mean corpuscular volume and counts of total white blood cells, red blood cells, hemoglobin, and platelets.

Between 1967 and 1994 a cohort of 105 workers exposed to low levels of benzene (as measured by personal monitors) was studied at a small petroleum company in Texas (Khuder et al., 1999). The exposure ranged from 0.14 ppm to 2.08 ppm (0.46 to 6.78 mg/m³) (8-hour TWA) (mean \pm 1 SD = 0.81 \pm 0.72 ppm). The mean complete blood counts (CBC) were within the normal range, as were the WBC. Other CBC values (RBC, hemoglobin level, MCV, and platelet count) were significantly reduced during the follow-up period. Duration of employment was significantly related to the changes in MCV and platelet counts. The reductions in MCV were statistically significant only among workers employed for more than 10 years. The study suggests that low levels of benzene may affect some CBC values.

A collaboration among the National Cancer Institute, the Shanghai Hygiene and Anti-Epidemic Center, the University of California Berkeley, and other institutions has produced an impressive amount of data on levels of benzene exposure and their effects on nearly 75,000 Chinese workers in 672 factories in 12 cities (Dosemeci et al., 1994; Yin et al., 1994; Hayes et al., 1996; Rothman et al., 1996b; Qu et al., 2002; Lan et al., 2004). The initial studies were on exposure between 1949 and 1987 (Dosemeci et al., 1994), but subsequent reports include later years. Chronic benzene poisoning, defined by a WBC level less than 4000 cells per microliter over several months duration, is a compensable adverse health effect for workers in China and a precursor of chronic disease.

In a cross-sectional study, hematologic outcomes were assessed in 44 (23 male and 21 female) workers heavily exposed to benzene (median = 31 ppm (101 mg/m³) as an 8-hr TWA) for six months to 16 years (mean = 6.3 years) at three workplaces in Shanghai (Rothman et al., 1996a). Controls were 44 age and gender-matched unexposed workers at two other workplaces. Hematologic parameters (total WBC, absolute lymphocyte count, platelets, RBC, and hematocrit) were decreased among exposed workers compared to controls (Table 6.1); an exception was the red blood cell mean corpuscular volume (MCV), which was higher among exposed subjects. In a subgroup of 11 workers with a median 8 hr TWA of 7.6 ppm (24.8 mg/m³) (range = 1-20 ppm) and not exposed to more than 31 ppm (101 mg/m³) on any of 5 sampling days, only the absolute lymphocyte count was significantly different between exposed workers and controls ($p = 0.03$). Among exposed subjects, a dose response relationship with various measures of current benzene exposure (i.e., personal air monitoring, benzene metabolites in urine) was present only for the total WBC count, the absolute lymphocyte count, and the MCV. The results support the use of the absolute lymphocyte count as the most sensitive indicator of benzene-induced hematotoxicity.

Table 6.1. Selected peripheral blood cell count data from Rothman *et al.* (1996)

	Controls (N=44)	≤ 31 ppm benzene Median (8 h TWA) = 13.6 ppm (N=22)	> 31 ppm benzene Median (8 h TWA) = 91.9 ppm (N=22)
WBC (mean (SD)/ μ L)	6800 (1700)	6400 (1800)	5600 (1900) ^a
Absolute lymphocyte count (mean (SD)/ μ L)	1900 (400)	1600 (300) ^a	1300 (300) ^c
RBC(mean $\times 10^3$ (SD)/ μ L)	4700 (600)	4600 (460) ^b	4200 (600) ^c
Platelets (mean (SD)/ μ L)	166 (59)	132 (45) ^b	121 (43) ^a
MCV (μ m ³)(mean(SD))	88.9 (4.9)	89.8 (3.9)	92.9 (3.4) ^c

^a p < 0.01; ^b p < 0.05; ^c p < 0.001

Subsequently the research group reported that in a case-control (50 cases, 50 controls) study within the Shanghai worker cohort, benzene poisoning was two to three times more likely if a person had either high CYP450 2E1 activity or no NAD(P)H:quinone reductase (NQO1) activity (NQO1*2/*2 genotype), and seven to eight times more likely with both high CYP450 2E1 activity and no NQO1 activity (Table 6.2) (Rothman *et al.*, 1997). The percentage of Chinese with the null activity NQO1*2/*2 genotype is five times that of non-Hispanic whites (22.4 % vs 4.4 %) (Ross, 2005). The data support the role of benzoquinone as a key metabolite in benzene-induced toxicity.

Table 6.2. Joint effects of CYP2E1 activity and NQO1 genotype on benzene poisoning from Rothman *et al.* (1997)

CYP2E1 activity	NQO1 genotype	Odds ratio	95% CI	No. cases
Slow	+/+ and +/-	1	-	8
Slow	-/-	2.4	0.6-9.7	6
Rapid	+/+ and +/-	2.9	1.0-8.2	21
Rapid	-/-	7.6	1.8-31.2	13

In a study that partially confirmed and extended the study of Rothman and colleagues, Chen and coworkers studied single nucleotide polymorphisms (SNPs) in CYP2E1, NQO1, MPO, GSTM1 and GSTT1 in 100 benzene-exposed workers diagnosed with chronic benzene poisoning and 90 benzene-exposed matched controls (Chen *et al.*, 2007). There was a 2.82-fold (95% CI = 1.42-5.58) increased risk of benzene poisoning in the workers with the NQO1 609C > T mutation genotype (T/T) compared with the heterozygote and the wild-type (C/C). Workers with the GSTT1 null genotype had a 1.91-fold (95% CI = 1.05-3.45) increased risk of poisoning compared with those with GSTT1 non-null genotype. A three genes' interaction revealed a 20.41-fold (95% CI = 3.79-111.11) increased risk of poisoning in subjects with the NQO1 609C > T T/T genotype and with the GSTT1 null genotype and the GSTM1 null genotype compared with those carrying the NQO1 609C > T C/T and C/C genotype, GSTT1 non-null genotype, and GSTM1 non-null genotype. These authors found no association of

CYP2E1 and MPO genotype with chronic benzene poisoning in this benzene-exposed population.

Personal benzene exposure and blood cell counts were measured in 130 exposed workers (62 men and 68 women) in three factories in Tianjin, China and in 51 age- and gender-matched unexposed subjects (Qu et al., 2002). Benzene air levels on the day of blood sampling ranged from 0.06 to 122 ppm (0.2 to 398 mg/m³). The 4-week average exposure levels were 0.08 to 54.5 ppm (0.26 to 178 mg/m³). Significant decreases of RBC, WBC, and neutrophils were observed (Table 6.3). The decreases correlated with both personal benzene exposures and levels of biomarkers: the urinary metabolites S-phenylmercapturic acid and trans,trans-muconic acid, and the albumin adducts of benzene oxide and 1,4-benzoquinone. The depressions in RBC, WBC, and neutrophils were exposure dependent and were also significantly different in the lowest exposed group (≤ 0.25 ppm) compared with unexposed subjects. The results suggested to the authors that lymphocytes may not be more sensitive than neutrophils to chronic benzene exposure. Unfortunately the results may be confounded since the workers were also exposed to toluene, which protects against the adverse effects of benzene (Snyder et al., 1989). The statement that some cell counts were significantly depressed at ≤ 0.25 ppm benzene is potentially important, but it was difficult to ascertain from the presentation of the data how many workers were exposed at that level.

Table 6.3. Selected peripheral blood cell count data from Qu et al. (2002)

Workers (N)	51	54	36	29	11
Mean (SD) cumulative exposure (ppm-yr)	0	32 (21)	74 (51)	123 (65)	237 (188)
Current exposure (ppm) (last four weeks)	0.004 (0.003)	3.07 (2.9)	5.89 (4.8)	17.4 (15.5)	50.6 (55.4)
RBC ($\times 10^4/\mu\text{L}$)*	463 (52)	403 (62) [#]	396 (57)	404 (51)	391 (39)
WBC (per μL)*	6671 (1502)	6383 (1330)	6089 (1455)	6103 (1560)	4727 (548)
Neutrophils (per μL)*	4006 (1108)	3377 (868) [#]	3491 (1121)	3501 (1314)	2480 (451)

*p < 0.001, test for exposure-response trend

[#]p < 0.01 vs. control by t test for difference between the means (only lowest benzene tested)

A cross-sectional survey studied 250 (86 male and 164 female) Chinese workers exposed in two shoe manufacturing facilities to glues containing 0.6 to 34 percent benzene for 6.1 ± 2.1 years (Lan et al., 2004). For each worker, individual benzene (and toluene) exposure was monitored repeatedly (up to 16 months) before blood samples were drawn. WBC and platelet counts were significantly lower than in the 140 control garment workers, even for exposure below 1 ppm benzene in air (mean = 0.57 ± 0.24 ppm) (1.86 ± 0.78 mg/m³) (Table 6.4). Progenitor cell colony formation declined significantly with increasing benzene exposure and was more sensitive to the effects of

benzene than was the number of mature blood cells. Genetic variants in myeloperoxidase (MPO) and NQO1 influenced susceptibility to benzene hematotoxicity. Increased myeloperoxidase activity and decreased NQO1 activity were associated with increased hematotoxicity. The authors concluded that hematotoxicity from benzene exposure may be evident among genetically susceptible subpopulations. A confounder is the co-exposure of the workers to toluene, a competitive inhibitor of benzene metabolism.

