

1.1.7 Medical Aspect

Industrial monitoring programs should be continually evaluated. Where excursion values are found, biologic monitoring and follow-ups should be carried out in addition to the regular periodic health examination programs. According to a Polish survey, workers with long-term exposure to some aromatic hydrocarbons have shown changes in leukocyte alkaline phosphatase (21).

1.2 Benzene

1.2.1 General Aspects

Benzene is the simplest aromatic compound known. However, benzene should not be confused with *benzin*, which is a low-boiling petroleum fraction of predominantly aliphatic compounds, similar to gasoline. Benzin, in many non-English-speaking European countries, indicates gasoline. In industry and commerce, benzene has been one of the most important industrial chemicals. However, currently as an analytic agent and in household and many industrial products, benzene has been replaced largely by other solvents, such as toluene.

1.2.2 Chemical and Physical Data

Benzene, benzol, phené, CH:CHCH:CHCH:CH , is a clear, colorless liquid with a characteristic pleasant odor at low concentrations, disagreeable at high levels. Some data are summarized in Table 47.1. Benzene forms a highly flammable (DOT) (22) and explosive mixture with air at 1.4 to 8.0 percent. It is an excellent solvent. Chemically, it is fairly stable, but readily undergoes substitution reactions to form halogen, nitrate, sulfonate, and alkyl derivatives. Commercial benzene, of which three grades have been standardized (ASTM D-16), usually contains varying concentrations of toluene, xylene, and phenol, and traces of carbon disulfide, thiophene, olefins, naphthalene, and related compounds.

The photochemical formation of nitrobenzenes and nitrophenols from benzene has been observed in the presence of nitrogen oxides. Benzene also combines photochemically with halogens to produce eye and mucous membrane irritants (23). Ozone reacts 10 to 20 times more slowly with benzene than with its methyl-substituted derivatives (24).

1.2.3 Occurrence, Industrial Sources, and Preparation

Benzene occurs in coal tar and petroleum naphtha from which it is commercially prepared (5). Benzene is a constituent of gasoline. A report from the U.S. Army in 1972 showed that their 37 unleaded and leaded gasolines averaged 0.8 percent benzene, with the highest value 2 percent. European gasolines even

evaluated. Where ex-
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carbons (21).

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thallene, and related

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recently still contained up to 5 percent benzene, being produced from higher aromatic reformates (25). The benzene concentration in the gasoline vapor phase is lower than that in the liquid, but depends somewhat on the concentration of other hydrocarbon and metal additives (25). Some consumer products, such as paint removers, in the past have contained 50 percent benzene or more (26). Products with a benzene content above 0.1 percent now have to be adequately labeled with warnings to the consumer (27).

Benzene occurs in thermal degradation gases from high density polystyrene (28) and in solid waste gasification products (29). It has been identified as well in condensates of tobacco smoke (12).

Benzene has been detected in the expired air in three nonsmokers and two smokers of eight human volunteers (30). It occurs as a degradation product in the rat after administration of triphenyllead acetate (31) and *p*-toluic acid phenylhydrazide (32).

About 150 air samples analyzed from the areas around Houston, Texas over a period of 15 months revealed an average benzene concentration of 1.3 to 15 ppb (33). In the Toronto atmosphere, average benzene concentrations were detected at 2 to 98 ppb (34), and near Los Angeles 60 to 70 ppb in 1962 (35).

Benzene is produced in billion gallon quantities per year, mainly by fractionated distillation from crude oil, solvent extractions or crystallization, catalytic dehydrogenation, and re-forming of light naphthas, paraffins, and cycloolefins (36). It is produced as well in the coke-oven industry as a by-product.

1.2.4 Utilization

The benzene's ubiquitous use stems from its ready availability at relatively low cost. It is used mainly in chemical processes as a raw material and as a solvent in industry and commerce. Some important processes include the manufacture of ethylbenzene, styrene, cumene, phenolic resins, ketones, adipic acid, caprolactam, nylon (5), and various dyes (37). However, in most consumer products, benzene has been substituted, unless so labeled (27). Benzene comprises a high energy component of aviation and motor gasolines.