In response to criticism (Lamm and Grunwald, 2006) of the adequacy of their dose-response data, the authors (Lan et al., 2006) confirmed the monotonicity of their data by spline regression analysis of benzene exposure and WBC counts. They found no apparent threshold in their exposure range of 0.2 to 75 ppm (0.65 to 245 mg/m³) benzene.

Table 6.4. Selected peripheral blood cell count data from Table 1 of Lan et al. (2004)

	Controls (< 0.04 ppm) (n = 140)	Low exposure 0.57 ppm (n = 109)	Medium 2.85 ppm (n = 110)	High 28.73 ppm (n = 31)	p for 0.57 ppm vs. controls
WBC	6480 (1710)*	5540 (1220)	5660 (1500)	4770 (892)	< 0.0001
Granulocytes	4110 (1410)	3360 (948)	3480 (1170)	2790 (750)	< 0.0001
Lymphocytes	2130 (577)	1960 (541)	1960 (533)	1800 (392)	0.018
CD4 ⁺ T cells	742 (262)	635 (187)	623 (177)	576 (188)	0.003
B cells	218 (94)	186 (95)	170 (75)	140 (101)	0.003
Monocytes	241 (92)	217 (97)	224 (93)	179 (74)	0.018
Platelets	230 (60) x 10 ³	214 (49) x 10 ³	200 (53) x 10 ³	172 (45) x 10 ³	0.023

* Unadjusted mean cell number (\pm 1 SD) per microliter of blood

In order to identify specific genes involved in the cell count changes above, the authors used a commercial assay (Golden Gate assay by Illumina) to analyze 1,395 single nucleotide polymorphisms (SNPs) in 411 genes in the 250 benzene-exposed workers and the 140 unexposed controls (Lan et al., 2009). Highly significant findings clustered in five genes (BLM, TP53, RAD51, WDR79, and WRN) that have important roles in DNA repair and genomic maintenance. TP53 (tumor protein p53), the “guardian of the genome,” is a tumor suppressor gene and is mutated in up to 50 percent of human cancers. WDR79 is located next to TP53 on chromosome 17 and is also known as TCAB1 and WRAP43 (for WD40-encoding RNA antisense to p53). BLM (for Bloom syndrome) codes for a member of the RecQ helicase family which is involved in DNA replication fork repair processes. WRN (Werner’s syndrome) codes for an enzyme(s) with helicase, exonuclease, and ATPase properties. RAD is a homologue of RecA involved in homologous recombination and repair. One or more SNPs in each gene were associated with statistically significant reductions of 10-20 percent in the WBC count among benzene-exposed workers ($p = 0.0011$ to 0.0002) but not among controls.

A further study of this cohort involved 1,023 tag SNPs in 121 gene regions important for benzene effects (Hosgood et al., 2009). Linear regression was used to relate genetic polymorphisms and total white blood cell (WBC) count and its subtypes. The minp (minimal p value) test assessed the association on the gene region level. The false discovery rate (FDR) method was used to control for multiple comparisons.¹ Vascular endothelial growth factor (VEGF) (minp = 0.0030) and ERCC3 (a gene involved in nucleotide excision repair of DNA) (minp = 0.0042) were the gene regions most significantly associated with altered WBC counts among benzene-exposed workers, after accounting for multiple comparisons. Statistically significant changes were also found for WBC subtype counts, including granulocytes, CD4+ T cells, and lymphocytes for VEGF, and granulocytes and NK cells for ERCC3. Further, in workers exposed to <1 ppm benzene, a SNP in VEGF was associated with changes in WBC and granulocyte counts, and SNPs in ERCC3 were associated with changes in WBC, NK cell, and granulocyte counts.

A cross-sectional study of the same workers evaluated the association between SNPs in genes involved in innate immunity and benzene hematotoxicity. A total of 1,236 tag SNPs in 149 gene regions of six pathways were analyzed (Shen et al., 2011). Six regions were significant for their association with WBC counts based on gene-region ($p < 0.05$) and SNP analyses (False Discovery Rate < 0.05): MBP (myelin basic protein), VCAM1 (vascular cell adhesion molecule 1), ALOX5 (arachidonate 5-lipoxygenase), MPO (myeloperoxidase), RAC2 (a member of a group of small GTPases), and CRP (C reactive protein). Specific SNPs for VCAM1, ALOX5, and MPO were the three most significant SNPs and showed similar effects on WBC subtypes: granulocytes, lymphocytes, and monocytes. A 3-SNP block in ALOXE3 showed a global association (omnibus $P = 0.0008$) with WBCs, but the SNPs were not individually significant.

Other recent studies have not found effects of benzene on blood cells at such low levels. Swaen and colleagues studied hematological effects at low benzene air levels among Dow Chemical Company employees in the Netherlands (Swaen et al., 2010). They compared 8,532 blood samples from 701 male workers with low benzene exposure to 12,173 samples from 1,059 male workers with no occupational benzene exposure for the years 1981-2007. A Job Exposure Matrix was constructed using 21,584 benzene air measurements for the exposed employees. The Matrix was used to estimate benzene exposure in the year in which each blood sample was collected. The average lymphocyte counts for the unexposed and exposed group were similar: 2,090.29 and 2,097.02 cells per microliter, respectively. Adjustments for smoking, age, and month of blood sample did not change the results. No adverse effect on any blood parameters was seen. Stratification into three exposure subgroups (<0.5 ppm (1.63 mg/m³), 0.5-1 ppm, and >1 ppm (3.26 mg/m³)) showed no significant differences for any of the blood parameters among the exposure categories including the non-exposed.

¹ The False Discovery Rate (FDR) of a set of predictions is the expected percent of false predictions in the set. If the algorithm returns 100 genes with an FDR of 0.3, expect 30 of them to be false and 70 to be correct.

In 1981, South Korea banned use of glues or solvents containing more than 1 percent benzene from the workplace, except where benzene is used in a completely sealed process. After the ban (in 2000), the number of workers possibly exposed to benzene was estimated to be 196,182 workers in 6,219 factories. Benzene exposure in different industries was assessed by reviewing the claimed cases for workmen's compensation due to hematopoietic diseases related to benzene which were investigated by Korea OSHA between 1992 and 2000 (Kang et al., 2005). Six factories were evaluated for benzene exposure. Personal air monitoring was performed in 61 workers; urine samples were collected from 57 to measure trans,trans-muconic acid (t,t-MA); and hematologic examination was performed. The geometric mean of benzene in air was 0.094 ppm (range = 0.005-5.311 ppm) (0.3 mg/m³; range = 0.02-17 mg/m³). Seven samples were higher than 1 ppm but less than 10 ppm, the occupational exposure limit in Korea. The geometric mean of t,t-MA in urine was 0.966 mg/g creatinine (range = 0.24-2.74). The benzene exposure level was low except in a factory where benzene was used to polymerize other chemicals. Ambient benzene from 0.1 to 1 ppm (0.326 to 3.26 mg/m³) was significantly correlated with urinary t,t-MA concentration (r=0.733, p<0.01). Hematologic parameters did not show any significant differences among groups divided by the level of exposure. Korean workers were not highly exposed to benzene and the level of exposure was mostly less than 1 ppm.

6.2 Chronic Toxicity to Infants and Children

Several epidemiological and ecological studies have been published, which examine the association between benzene exposure and health outcomes in children, notably leukemia (Brosselin et al., 2009). However, these studies use proximity to gasoline stations or roads with high traffic volumes as proxies for benzene exposure. Since the resulting exposures are to complex mixtures of VOCs and/or vehicular exhaust of which benzene is one component among many, the role of benzene in the reported health effects is not clear. Nonetheless, we briefly describe some below.

In France, Brosselin and colleagues studied the association between acute childhood leukemia and residing next to gas stations and automotive repair shops for 2003-2004 (the ESCALE study (SFCE))(Brosselin et al., 2009). A total of 765 cases of acute leukemia and 1,681 controls under 15 years of age was studied. Acute leukemia was significantly associated with residence next to either gas stations or automotive-repair garages (odds ratio (OR) = 1.6 [95% CI = 1.2-2.2]) and next to a gas station only (OR = 1.9 [95% CI = 1.2-3.0]). The OR did not show a tendency to increase with exposure duration. The results were not modified by adjustment for potential confounders including urban/rural status and type of housing (Brosselin et al., 2009). In a further study of this population, acute leukemia was significantly associated with higher estimates of traffic NO₂ levels at the home (> 27.7 µg/m³) compared with lower NO₂ levels (< 21.9 µg/m³) [OR = 1.2; 95% CI = 1.0-1.5] and with the presence of a heavily-trafficked road within 500 meters (m) of the home compared with the absence of such a road in the same area (OR=2.0; 95% CI, 1.0-3.6). There was a significant association between acute leukemia and a high density of heavy-traffic roads within 500 m

compared with the reference category with no heavy-traffic road within 500 m (OR = 2.2; 95% CI, 1.1-4.2), and a significant positive linear trend of the association of acute leukemia with the total length of heavy-traffic road within 500 m (Amigou et al., 2011).

An earlier case-control study in France for the years 1995-1999 involved 280 leukemia cases and 285 controls. There was no association between the mothers' work exposure to hydrocarbons during pregnancy and leukemia, or between residential traffic density and leukemia. There was a statistically significant association between residences near a gas station or an automotive repair shop during childhood and the risk of childhood leukemia (OR = 4.0, 95% CI = 1.5-10.3), with a positive duration trend. The association was strong for acute non-lymphocytic leukemia (OR = 7.7, 95% CI 1.7-34.3) and was not altered by adjustment for potential confounders (Steffen et al., 2004).

An ecologic analysis studied 977 cases of childhood lymphohematopoietic cancer diagnosed from 1995-2004 in Texas (Whitworth et al., 2008). Exposure values were the U.S. Environmental Protection Agency's 1999 modeled estimates of benzene and 1,3-butadiene for 886 census tracts surrounding Houston. Census tracts with the highest benzene levels had elevated rates of all leukemia [rate ratio (RR) = 1.37; 95% CI = 1.05-1.78]. The association was higher for acute myeloid leukemia (RR = 2.02; 95% CI, 1.03-3.96) than for acute lymphocytic leukemia (RR = 1.24; 95% CI, 0.92-1.66). Among census tracts with the highest 1,3-butadiene levels, the authors observed RRs of 1.40 (95% CI, 1.07-1.81), 1.68 (95% CI, 0.84-3.35), and 1.32 (95% CI, 0.98-1.77) for all leukemia, acute myeloid leukemia, and acute lymphocytic leukemia, respectively.

6.3 Chronic Toxicity to Experimental Animals

A number of animal studies have demonstrated that benzene exposure can induce bone marrow damage, changes in circulating blood cells, developmental and reproductive effects, alterations of the immune response, and cancer. With respect to chronic toxicity, hematological changes appear to be the most sensitive indicator (Table 6.5).

Wolf and coworkers conducted repeat benzene exposures (7-8 h/day, 5 days/week) in several species. Rabbits were exposed to 80 ppm (261 mg/m³) for 175 total exposures; rats were exposed to 88 ppm (287 mg/m³) for 136 total exposures; and guinea pigs were exposed to 88 ppm (287 mg/m³) for 193 total exposures (Wolf et al., 1956). The observed effects included leukopenia, increased spleen weight, and histological changes to the bone marrow.

Hematologic effects, including leukopenia, were observed in rats exposed to mean concentrations of 44 ppm (143 mg/m³) or greater for 5 to 8 weeks. Exposure to 31 ppm (100 mg/m³) benzene or less did not result in leukopenia after 3 to 4 months of exposure (Deichmann et al., 1963).

Among Sprague-Dawley rats and AKR/J mice exposed to 300 ppm (972 mg/m³) benzene, 6 hours/day, 5 days/week for life, Snyder and coworkers found lymphocytopenia, anemia, and decreased survival time (Snyder et al., 1978).

Male mice exposed to 400 ppm (1,304 mg/m³) benzene, 6 hours/day, 5 days/week for 9.5 weeks showed depressed bone marrow cellularity, decreased stem cell count, and altered morphology in spleen colony-forming cells (Cronkite et al., 1982)

Mice are more sensitive than rats or rabbits to the hematologic and leukemic effects of benzene (IARC, 1982; Sabourin et al., 1989). Metabolism of benzene to hydroquinone, muconic acid, and hydroquinone glucuronide is much greater in mice than rats, whereas the detoxification pathways are approximately equivalent between the two species (Sabourin et al., 1988).

A study on the chronic hematological effects of benzene exposure in C57 Bl/6 male mice (5 or 6 per group) showed that peripheral lymphocytes, red blood cells and colony-forming units (CFUs) in the bone marrow and spleen were significantly decreased in number after treatment with 10 ppm (32.4 mg/m³) benzene for 6 hours/day, 5 days/week for 178 days compared to unexposed controls (Baarson et al., 1984). Ten ppm, the only concentration studied, was the workplace exposure standard at the time.

Male and female mice (9 or 10 per group) were exposed to 10, 25, 100, 300 and 400 ppm benzene for 6 hours/day, 5 days/week for 2 to 16 weeks (Cronkite et al., 1985). After 2 weeks at 100 ppm (326 mg/m³) benzene and higher, mice showed both decreased bone marrow cellularity and a reduction of pluripotent stem cells in the bone marrow. The decrease in marrow cellularity continued for up to 25 weeks following a 16-week exposure to 300 ppm (972 mg/m³) benzene. Peripheral blood lymphocytes (PBLs) were dose-dependently decreased with benzene exposures of greater than 25 ppm (81 mg/m³) for 16 weeks, but recovered to normal levels following a 16-week recovery period.

Fifty Sprague-Dawley rats and 150 CD-1 mice of both sexes were exposed to 0, 1, 10, 30, or 300 ppm (0, 3.26, 32.6, 97.2, or 972 mg/m³) benzene, 6 hours/day, 5 days/week for 13 weeks (Ward et al., 1985). Serial necropsies were conducted at 7, 14, 28, 56, and 91 days (20 percent of each group of rodents at each time point). No hematological changes were found for mice and rats at 1, 10, or 30 ppm. In male and female mice significant increases in mean cell volume and mean cell hemoglobin values and decreases in hematocrit, hemoglobin, lymphocyte percentages, and decreases in red cell, leukocyte and platelet counts were observed at 300 ppm. The changes were first observed after 14 days of exposure. Histological changes in mice included myeloid hypoplasia of the bone marrow, lymphoid depletion in the mesenteric lymph node, increased extramedullary hematopoiesis in the spleen, and periarteriolar lymphoid sheath depletion. Effects were less severe in the rats. In this subchronic study 30 ppm (97.2 mg/m³) was a NOAEL.

The National Toxicology Program (NTP, 1986) conducted a chronic (2 year) toxicity “bioassay” in F344 rats and B6C3F1 mice of benzene by gavage in corn oil. Doses were 0, 25, 50, and 100 mg/kg-day for females and 0, 50, 100, and 200 mg/kg-day for males. Dose-related lymphocytopenia and leukocytopenia were observed in both species in all dosed groups. Mice exhibited lymphoid depletion of the thymus and spleen and hyperplasia of the bone marrow.

Investigators at Brookhaven National Laboratory exposed CBA/Ca mice to 10, 25, 100, 300, 400 and 3,000 ppm (32.6, 82, 326, 972, 1,304 and 9,720 mg/m³) benzene 6 hours/day, 5 days/week for up to 16 weeks (Cronkite et al., 1989). No effects were observed at 10 ppm. Lymphopenia was observed in the 25 ppm exposure group. Higher concentrations of benzene produced dose-dependent decreases in blood lymphocytes, bone marrow cellularity, spleen colony-forming units, and an increased percentage of CFU-S in S-phase synthesis.

Farris et al. exposed B6C3F₁ mice to 1, 5, 10, 100, and 200 ppm (3.26, 16.3, 32.6, 326, and 652 mg/m³) benzene for 6 hr/day, 5 days/week, for 1, 2, 4, or 8 weeks (Farris et al., 1997). In addition some animals were allowed to recover from the exposure for up to 25 days. There were no significant effects on hematopoietic parameters from exposure to 10 ppm benzene or less. Exposure to higher levels reduced the number of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, and most blood parameters. The replication of primitive progenitor cells was increased. The authors suggested that this last effect, in concert with the genotoxicity of benzene, could play a role in the carcinogenicity of benzene.

Table 6.5. Important non-acute animal inhalation studies of benzene

Study	Animal	Exposure	Duration	Effect	NOAEL	LOAEL
(Farris et al., 1997)	Mice	6 h/d, 5 d/wk	1, 2, 4, or 8 wk	hematopoiesis	10 ppm	100 ppm
(Cronkite et al., 1989)	Mice	6 h/d, 5 d/wk	Up to 16 wk	hematopoiesis	10 ppm	25 ppm
(Aoyama, 1986)	Mice	6 h/d	14 days	hematopoiesis	not found	50 ppm
(Ward et al., 1985)	mice & rats	6 h/d, 5 d/wk	13 weeks	hematopoiesis	30 ppm	300 ppm
(Cronkite et al., 1985)	Mice	6 h/d, 5 d/wk	2-16 wk	hematopoiesis	25 ppm	100 pm
(Baarson et al., 1984)	Mice	6 h/d, 5 d/wk	178 d	hematopoiesis	not found	10 ppm
(Deichmann et al., 1963)	Rats	5 h/d, 4 d/wk	5 wk-4 mo	hematopoiesis	31 ppm	44 ppm
(Wolf et al., 1956)	rabbits, rats, guinea pigs	7 h/d	136-193 exposures	hematopoiesis	not found	80-88 ppm

7 Developmental and Reproductive Toxicity

7.1 Human Studies

Based on blood samples taken at birth from mother and infant, benzene can cross the human placenta and be in the umbilical cord at a level equal to or greater than in maternal blood (Dowty et al., 1976). The database of benzene effects on human reproductive and developmental toxicity is limited to a few reports which usually have small samples, unmeasured exposure to benzene, and exposure to other chemicals. CYP2E1, which is a key enzyme in the pathway from benzene to its toxic metabolites, was not detected in human livers in the early fetal period (Vieira et al., 1996) but was detectable at low levels in some fetuses beginning in the second trimester (Johnsrud et al., 2003). In the third trimester CYP2E1 is present in most fetuses at 10-20 percent of adult levels (Table 7.1). However, many phase II enzymes which detoxify benzene metabolites are also low in the fetal period (McCarver and Hines, 2002). For example, for seven of eight substrates of UDP-glucuronyltransferase in human liver, the level during the fetal period ranged from less than 1 to 30 percent of adult levels and the level at the end of the fetal period ranged from 6 to 31 percent of adult levels. For only one substrate (5-hydroxytryptamine) were adult levels of UDP-glucuronyltransferase present in the fetal period (Leakey et al., 1987).