1.2.5 Physiologic Response

The physiological and toxicologic effects of benzene differ with the type of exposure. Some of the available data are summarized in Tables 47.2 to 47.5 (15-17, 38-100).

Response to Human Acute Exposure. Oral Toxicity. Oral ingestion of 9 to 12 g benzene (38) has caused signs of staggering gait, vomiting, somnolence, shallow, rapid pulse, loss of consciousness, and later delirium, with subsequent chemical pneumonitis, serious collapse due to initial stimulation, then abrupt CNS depression. Generally, at moderate concentrations, symptoms are dizziness,

Table 47.2. Human Acute Benzene Exposure

Route	Dose or Concentration	Results, Signs, or Symptoms	Ref.
Oral	9-12 g	Staggering gait, vomiting, somnolence, shallow, rapid pulse, loss of consciousness, delirium, death	38
	10 ml	May be approximate fatal dose	39
	30 g	May be approximate fatal dose	40
Inhalation	1.5 ppm (5 mg/m ³)	Olfactory threshold	15
	25 ppm (0.08 mg/liter)/480 min	No effect, detectable in blood	17
	50-150 ppm (0.16-0.48 mg/liter)/300 min	Headache, lassitude, weariness	17
	500 ppm (1.6 mg/liter)/60 min	Headache	17
	1500 ppm (4.8 mg/liter)/60 min	Symptoms of illness	17
	3000 ppm (9.6 mg/liter)/30 min	May be tolerated for 0.5-1 hr	17, 38
	7500 ppm (24.0 mg/liter)/60 min	Signs of toxicity in 0.5-1 hr	38
	3100-5000 ppm (10-16 mg/liter)	Subtle signs of intoxication, absorbed 79.8-84.8%	38
	19,000-20,000 ppm (61-64 mg/liter)/5-10 min	May be fatal in 5 to 10 min	17

excitation, and pallor, followed by flushing, weakness, headache, breathlessness, constriction of the chest, and fear of impending death. Visual disturbances and convulsions are frequent. At higher concentrations, as shown above, the symptoms are excitement, euphoria, and hilarity, then quite suddenly change to weariness, fatigue, and sleepiness, followed by coma and death (101). Conversely, benzene was used therapeutically between 1910 and 1920 to reduce leukocytes in leukemia (38).

Dermal Effects. Dermal contact presents a possible route of absorption, but generally at a much lower rate than through the mucous membranes of the respiratory system. Benzene is irritant to the skin (38), and by defatting the keratin layer may cause erythema, vesiculation, and dry and scaly dermatitis (102).

Inhalation Effects. About 130 years ago, when benzene first was obtained in pure form, attempts were made to use this agent to produce anesthesia in man. However, owing to the unpleasant side and aftereffects, the practice was quickly abandoned (38).

Generally, as a consequence of benzene's high volatility, the inhalation route has been the most prevalent means of intoxication (103). Acute human exposure has occurred mainly during excessive promulgation of fumes in accidental spills, drying of soiled clothing in poorly ventilated areas in dry-cleaning businesses, in the home, or confined spaces where benzene was used as a solvent or product component.

Low benzene concentrations such as 25 ppm inhaled for 2 hr appeared quickly in the blood but cleared within 300 min (104). Absorption was determined as 79.8 to 84.8 percent by the human system (38) when inhaled at concentrations of 10 to 16 mg/liter. Some portion is normally exhaled in

Symptoms	Ref.
involence, shallow,	38
ness, delirium, death	39
st	40
st	15
id	17
ess	17
	17
nt	17, 38
	38
absorbed 79.8-84.8%	38
	17