Table 7.1. Changes of CYP2E1 with age in human liver (Hines, 2007)

Age	N	pmol CYP2E1/mg protein
1 st trimester fetus: 8-13.4 weeks	14	- (not detectable)
2 nd trimester fetus: 13.6-25 weeks	45	0.3 ± 0.6 (mean ± SD)
3 rd trimester fetus: 27-40 weeks	14	5.8 ± 4.6
Neonate: 0-29 days	42	13.4 ± 16.0
Infant: 1.1-11.3 months	64	36.2 ± 20.3
Prepubertal: 1.1-10.0 years	41	43.1 ± 20.6
Adolescent: 11.0-17.7 years	20	~68 (median)
Adult	-	~50 (median)

The EDEN Mother-Child Cohort Study Group assessed the relation between personal exposure to airborne benzene in non-smoking pregnant French women and fetal growth (Slama et al., 2009). A group of 271 mothers recruited from the University Hospitals of Nancy and Poitiers from September 2003 through June 2006 carried a diffusive air sampler during week 27 of gestation to assess benzene exposure. The authors estimated head circumference of the offspring by ultrasound measurements during the second and third trimesters of pregnancy and at birth. Median benzene exposure was 1.8 µg/m³ (0.5 ppb) (5 and 95th percentiles, 0.5 and 7.5 µg/m³). An increase of 1 in log-transformed benzene exposure was associated with a gestational age-adjusted decrease of 68 g in mean birth weight (95% CI, -135 to -1 g; p = 0.05) and of 1.9 mm in mean head circumference at birth (95% CI, -3.8 to 0.0 mm; p = 0.06). Similarly, this differential in exposure was also associated with an adjusted decrease of 1.9 mm in head circumference during the third trimester (95% CI, -4.0 to 0.3 mm; p = 0.09) and of 1.5 mm in head circumference at the end of the second trimester (95% CI, -3.1 to 0 mm;

$p = 0.05$). The association cannot necessarily be attributed solely to benzene, particularly since the benzene exposure may reflect exposure to a mixture of associated traffic-related air pollutants. Traffic-related air pollutants have been associated in a number of studies with adverse birth outcomes including low birth weight.

Lindbohm and colleagues reported a statistically significant increase in spontaneous abortions in women whose husbands worked at petroleum refineries or with petroleum derived solvents including benzene (16 spontaneous abortions among 93 pregnancies; odds ratio = 2.2; 95% CI = 1.3-3.8) (Lindbohm et al., 1991).

The Texas Birth Defects Registry contained data on neural tube defects (533 cases of spina bifida and 303 cases of anencephaly) in babies delivered between 1999 and 2004 (Lupo et al., 2011). Census tract-level estimates of annual benzene, toluene, ethylbenzene, and xylene (BTEX) levels were obtained from the U.S. EPA's 1999 Assessment System for Population Exposure Nationwide. Mothers living in census tracts with the highest benzene levels were more than twice as likely to have offspring with spina bifida than were women living in census tracts with the lowest levels (odds ratio = 2.30; 95% CI = 1.22-4.33). No other significant associations were observed for benzene and no associations were found for toluene, ethylbenzene, and xylene. A variety of confounders such as race/ethnicity, maternal age, and socioeconomic status were taken into account.

Ghosh and colleagues investigated the effect of ambient air pollution in Los Angeles County on birth weight among 8,181 term (≥ 37 wk gestation), low birth weight (LBW; $<2,500$ g) children and 370,922 term normal birth weight children born from 1995 through 2006; all mothers lived within 5 miles of at least one of four stationary toxic air contaminant monitors (Ghosh et al., 2012). The influence of local variation in traffic pollution was assessed by land-use-based, regression-modeled estimates of oxides of nitrogen. Adjustments were made for maternal age, race/ethnicity, education, and parity, and for infant gestational age (and gestational age squared). Logistic regression indicated that the odds of term LBW increased 2–5 percent (range of 95% CI = 0%-9%) per interquartile-range increase in modeled traffic pollution estimates and in monitoring-based air toxics exposure estimates for (1) the entire pregnancy, (2) the third trimester, and (3) the last month of pregnancy. Models stratified by monitoring station (to investigate air toxics associations based solely on temporal variations) resulted in 2-5 percent increased odds per interquartile-range increase in third-trimester exposures to benzene, toluene, ethyl benzene, and xylene (BTEX); some confidence intervals indicated statistically significant effects. However, benzene was not a better predictor of LBW than toluene, ethyl benzene, xylene, or PAHs (Polycyclic Aromatic Hydrocarbons excluding naphthalene).

In the latter 1990s, the level of benzene in gasoline sold in the United States decreased as the result of regulation. Zahran and coworkers investigated the relationship between maternal exposure to benzene and birth weight outcomes among US residents in 1996 and 1999. A total of 3.1 million singleton births registered with the U.S. National Center for Health Statistics were included (Zahran et al., 2012). Maternal benzene

concentrations were estimated at the county level using data from the US EPA's National Air Toxics Assessment. Regression analysis estimated that a 1 $\mu\text{g}/\text{m}^3$ (0.3 ppb) increase in maternal exposure to benzene (1) reduced birth weight by 16.5 g (95% CI, 17.6-15.4), (2) increased the odds of a low birth weight (LBW) child by 7 percent, and (3) increased the odds of a very LBW child by a multiplicative factor of 1.23 (95% CI, 1.19-1.28). In counties where benzene levels decreased 25 percent from 1996 to 1999, birth weight increased by 13.7 g (95% CI, 10.7-16.8) and the risk of low birth weight (LBW) decreased by a factor of 0.95 (95% CI, 0.93-0.98). The authors admit that concentrating on benzene is a limitation since PM also affects birth weight.

Xing and coworkers used multicolor fluorescence in situ hybridization (FISH) to measure the incidence of sperm with numerical abnormalities of chromosomes X, Y, and 21 among 33 benzene-exposed men and 33 unexposed men from Chinese factories (Xing et al., 2010). Passive air monitors were used to measure benzene as well as toluene and xylene. Benzene levels for the exposed ranged from zero (i.e., limit of detection) to 24 ppm (78 mg/m^3); nine had levels ≤ 1 ppm (≤ 3.26 mg/m^3). Exposed men were grouped into low and high exposure based on levels of urinary t,t-muconic acid. Compared to controls, sperm aneuploidy increased across low- and high-exposed groups for disomy X [incidence rate ratio (IRR) for low = 2.0; 95% CI = 1.1-3.4; and IRR for high = 2.8; 95% CI = 1.5-4.9], and for overall hyperhaploidy for X, Y and 21 chromosomes (IRR for low = 1.6; 95% CI, 1.0-2.4; and IRR for high = 2.3; 95% CI, 1.5-3.6, respectively). Even for the nine exposed to ≤ 1 ppm, the authors found statistically significantly elevated disomy X (IRR = 1.8; 95% CI = 1.1-3.00) and hyperhaploidy (IRR = 2.0; 95% CI = 1.1-3.9) compared with the 33 unexposed men. In this study benzene increased the frequencies of aneuploid sperm for chromosomes associated with chromosomal abnormality syndromes in human offspring at surprisingly low levels. Further studies with this cohort using chromosome 1 and low, moderate and high exposure groups yielded IRRs and 95% CIs for all structural aberrations combined of 1.42 (95% CI = 1.10-1.83), 1.44 (CI = 1.12-1.85), and 1.75 (CI = 1.36-2.24) and for deletion of 1p36.3 alone of 4.31 (CI = 1.18-15.78), 6.02 (CI = 1.69-21.39), and 7.88 (CI = 2.21-28.05) for men with low, moderate, and high exposure, respectively, compared with unexposed men (Marchetti et al., 2012).

7.2 Animal Studies

Inhalation of ^{14}C -benzene by pregnant mice resulted in labeled material in the fetuses (Ghantous and Danielsson, 1986).

Groups of 40 female Sprague-Dawley rats were exposed to 0, 1, 10, 40, and 100 ppm (0, 3.26, 32.6, 129.6, or 326 mg/m^3) benzene for 6 hours/day during days 6-15 of gestation (Coate et al., 1984). At least 80 percent of the 40 females in each group littered (mean litter size = 13 fetuses). The viscera and skeletons of the fetuses were evaluated for variants and the fetal body weight and length were measured. No increase in variants was noted. A significant decrease was noted in the body weights of fetuses from dams exposed to 100 ppm (326 mg/m^3) benzene (Table 7.2). No effects were observed at 40 ppm (130 mg/m^3), the NOAEL for this experiment.

Table 7.2. Fetal body weights from Table 5 of Coate et al. (1984)

Group	Litters	ppm benzene	Fetal male bw (mean \pm SD)	Fetal female bw	Live† fetuses per litter (mean \pm SD)
1	32/40	0	4.02 \pm 0.349 g	3.78 \pm 0.303 g	13.0 \pm 3.10
2	33/40	0	4.06 \pm 0.430	3.85 \pm 0.477	12.5 \pm 2.98
3	37/40	1	3.86 \pm 0.381	3.69 \pm 0.350	13.8 \pm 2.47
4	37/40	10	3.88 \pm 0.303	3.70 \pm 0.385	13.3 \pm 2.56
5	37/40	40	3.91 \pm 0.492	3.64 \pm 0.382	12.9 \pm 2.90
6	35/40	100	3.77 \pm 0.226*	3.56 \pm 0.274*	13.8 \pm 2.43

* significantly lower than 0 ppm groups; $p < 0.05$

† There were no dead fetuses.

Exposure of pregnant Swiss Webster mice to concentrations as low as 5 ppm (16 mg/m^3) benzene on days 6-15 of gestation (6 hr/day) resulted in bone-marrow hematopoietic changes in the offspring that persisted into adulthood (Keller and Snyder, 1986). However, the hematopoietic effects (e.g., bimodal changes in erythroid colony-forming cells) were of uncertain clinical significance.

In a subsequent, similar study, the authors (Keller and Snyder, 1988) found that exposure of mice in utero for 6 h/day to 5, 10 and 20 ppm (16.3, 32.6, and 65.2 mg/m^3) benzene on days 6-15 of gestation resulted in suppression of erythropoietic precursor cells and persistent, enhanced granulopoiesis in peripheral blood cells of 2-day neonates (Table 7.3) and increased granulocytes in the livers of 2-day neonates and the spleens of adults at 6 weeks (data not shown). There was a dose-dependent decrease in early nucleated red cells (basophilic normoblasts) (Table 7.3). The authors considered these effects to be significant bone-marrow toxicity. OEHHA staff previously used this study to develop a Maximum Allowable Daily Level (MADL) for Proposition 65 (OEHHA, 2001). The benzene MADL is 49 $\mu\text{g}/\text{day}$ for an inhalation exposure and 24 $\mu\text{g}/\text{day}$ for an oral exposure.

Table 7.3. Differential peripheral blood cell counts in fetuses of benzene-exposed pregnant mice.#

Exposure	Blasts	Dividing Granulo-cytes	Nondividing granulocytes	Early nucleated red cells	Late nucleated red cells	Primitive nucleated red cells	Lympho-cytes
16-day fetuses							
Air	0.00±0.00	0.50±0.16	1.60±0.50	5.10±1.34	0.40±0.22	92.4±1.95	
5 ppm	0.00±0.00	2.10±0.67	3.60±1.57	5.80±1.88	0.80±0.25	87.4±4.11	
10 ppm	0.10±0.00	0.90±0.28	1.30±0.33	4.00±0.60	1.20±0.39	92.4±1.20	
20 ppm	0.10±0.00	1.50±0.50	2.20±0.63	3.90±0.79	1.50±0.34	90.7±1.48	
2-day neonates							
Air	0.00±0.00	3.80±0.66	67.60±2.44	7.30±1.36	6.20±1.79		14.0±3.1
5 ppm	0.20±0.14	3.10±0.57	72.30±3.09	1.70±0.62*	3.60±0.88		17.9±2.4
10 ppm	0.10±0.10	5.90±1.04	67.90±2.88	0.50±0.22*	7.30±0.83		16.9±2.0
20 ppm	0.10±0.10	2.10±0.62	80.40±2.67*	0.00±0.00*	1.60±0.45*		14.2±2.5
6-week adults							
Air	0.00±0.00	2.20±0.47	19.3±2.28	0.00±0.00	0.20±0.14		75.0±3.0
5 ppm	0.00±0.00	1.20±0.47	22.0±2.47	0.10±0.10	0.20±0.13		72.3±3.1
10 ppm	0.00±0.00	0.60±0.22	24.2±2.59	0.00±0.00	0.10±0.10		75.1±2.9
20 ppm	0.10±0.10	2.20±0.63	16.7±2.27	0.10±0.10	0.20±0.13		77.6±2.4

100 cells were counted from 1 male and 1 female from each of 5 litters per treatment (n=10)

*p < 0.05 vs corresponding control by Dunnett's test. Values are mean±SE.

An exposure of 500 ppm (1,600 mg/m³) benzene for 7 hours per day through days 6-15 of gestation was teratogenic in the fetal brain of Sprague-Dawley rats, while 50 ppm (160 mg/m³) and 500 ppm resulted in reduced fetal weights on day 20 of gestation (Table 7.4) (Kuna and Kapp, 1981). The higher exposure levels also had significantly more fetuses with skeletal variants. No fetal effects were noted at an exposure of 10 ppm (32.6 mg/m³), which is the NOAEL for this study.

Table 7.4. Fetal body weight and length (Kuna and Kapp, 1981)

Benzene	0 ppm	10 ppm	50 ppm	500 ppm
Inseminated rat dams (n)	17	18	20	19
Live fetuses/implants (n)	107/119	188/197	127/131	151/165
Mean body weight of live fetuses (g)	4.4 ± 0.6	4.4 ± 0.5	3.8 ± 0.7*	3.6 ± 0.8*
Mean crown-rump length (cm) in live fetuses	4.1 ± 0.2	4.1 ± 0.2	3.9 ± 0.3	3.8 ± 0.4*
Fetuses (litters) with skeletal or visceral variants (n)	3 (3)	2 (1)	23** (6)	30** (6)
Fetuses with brain anomalies or variants	0/35	0/56	5/35	7/44***

* statistically significant difference from control ($p < 0.05$); values are mean \pm 1 SD.

** significantly different by chi-square test

*** statistically different from control ($p < 0.05$) by Fisher Exact Test (2-tailed)

Inhalation of 500 ppm benzene (the only concentration tested) for 7 hours/day on gestational days 6 to 15 in CF-1 mice and days 6 to 18 in white New Zealand rabbits induced minor skeletal variations that the authors did not consider to be teratogenic (Murray et al., 1979).

Exposure of CFY rats to continuous benzene inhalation (24 h/day) at 150, 450, 1500, or 3000 mg/m³ (50, 150, 500, or 1000 ppm) from days 7-14 of gestation led to decreased fetal body weights, elevated liver weights, and signs of skeletal retardation at 150 mg/m³ (50 ppm) benzene, the lowest concentration tested (Tatrai et al., 1980).

Female CFLP mice and NZ rabbits were exposed by inhalation to 0, 500, or 1,000 mg/m³ (0, 153, or 307 ppm) benzene for 24 h/day from day 6 to day 15 of pregnancy (Ungvary and Tatrai, 1985). Maternal toxic effects were moderate and dose dependent. Benzene induced skeletal variations and weight retardation in fetuses of rabbits at 1,000 mg/m³ and in fetuses of mice at 500 and 1,000 mg/m³. Benzene increased the post-implantation loss (percent fetuses dead or resorbed) in rabbits at 1,000 mg/m³. Benzene induced spontaneous abortion in rabbits at 1,000 mg/m³.

In order to determine if prenatal exposure to benzene induces neurobehavioral changes in offspring, 0.1 mg/kg benzene was injected subcutaneously on gestation day 15 into four pregnant female Sprague-Dawley rats (Lo Pumo et al., 2006). There were no changes in total number of neonates, body weight, and eye opening time between progeny of benzene-exposed dams and controls, and no malformations. At birth, neonatal reflexes (cliff aversion, forelimb, placing, bar holding, forelimb grasping, startle) were scored in benzene-exposed pups. More benzene-exposed pups exhibited reflexes each day compared to controls. Also, the completion (maximum appearance, i.e. 100 percent of the litter exhibited each reflex) of neonatal reflexes in benzene-exposed animals preceded that of controls. Beginning at 2 months after birth, cognitive and motor performance was assessed in males of the prenatally benzene-exposed progeny. Motor activity in the open-field test showed reduced ambulation in benzene-

exposed rats compared to controls. Acquisition of active avoidance responses in the shuttle-box test was impaired in benzene-exposed rats vs. controls. Prenatal benzene exposure was associated with reduced retention latency in a step-through passive avoidance task. The authors concluded that acute exposure to benzene during gestational organogenesis may cause long-lasting changes in motor behavior and cognitive processes. It is problematic to extrapolate this acute subcutaneously administered dose to an equivalent inhalation exposure.

Exposure of rabbits to 80 ppm (261 mg/m³) and of guinea pigs to 88 ppm (277 mg/m³) benzene 7 hours/day, 5 days a week for 8 months caused testicular degeneration (Wolf et al., 1956).