Table 47.3. Acute Animal Experiments with Benzene

Symptoms	Ref.	Acute	Species	Dose or Concentration	Results, Signs, or Symptoms	Ref.	
involence, shallow,	38	Oral	Rat	3.4 g/kg	LD ₅₀	41	
ness, delirium, death	39		Rat	5.6 g/kg	LD ₅₀	42	
se	40		Rat	<1.0-5 g/kg	LD ₅₀ , variation, age and strain dependent	41	
ost	15		Rabbit	150-300 mg/kg (2.5-11.0 µCi)	Use of cold and radioactive benzene, no effects; 48.5% exhaled unchanged, 51.5% urinary excretion; 18.2-21.2% as phenol, 4.8% as quinol, 4.4% catechol	43	
id	17	Intrapulmonary instillation	Mouse	4.70 g/kg	LD ₅₀	44	
ess	17		Dog	2.00 g/kg	Lowest reported lethal dose	47	
	17		Rat	0.25 ml	LD ₁₀₀ , cardiac arrest, death	16	
nt	17, 38						
absorbed 79.8-84.8%	38	Eye	Rabbit	0.10 ml	Irritancy, moderate conjunctival irritant, causes transient corneal injury	42	
	17		SC	Mouse	0.088 g/kg	No effect	45
					0.44 g/kg	27% inhibition of circulatory erythrocytes	45
					2.20 g/kg	50% inhibition of circulatory erythrocytes	45
				2.70 g/kg	Found to be a teratogen	46	
			Frog	1.40 g/kg	Lethal dose	47	
			Rat	1.15 g/kg	Lethal dose	48	
			Mouse	0.468 g/kg	LD ₅₀	49	
			Guinea pig	0.527 g/kg	Lethal dose	50	
		Inhalation	Rat	10,000 ppm (31.9 mg/liter)/7 hr	LC ₅₀	17	
				13,700 ppm (43.7 mg/liter)	LC ₅₀ , high levels of liver and lung congestion	51	
				16,000 ppm (51.0 mg/liter)/4 hr	LC ₅₀	52	
			Mouse	2,195 ppm (7.0 mg/liter)	Narcosis	38	
				9,980 ppm (31.8 mg/liter)	LC ₅₀	53	
			Rabbit	4,000 ppm (12.8 mg/liter)	Narcosis	54	
				10,000 ppm (31.9 mg/liter)	Death	54	
				35,000-45,000 ppm (111.6-144 mg/liter)			
				4-71 min	Slight anesthesia in 4 min and death in 22-71 min	55	
				3.7 min	Light anesthesia, relaxed	55	
				5.0 min	Excitation, tremors, running movements	55	
				6.5 min	Loss of pupil reflex	55	
				11.4 min	Loss of blinking reflex	55	
				12.0 min	Pupillary contraction	55	
				15.6 min	Involuntary blinking	55	
				36.2 min	Death	55	
			Guinea pig	6,270 ppm (20 mg/liter)/30 min peak	Benzene blood level 3.2 mg/100 ml, 1.7 mg/m ³ phenol and clearing	56	
				15,675 ppm (50 mg/liter)/30 min peak	Benzene blood level 8.0 mg/100 ml, 1.8 mg/m ³ phenol and clearing	56	
			Dog	45,800 ppm (146 mg/liter)	Lethal dose	50	
			Cat	53,300 ppm (170 mg/liter)	Lethal dose	50	
		Aquatic	Striped bass	100-10 ppm	TLm96	57	
				10.9 µl/liter	LC ₅₀ /96 hr toxicity similar to that of crude oil	57	
			<i>T. Calif-ornicus</i>	0.1 ml/liter	Lethal and more toxic than other petroleum products	58	

Table 47.4. Human Chronic Inhalation of Benzene

Years of Exposure	Year Published	No. of Cases	Type of Exposure	Exposure Conc.	Findings	Ref(s)
3-54 yr	1939	286/332	Rotogravure ink solvent application and personal dry-cleaning agent	11-1060 ppm/4-7 days/wk	Lowest concentration: fatigue and dizziness Medium concentration: fatigue, dryness of mucous membranes, hemorrhaging, nausea or vomiting, and lethargy Highest concentration: weakness, fatigue, epistaxis, dryness of mucous membranes, loss of appetite, nausea or vomiting, shortness of breath, dizziness, insomnia, and lethargy	5910 3
1946-1956	1956	107/147	Shoe manufacturing	~400 ppm	Hematopathy and thrombocytopenia most common	60
Prior to 1964	1964	6/47	Paint thinners, printing inks, adhesives		Six myeloid, hemocytoblastic, or lymphatic effects, some reversible, of 47 cases with hemopathy	61
1945-1965	1965	20			Aplastic anemia	62
Prior to 1966	1966	3	Benzene vapor	—	Aplastic anemia with osmotic fragility	63
Prior to 1955	1966	125/147	Shoe adhesive solvents	~400 ppm 1955-1966	Nine-year follow-up of 125-147 cases with some decreased thrombocytosis from previous benzene exposure of 100/147 with abnormal hematology	64
1938-1968	1968	1	Occupational varnishing	—	Rare case of acute erythromyelosis	62