In a one-generation reproduction study, groups of 26 female Sprague-Dawley rats were exposed for 6 hours per day by inhalation to 1, 10, 30, and 300 ppm benzene during a 10-week pre-mating period and during mating (to proven fertile males), gestation, and lactation (Kuna et al., 1992). There was no effect on female reproductive performance at any benzene level. Performance measures included number of litters (range = 19-24), mean gestation length (21.6-21.9 days), mean pup number per litter (11.7-12.6), and viability index (96.9-99.5%). At 30 and 300 ppm there was a trend for 21-day-old pups toward reduced body and organ weight but differences were statistically significant ($p < 0.05$) only for female pups at 300 ppm (32.59 ± 5.05 g vs. 36.3 ± 5.20 g in controls).

7.3 Genotoxicity

A review of the data from more than 1400 genotoxicity tests for benzene and its metabolites (Whysner et al., 2004) led to the conclusion that benzene and its metabolites do not produce reverse mutations in *Salmonella typhimurium* but are clastogenic and aneugenic, producing micronuclei (MN), chromosomal aberrations (CA), sister chromatid exchanges (SCE), and DNA strand breaks.

The International Agency for Research on Cancer (IARC) recently summarized the genotoxicity of benzene (IARC, 2012): "There is strong evidence that benzene metabolites, acting alone or in concert, produce multiple genotoxic effects at the level of the pluripotent haematopoietic stem cell resulting in chromosomal changes in humans consistent with those seen in haematopoietic cancer. In multiple studies in different occupational populations in many countries over more than three decades a variety of genotoxic changes, including chromosomal abnormalities, have been found in the lymphocytes of workers exposed to benzene."

In the most sensitive inhalation study of genotoxicity in animals, inhalation of 3, 10, and 30 ppm (9.7, 32.6, and 970 mg/m³) benzene for 6 hours by adult male Sprague-Dawley rats resulted in a significant increase over controls in the frequency of sister chromatid exchanges (SCE) in peripheral blood lymphocytes (Erexson et al., 1986). One ppm (3.26 mg/m³) was a tentative NOAEL for the effect. Male DBA/2 mouse peripheral blood lymphocytes showed a significant concentration-related increase in SCE frequency over controls at 10, 100, and 1,000 ppm (32.6, 326, and 3,260 mg/m³) benzene, the three concentrations tested. Mouse femoral bone marrow also showed a significant concentration-dependent increase in micronuclei at 10, 100, and 1,000 ppm over controls (Erexson et al., 1986).

7.4 Toxicogenomics

In order to study hematotoxicity at the level of altered multigene expression, cDNA microarray analyses were performed on mouse bone marrow tissue extracts during and after a 2-week exposure to 300 ppm benzene (Yoon et al., 2003). Expression of fifteen genes was at least doubled by benzene exposure compared to controls. Two of these were increased nearly five-fold (a polycomb binding protein and Metallothionein 1). CYP2E1 expression was increased 2.13 fold. Conversely this high-level benzene exposure decreased expression of a G-protein coupled receptor to 1 percent of its normal output.

One of the cohorts of Chinese workers described above (Lan et al., 2004) was analyzed by microarray analysis for global gene expression in the peripheral blood mononuclear cells (WBC) of 83 workers exposed to benzene levels ranging from < 1 ppm to > 10 ppm. The workers were divided into 4 exposure groups and compared to a group of 42 controls (Table 7.5) (McHale et al., 2011). Changes in many metabolic pathways and extensive increases (and probably decreases, which are not discussed) of the expression of specific genes were found at all benzene exposure levels (McHale et al., 2011). The AML (acute myeloid leukemia) pathway was among the pathways most significantly associated with benzene exposure. Alterations in immune response pathways (e.g., toll-like receptor signaling pathway, T-cell receptor signaling pathway) were associated with most exposure levels. A 16-gene increased expression signature (relative to no exposure) was associated with all levels of benzene exposure. The three genes with the highest increased expression were a serpin peptidase inhibitor, a tumor necrosis factor, and interleukin 1 alpha.

The above summaries of acute and chronic toxicity of benzene are intended to give an overview of the data and to analyze reports most relevant to developing Reference Exposure Levels (RELs) for benzene, i.e., inhalation studies. More comprehensive reviews of benzene toxicity are available (Sandmeyer, 1981b; World Health Organization, 1993; USEPA, 2002; ATSDR, 2007; Wilbur et al., 2008; Goldstein and Witz, 2009; IARC, 2012; Wang et al., 2012).

8 Derivation of Reference Exposure Levels

8.1 Acute Reference Exposure Level for Benzene

<i>Key study</i>	Keller and Snyder, 1988
<i>Study population</i>	pregnant female rats
<i>Exposure method</i>	inhalation of 0, 5, 10, or 20 ppm benzene
<i>Exposure continuity</i>	6 hours per day
<i>Exposure duration</i>	10 days (days 6-15 of gestation)
<i>Critical effects</i>	decreased early nucleated red cell counts (Table 7.3)
<i>LOAEL</i>	5 ppm (16 mg/m ³)
<i>NOAEL</i>	not found
<i>BMCL_{0.5SD}</i>	not used due to poor fit (Table 8.1)
<i>Human equivalent concentration</i>	5 ppm (RGDR* = 1)(systemic effect)
<i>Time adjustment factor</i>	Not done (see below)
<i>LOAEL uncertainty factor (UF_L)</i>	√10 (see below)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	2 (default) (OEHHA, 2008)
<i>Toxicodynamic (UF_{A-d})</i>	√10 (default)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	10 (default)
<i>Toxicodynamic (UF_{H-d})</i>	√10 (default)
<i>Database uncertainty factor</i>	1 (developmental studies are available)
<i>Cumulative uncertainty factor</i>	600
<i>Acute Reference Exposure Level</i>	8 ppb (27 µg/m³)

*The Regional Gas Dose Ratio (RGDR) is the ratio of the regional gas dose calculated for the respiratory region affected by the inhaled toxicant in the animal species to the regional gas dose in the corresponding region in humans. For a toxicant with a systemic effect, the default value is 1.

Reference Exposure Levels are based on the most sensitive, relevant health effect reported in the medical and toxicological literature. Acute Reference Exposure Levels are levels at which infrequent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (OEHHA, 2008)). Studies of developmental toxicity usually use repeat exposures in utero, either throughout gestation or during organogenesis. The acute REL for benzene is based on a developmental study (Keller and Snyder, 1988) in which pregnant rats were exposed 6 hours per day during days 6 through 15 of gestation. However, developmental toxicity may occur in response to just one exposure during a specific window of susceptibility. A literature search found 133 single-day exposure developmental toxicity studies involving 58 chemicals (Davis et al., 2009). The same endpoints observed in repeat dose studies are often observed with single exposures, an acute effect. The acute REL derived above is a level not to be exceeded in any one hour period, which is the default application for acute RELs based on developmental studies (OEHHA, 2008)

In the key study, which OEHHA earlier used to develop a Proposition 65 MADL for benzene (OEHHA, 2001), a monotonic dose response was seen for early nucleated red cells in 2 day neonates. The LOAEL was 5 ppm. A NOAEL was not detected. The several continuous models in BMDs version 2.2 were fit to the data. The Hill Model calculated a $BMCL_{0.5SD}$ of 0.0112 ppm, which was much smaller than the model's $BMC_{0.5SD}$ of 0.92 ppm, while other models had poor fits ($p < 0.1$) (data not shown). The poor results were in part due to the high adverse response (> 75 percent decrease in differential cell count) at 5 ppm, the lowest dose, and hitting a bound of 0 at 20 ppm, the highest dose. The data from the highest dose were omitted and the BMDs linear model was fit to the data. The value for fit was also below 0.1 (Table 8.1). The BMDs results were not used as the point of departure for the REL. However, despite the relatively poor fit to the data, the proximity of the $BMCL_{0.5SD}$ of 1.51 ppm to the LOAEL of 5 ppm provides some support for the use of $\sqrt{10}$ for the LOAEL to NOAEL UF.

Table 8.1. Benzene 2d neonate data (drop 20 ppm) in BMDs 2.2 Linear Model

Variance	Deviation	BMC(ppm)	BMCL(ppm)	p for fit	AIC* (fitted)
Constant	1 SD	4.14	3.01	0.0364	98.0831
Constant	0.5 SD	2.07	1.51	0.0364	98.0831
Constant	0.05 Relative	0.48	0.40	0.0364	98.0831
Not	1 SD	8.11	5.18	0.015	76.4167
Not	0.5 SD	4.06	2.59	0.015	76.4167
Not	0.05 Relative	0.548	0.512	0.015	76.4167

*Akaike Information Criterion

The default interspecies UF_{A-k} of 2 for residual pharmacokinetic differences was used. As indicated above PBPK models for benzene are available in mice, rats, and humans. The hematological effects in the key study have a plausible mechanism involving the toxic metabolites of benzene.

The default intraspecies UF_{H-k} of 10 was used. Screening PBPK modeling by OEHHA staff for toluene, xylene, and naphthalene (OEHHA, 2008) indicated that a factor of $\sqrt{10}$ may be adequate for benzene. However, the modeling did not include metabolism. Several benzene-specific reports supported the default value of 10 and possibly a greater value. In one study of Chinese workers, those with high CYP2E1 and no NQO1 had an odds ratio (OR) of 7.6 for benzene poisoning compared to those with low CYP2E1 and normal NQO1 activity (Table 6.1.2) (Rothman et al., 1996b). The prevalence of no NQO1 activity is five times greater (22.4% vs. 4.4%) in the Chinese population compared to non-Hispanic whites (Ross, 2005). A three genes' interaction revealed a 20.41-fold increased risk of poisoning in Chinese subjects with the NQO1 T/T genotype and with the GSTT1 null genotype and the GSTM1 null genotype compared with those carrying the NQO1 C/T and C/C genotype, GSTT1 non-null genotype, and GSTM1 non-null genotype (Chen et al., 2007). In a study not involving Chinese adults, at 1 ppm benzene continuous exposure, the PBPK model-estimated quantity of benzene metabolized in human bone marrow ranged from 2 to 40 mg/day, a 20-fold variation (Bois et al., 1996).