Table 47.4. (Continued)

Findings	Ref.	Years of Exposure	Year Published	No. of Cases	Type of Exposure	Exposure Conc.	Findings	Ref.
Lowest concentration: fatigue and dizziness	59	Prior to 1971 3 mo-17 yr	1971	51/217	Shoe adhesive solvents	30-210 ppm	Leukopenia 9.7%, pancytopenia 2.8%, eosinophilia 2.3%, thrombocytopenia 1.8%, basophilia 0.5%, giant platelets 0.5%; anemia was reversible	65
Medium concentration: fatigue, dryness of mucous membranes, hemorrhaging, nausea or vomiting, and lethargy		Benzene prior to 1953, then toluene	1971	34	Rotogravure benzene and toluene	125->525 ppm	Some higher chromosome aberration in peripheral blood lymphocytes	66
Highest concentration: weakness, fatigue, epistaxis, dryness of mucous membranes, loss of appetite, nausea or vomiting, shortness of breath, dizziness, insomnia, and lethargy		Prior to 1961 5-18 yr	1971	5/216	Watch industry	—	Ten-year follow-up: 3 thrombocytopenias, 1 thrombocytopenia and anemia, 1 death of aplastic anemia	67
Hematopathy and thrombocytopenia most common	60	4 mo-15 yr	1972	32	Shoe adhesive and solvents	150-650 ppm	Reversible pancytopenia, 28 thrombocytopenia, 14 macrocytic anemia, 3 megaloblastic erythropoiesis	68
Six myeloid, hemocytoblastic, or lymphatic effects, some reversible, of 47 cases with hemopathy	61	Prior to 1973	1973	299/1000	—	—	Absolute monocytosis in an average of 14%, 19 reticuloendothelial hyperplasia	69
Aplastic anemia	62	1 yr	1977	40	Shoe industry benzene and other solvents	—	Rate of leukemia-like effects of occupationally exposed workers 13.0/100,000 vs. 6.0/100,000 in general population	70
Aplastic anemia with osmotic fragility	63							
Nine-year follow-up of 125-147 cases with some decreased thrombocytosis from previous benzene exposure of 100/147 with abnormal hematology	64	Prior to 1950 and later	1978	350/594	U.S. industrial benzene production and use	—	Various hematopathies and several cases with leukemia-like effects	71
Rare case of acute erythromyelosis	62							

Table 47.5. Chronic Animal Experiments with Benzene

Route	Species	Dose or Conc.	Time	Results or Findings	Ref.
Oral	Rat (F)	1 mg/kg/day	187 days	No effect	72
		10 mg/kg/day	187 days	Very slight leukopenia	72
		50 mg/kg/day	187 days	Leukopenia and erythrocytopenia	72
		100 mg/kg/day	187 days	Leukopenia and erythrocytopenia	72
		100 mg/liter ^a	4 mo	Development of leukopenia during 1st month, CNS disruption after 4 mo	72
		250 mg/liter ^a	40-50 days	Temporary changes in leukocyte counts and cholinesterase activity	73
		250 mg/liter ^a + 10 mg/m ³	20 days	Temporary changes in leukocyte counts and cholinesterase activity	73
		1.6 ml/kg	3 days	Increased liver weight, decreased protein weight and enzyme activities	74
		1.5 mg/kg + 0.51 mg/m ³	100 days	Sensitizing effect in 30-40 days	75
		9.0 mg/kg + 6.63 mg/m ³	100 days	Very rapid sensitizing and intoxicating effect	75
	Rabbit	1 mg/kg	6 mo	Mild leukopenic, splenic, and testicular degeneration	72
Dermal	Rat	0.6 g/kg	4 hr/day × 4 mo	Plasmic cell increase in bone marrow, disruption of erythropoietic element maturation	76
	Mouse (hairless)	To cover dorsal skin	2 yr	Only slightly above spontaneous frequency of several tumor types, but some skin papillomas	77
	Rabbit	2 µl/appln. 25% in vaseline	3 ×/day × 3 days 2 ×/wk	Epidermal hyperplasia with neural invasion Tremors, excitement, cachexia	78 78
Dermal	Rabbit (ear)	Undiluted	10-20 appln.	Slight to moderate irritation, moderate necrosis	79
SC	Rat	1 mg/kg	12 days	Chromosomal damage in 50.94% of bone marrow cells	79
		2.5 mg/kg	30 days	Prolonged generation and transit time of granulocytes and reticulocytes, and decreased maturation time of neutrophilic granulocytes and erythroblasts	80
		0.2 g/kg	12 days	Lymphopenia, with toluene increased neutrophils and bacillonuclear neutrophils	81
		1 g/kg	12 days	Chromosomal analysis at metaphase: chromatid breaks 50.9%, gaps 44.7%, isochromatid breaks 4.34%, somewhat higher than from toluene	79
		2 ml/kg	21 days	Cellular injury, mitochondrial swelling, severe dilation of membrane system, rough and smooth endoplasmic reticulum; severely disintegrated hematopoietic cells	82
		1 ml/kg/day	14 days	Leukopenia, no significant change in hematocrit	83
		1 ml/kg/day 2 ml/kg/day	5 wk 3wk	Rapid decrease in femoral marrow nucleated cell count and DNA phosphorus % (of bone marrow dry wt.)	84
	Mouse	0.025-0.1 ml	54 wk, 104 wk	Not a carcinogen	85
		0.5 ml/kg	2 appln./day × 1-10 days	Dose-dependent binding to liver, residue increasing with time; initial binding to bone marrow, disassociation after day 6	86
SC	Rabbit	0.1 ml/kg/day × 13 mo		Increased pseudoeosinopoiesis	86
		0.5 ml/kg/day × 10-15 days		Initial stimulation of pseudoeosinopoiesis, bone marrow promyelocyte, and myelocyte count	86

Observations or Findings	Ref.
Leukopenia	42
Erythrocytopenia	42
Erythrocytopenia	42
Leukopenia during 1st disruption after 4 mo	72
Changes in leukocyte counts	73
Enzyme activity	73
Changes in leukocyte counts	73
Enzyme activity	73
Weight, decreased protein	74
Enzyme activities	75
Death in 30-40 days	75
Anizing and intoxicating	75
Splenic, and testicular	42
Decrease in bone marrow, dis-	76
Thrombopoietic element	76
Spontaneous frequency	77
For types, but some skin	77
Hyperplasia with neural invasion	78
Weight, cachexia	38
Gut irritation, moderate	42
Damage in 50.94% of bone	79
Retention and transit time of	80
And reticulocytes, and de-	80
Termination time of neutrophilic	80
And erythroblasts	80
With toluene increased neu-	81
Acidophilic neutrophils	81
Analysis at metaphase; chro-	79
mosomes 0.9%, gaps 44.7%, iso-	79
chromosomes 4.34% somewhat	79
From toluene	82
Mitochondrial swelling, se-	82
miotic membrane system, rough	82
Endoplasmic reticulum; se-	82
parated hematopoietic cells	82
Significant change in	83
In femoral marrow nu-	84
cleic acid and DNA phosphorus	84
content (dry wt.)	85
Binding to liver, residue	86
Time: initial binding to	86
Disassociation after day 6	86
Neutropoiesis	86
Of pseudoneutropoiesis,	86
Neutrophilic, and neutro-	86

Table 47.5. (Continued)