CYP2E1, a principal enzyme in the pathway of benzene metabolism which produces toxic metabolites, has not been detected early in human fetal liver (Vieira et al., 1996) and rises to only 10-20 percent of the adult level by the third trimester (Johnsrud et al., 2003)(Table 7.1). However, since many detoxifying enzymes are also low during this period (McCarver and Hines, 2002), bone marrow toxicity from benzene metabolites can occur in the fetus. The 8-fold variation in CYP2E1 levels between the third trimester fetus and the adult (Table 7.1) is also compatible with the default value of 10 for toxicokinetic variability among humans.

The default intraspecies UF_{H-d} (toxicodynamics) of $\sqrt{10}$ was used to account for pharmacodynamic variability among pregnant women and their fetuses and among infants, children, and adults (OEHHA, 2008). During embryonic and fetal development, hematopoiesis occurs first (mesoblastic period) in the extraembryonic yolk sac beginning in the 2nd week and ceasing by the eighth week of gestation, then in the liver (hepatic period) and to a lesser extent in thymus and spleen beginning at the 5th-6th week of gestation, and finally in the bone marrow (myeloid period) beginning at the 16th-18th week of gestation or even earlier (Charbord et al., 1996; Peault, 1996; Brugnara and Platt, 2003). The bone marrow volume increases linearly with body weight between 29-33 weeks of gestation and term (Hudson, 1965).

Blood cell type and rates of formation change with age (e.g., Table 7.3) and hematopoiesis would be expected to be more dynamic during developmental (growth) stages than in the adult. The erythroblast count, a marker of the contribution of the liver to erythropoiesis, decreases exponentially from 83/100 leukocytes at 17 weeks gestation to 4/100 at term (Nicolaidis et al., 1989). The third trimester fetus is said to produce red cells at three to five times the rate in adults at steady-state (Palis and Segel, 1998). The estimate is based on the linear decline in reticulocytes per 100 red blood cells from ~10 percent at 17-24 weeks of gestation to ~4 percent at term (Matoth et al., 1971; Zaizov and Matoth, 1976; Nicolaidis et al., 1989), a comparison of reticulocytes/1000 red cells in newborns (mean = 51.9) versus adults (mean = 15.7) (Seip, 1955), and a computerized simulation analysis of total red cell volume and life span at different life stages (Bratteby et al., 1968).

In a study of infants born prematurely, the body weight steadily increased (tripled) with gestational age, the percent reticulocytes declined overall, and the red blood cells (RBC) per volume was fairly constant (Table 8.2) (Zaizov and Matoth, 1976). Since both the bone marrow volume (Hudson, 1965) and the red blood cell volume (Bratteby, 1968) increase with gestational age and body weight, the net formation of RBCs must be substantial.

At birth a large drop in red cell production results in a transient physiological anemia of clinical concern (Palis and Segel, 1998). which reaches a low point in total red cells and hematocrit at 6-9 weeks of life after which red cell counts increase again (Matoth et al., 1971).

Table 8.2. Red cell values on first postnatal day in infants born prematurely (Zaizov and Matoth, 1976)

Gestation (wk)	N (infants)	Body wt (g) ^a	% retics.	RBC x 10 ⁴
24-25	7	725 ± 185	6.0 ± 0.5	4.65 ± 0.43
26-27	11	993 ± 194	9.6 ± 3.2	4.73 ± 0.45
28-29	7	1174 ± 128	7.5 ± 2.5	4.62 ± 0.75
30-31	25	1450 ± 232	5.8 ± 2.0	4.79 ± 0.74
32-33	23	1816 ± 192	5.0 ± 1.9	5.0 ± 0.76
34-35	23	1957 ± 291	3.9 ± 1.6	5.09 ± 0.5
36-37	20	2245 ± 213	4.2 ± 1.8	5.27 ± 0.68
term ^b	19		3.2 ± 1.4	5.14 ± 0.7

^a mean ± standard deviation

^b term data based on (Matoth et al., 1971)

Although hematopoiesis is dynamic during development, we were unable to find pertinent quantitative data justifying a UF_{H-d} factor greater than the default factor of $\sqrt{10}$.

In the study (Coate et al., 1984) that was the basis of OEHHA's previous acute REL for benzene (OEHHA, 1999), statistically significant decreased fetal body weight was seen only at 100 ppm, the highest dose tested (Table 7.2). A mechanism for the fetal effect of decreased body weight in the study is not obvious. The effects on fetal body weight may be due to the parent compound, which can cross the placental wall, and/or to one of more benzene metabolites. The Keller and Snyder (1988) study is a much more sensitive study than the Coate et al. study showing effects on the hematopoietic system in neonates at much lower gestational exposure levels than Coate et al. found affecting fetal body weight.

8.2 8 hour Reference Exposure Level for Benzene

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 of the Technical Support Document (OEHHA, 2008)).

For a health protective approach, OEHHA determined that the 8 hour REL should be the same as the chronic REL: 0.002 ppm (7 $\mu\text{g}/\text{m}^3$). It is unclear whether the adverse effects of repeated benzene exposure are reversed by overnight or over-the-weekend periods of non-exposure and they are likely to continue to worsen with additional exposure.

8.3 Chronic Reference Exposure Level for Benzene

<i>Study</i>	Lan et al. (2004)
<i>Study population</i>	250 male and female Chinese shoe workers aged 29.9 ± 8.4 years (vs. 140 controls)
<i>Exposure method</i>	Discontinuous occupational exposure
<i>Exposure continuity</i>	8 hr/day (10 m^3 per 20 m^3 day), 6 days/week
<i>Exposure duration</i>	6.1 ± 2.1 years
<i>Critical effects</i>	Decreased peripheral blood cell counts (7 categories; see Table 6.4)
<i>LOAEL</i>	0.57 ± 0.24 ppm (1.86 ± 0.78 mg/m^3)
<i>NOAEL</i>	Not found
<i>BMCL_{0.5SD}</i>	0.476 ppm (Hill Model version 2.15)(Table 8.3)
<i>Average occupational exposure</i>	0.204 ppm ($0.476 \text{ ppm} \times 10/20 \times 6/7$)
<i>Human equivalent concentration</i>	0.204 ppm ($0.665 \text{ mg}/\text{m}^3$)
<i>LOAEL uncertainty factor (UF_L)</i>	Not applicable with BMC
<i>Subchronic uncertainty factor (UF_S)</i>	$\sqrt{10}$ (8- \leq 12% expected lifetime)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	1 (default, human study)
<i>Toxicodynamic (UF_{A-d})</i>	1 (default, human study)
<i>Intraspecies uncertainty factor</i>	30 (see explanation below)
<i>Toxicokinetic (UF_{H-k})</i>	
<i>Toxicodynamic (UF_{H-d})</i>	
<i>Database uncertainty factor</i>	1 (developmental studies are available)
<i>Cumulative uncertainty factor</i>	100
<i>Chronic Reference Exposure Level</i>	2 ppb (7 $\mu\text{g}/\text{m}^3$)

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous chronic exposures (see Section 7 in the Technical Support Document (OEHHA, 2008)).

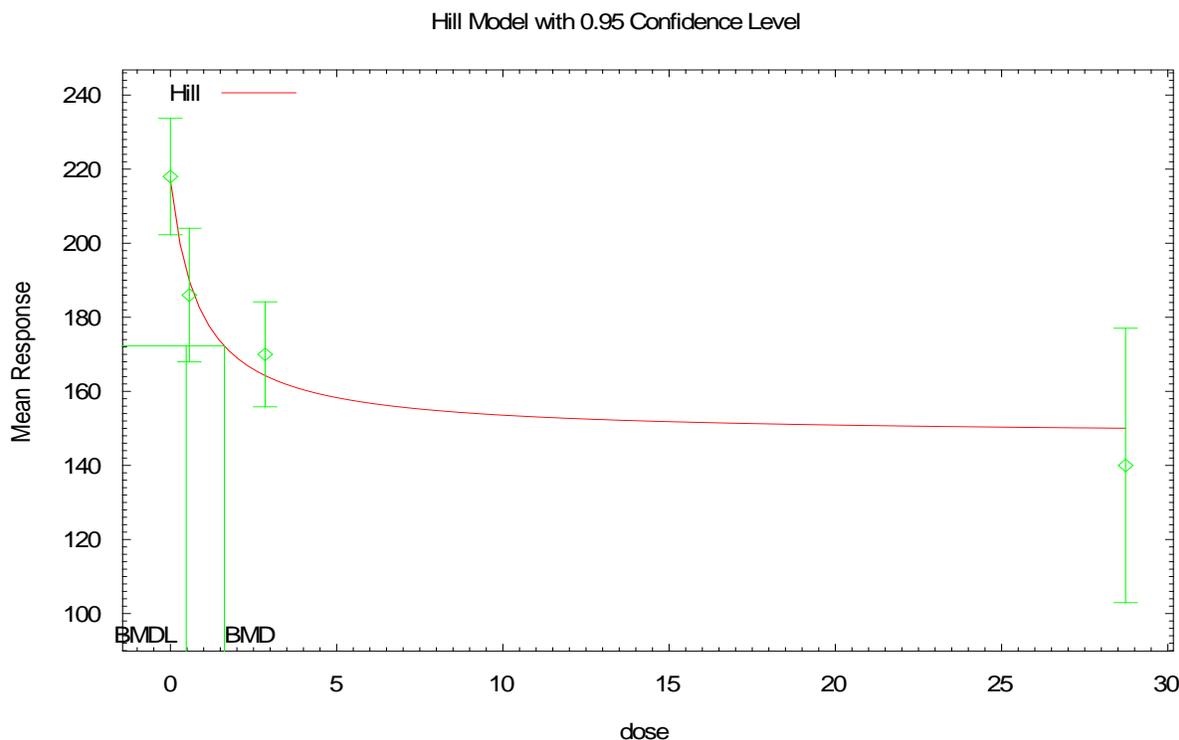
The point of departure for the REL was derived using the changes in B cell levels (Lan *et al.*, 2004), which were considered the most sensitive endpoint, as a function of benzene concentration (Table 6.1.4) and the continuous models in the BMDS software. The Hill model (version 2.15) in the BMDS software gave acceptable values for fit and the lowest AIC. We specified a risk of 0.5 estimated standard deviation from the control mean as the benchmark and obtained a BMC of 1.62 ppm and a BMCL of 0.476 ppm (p value for fit = 0.303) (Table 8.4). The U.S. Environmental Protection Agency (USEPA) has suggested use of 1 standard deviation from the control mean as a benchmark but a $BMCL_{1SD}$ could not be obtained with the B cell data using the Hill model (Table 8.4).

Table 8.4. Benzene B cell data in BMDS 2.2 (Hill Model Version: 2.12; 02/20/2007)

Model	BMC (ppm)	BMCL (ppm)	BMC/BMCL	p (test 4)	AIC (fitted)
Constant variance (rho = 0)					
Polynomial 0.5 SD	3.04	2.05	1.5	0.04099	3907.44
Exponential 0.5 SD (Models 4 and 5)	1.22	0.44	2.8	0.1105	3905.811
Power 0.5 SD	20.35	14.03	1.7	0.0006241	3916.02
Linear 0.5 SD	20.35	14.03	1.7	0.0006241	3916.02
Hill 0.1 SD	0.131	0.0299	4.4	0.303	3904.325
Hill 0.25 SD	0.422	0.1039	4.1	0.303	3904.325
Hill 0.5 SD	1.624	0.4764	3.4	0.303	3904.325
Hill 0.05 Relative Dev	0.164	0.038	4.3	0.303	3904.325
Hill 1.0 SD	Failed (BMR not in range of mean function)				
Variance not constant (rho ≠ 0)					
Hill 0.1 SD	0.129	Failed	-	0.663	3904.575
Hill 0.25 SD	0.427	Failed	-	0.663	3904.575
Hill 0.5 SD	1.813	Failed	-	0.663	3904.575
Hill 1.0 SD	Failed (BMR not in range of mean function)				

The Lan *et al.* (2004) study is more sensitive than both the Tsai *et al.* (1983) study, used previously by OEHHA for its chronic REL (OEHHA, 2000), and the Rothman *et al.* (1996) study used by USEPA for its Reference Concentration (RfC). Effects on the hematologic system are seen at a level where Tsai *et al.* did not find significant changes. However, as indicated above, at least half ($\geq 50\%$) of the exposures in Tsai *et al.* were less than 0.1 ppm, the median concentration. In Lan *et al.*, 0.09 ppm is two standard deviations below the mean of 0.57 ppm indicating that in Lan *et al.* only 2.5 percent of the exposures were below 0.09 ppm. The findings of significant depression in some blood cell counts at ≤ 0.25 ppm by Qu *et al.* (2002) and at 0.79 ppm by Khudar *et al.* (1999) are compatible with the results of Lan *et al.* (2004).

A combined intraspecies uncertainty factor (UF_H) of 30 was used as a compromise instead of separate toxicokinetic and toxicodynamic factors since there were reasons for and against higher and lower values for each subfactor.

Figure 8.3. The continuous Hill model fit to the B cell data of Lan et al. (2004).

As discussed for the acute REL, several benzene-specific reports supported the default value of 10 for UF_{h-k} (and possibly a greater value). Chinese workers with high CYP2E1 and no NQO1 had an odds ratio (OR) of 7.6 for benzene poisoning compared to those with low CYP2E1 and normal NQO1 activity (Table 6.1.2) (Rothman et al., 1996b). The prevalence of no NQO1 activity is five times greater (22.4% vs. 4.4%) in the Chinese population compared to non-Hispanic whites (Ross, 2005). A three genes' interaction revealed a 20.41-fold increased risk of poisoning in subjects with the NQO1 T/T genotype and with the GSTT1 null genotype and the GSTM1 null genotype compared with those carrying the NQO1 C/T and C/C genotype, GSTT1 non-null genotype, and GSTM1 non-null genotype (Chen et al., 2007). Some of these variations may already be incorporated in the population of Chinese workers studied in Lan et al. (2004), which was used as the basis of the 8-hour and chronic RELs, so that a UF_{h-k} less than 10 might be applied. However, in a study not limited to Chinese adults, at 1 ppm benzene continuous exposure the PBPK model-estimated quantity of benzene metabolized in human bone marrow ranged from 2 to 40 mg/day, a 20-fold variation (Bois et al., 1996; Lan et al., 2004). The observation that CYP2E1 levels vary 8-fold between third trimester fetus and adult (Table 7.1) also supports use of the value of 10.

The intraspecies UF_{H-d} (toxicodynamics) was used to account for pharmacodynamic variability among pregnant women and their fetuses and among infants, children, and adults (OEHHA, 2008). During embryonic and fetal development hematopoiesis first

occurs in the extraembryonic yolk sac, then in the liver and thymus, and finally in the bone marrow during the second and third trimesters (Peault, 1996). Blood cell type and rates of formation change with age (e.g., Table 7.3) and would be expected to be more dynamic during pre- and post-natal developmental stages than in adults. Further discussion of this subfactor can be found in the acute REL derivation above. However, unlike the acute REL where a sensitive developmental stage was the critical effect, the key study for the chronic REL involved only exposures and effects in adult workers.

The federal Agency for Toxic Substances and Disease Registry (ATSDR) developed a draft Minimal Risk Level (MRL) for chronic (365 days or more) exposure to benzene of 0.003 ppm (10 $\mu\text{g}/\text{m}^3$) based on the Lan *et al.* (2004) study (ATSDR, 2005; Wohlers *et al.*, 2006; Wilbur *et al.*, 2008). ATSDR also used the data on B cells (see Table 3 above) in the continuous Hill model. However, they defined the BMR as 0.25 standard deviation from the mean, and got a BMC of 0.42 ppm and a BMCL of 0.10 ppm.

In 2003, USEPA derived a Reference Concentration (RfC) for benzene of 10 ppb (30 $\mu\text{g}/\text{m}^3$) using the Rothman *et al.* (1996) study, a benchmark dose approach, and their judgment of appropriate uncertainty factors (IRIS, 2007). This RfC was derived prior to the publication of Lan *et al.* (2004).

8.4 Data Strengths and Limitations for Development of the RELs

Both the animal and human databases for benzene are extensive compared to many other chemicals in commerce. However, a major data gap is the absence of health effects data in infants and children and in young animals. The hematopoietic system of a growing child must not only keep up with blood cell turnover but must also supply extra cells for a growing body. Rapidly dividing cells are more prone to mutation and other cellular changes resulting from chemical exposure. Thus infants and children may be more sensitive to benzene and its metabolites.

A data gap of a specific test is that of a two-generation reproductive study (Chapin and Sloane, 1997). USEPA considered that this gap warranted an additional “database” uncertainty factor in its benzene RfC derivation. Due to the otherwise extensive animal and human data available for benzene, including developmental studies in animals, we did not apply such a factor. OEHHA’s acute REL is based on a developmental study in rats.

8.5 Benzene as a Toxic Air Contaminant Especially Affecting Infants and Children

In view of the wide-spread exposure to benzene, the documented toxicokinetic variability in benzene metabolism, and dynamic hematopoiesis during development, there is valid concern that benzene exposure may disproportionately impact infants and children. Benzene causes leukemia in exposed workers, including acute myeloid leukemia, and acute non-lymphocytic leukemia. A positive association has also been found between benzene exposure in workers and acute lymphocytic leukemia, chronic

lymphocytic leukemia, multiple myeloma and non-Hodgkin lymphoma. Acute lymphoblastic leukemia is the most common childhood cancer; other types of leukemia also occur in children. (IARC, 2012). OEHHA recommends that benzene be identified as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

9 References

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