Route	Species	Dose or Conc.	Time	Results or Findings	Ref.
		0.5 ml/kg/day × 3-4 wk		Decrease of blood leukocytes	86
		0.3 ml/kg/day × 1-9 wk		Panctopenia, hypoplasia of bone marrow, with severe inhibition of the DNA synthesis	87
		0.5 ml/kg/day × 2-3 wk		Decrease in peripheral blood leukocyte count, decreases mitochondrial respiration	88
		1.0 ml/kg/day × 12 days		Increased erythrocyte permeability induction of porphyrin biosynthesis in brain gray matter	89
Inhaln.	Rat	4.8 ppm (15 mg/m ³)	Continuous	Hyperplasia and vacuolization of smooth reticulum, selective destruction of olfactory basal cells	90
	Rat, guinea pig, dog, primate	17 ppm (56 mg/m ³) 30 ppm (98 mg/m ³) 256 ppm (817 mg/m ³)	8 hr/day × 5 day/ wk continuously 9-127 days	Histopathology of all organs essentially negative, some slight weight reduction at the highest level	91
	Rat	94 ppm (350 mg/m ³)	5.5 hr/day × 30 days	Increased α-aminobutyric acid level decreased in cerebellum but not mesencephalon, aspartic and glutamic acid decreased at both sites	92
		158 ppm (500 mg/m ³)	4 hr/day interm.	Leukopenia and muscle antagonistic chromaxy	93
		400 ppm (1260 mg/m ³)	13 wk	Increased thrombocytes, leukopenia, decreased segmented granulocytes	94
		158-1580 ppm (50-5000 mg/m ³)	Continuous	Leukopenia, leukocytosis, reduced blood cholinesterase, alteration of reversible chromaxy ratio of muscle antagonists	95
Inhaln.	Rat	88 ppm (0.28 mg/liter)	7-8 hr/day × 5 day/wk × 6 mo for all concentrations	Slight splenomegaly	42
		2200 ppm (7.00 mg/liter)		Narcosis and growth reduction	
		4400 ppm (14.00 mg/liter)		Above symptoms amplified	
		6600 ppm (21.00 mg/liter)		Liver histopathology and bone marrow changes	
		9400 ppm (30.00 mg/liter)		Liver histopathology and bone marrow changes	
		1650 ppm (5.22 mg/liter)	6 hr/day × 5 day/ wk × 12 wk	Leukopenia, partially reversed by phenobarbital	96
		8500 ppm (27.00 mg/liter)	6 hr/day × 10 days	Enzymatic changes in peripheral blood leukocytes, reduction of lymphocytes, granular lymphocytes	97
	Mouse	4680 ppm (14.80 mg/liter)	8 hr/day	Depletion of bone marrow colony-forming cells	98
	Rabbit	12,000 ppm (38.60 mg/liter)	1 hr/day × several days	Development of grayish white cornea, transient but persistent irritation	38
	Gpg	Vapor	6 hr/day × 5 wk	Increased blood serum aspartate and alanine aminotransferase activity, slightly decreased cholinesterase activity first week, decreased serum protein, increased prothrombin time	99
	Dog	500-800 ppm (1.6-2.55 mg/liter)	2-8 hr/day × 24-410 days	Base line benzene in blood increased from 5-6.5 times post-exposure	100

unchanged form (105). Inhaled benzene at a concentration of 0.340 mg/liter for 5 hr resulted in 33 to 65 percent body retention; 3.8 to 27.8 percent was exhaled unchanged through the lungs, and 0.1 to 0.2 percent excreted with body fluids. Retained benzene is converted 9.7 to 42 percent to urinary phenol, 0 to 5.4 percent to pyrocatechol, and 0.1 to 3.3 percent to hydroquinone (106), and via the hydroxy compound mainly to conjugated sulfates (106). Sato et al. (107) theorize that benzene distribution into tissues is high, owing to its cellular high water-fat partition coefficient.

Benzene intoxication, resembling that of gasoline (40) in the early acute stages, appears to be due primarily to CNS effects. The rate of recovery depends on the initial concentration and exposure time, but symptoms may persist for several weeks. The main signs of intoxication are manifested by drowsiness, dizziness, headache, vertigo, and delirium, and may proceed to loss of consciousness. Inhalation of a concentration of about 16 mg/liter may give a feeling of warmth (38). An acute first-time moderate exposure to benzene may produce typical prenarcois syndrome, with headache, giddiness, and sometimes transient mild irritation of the respiratory and alimentary tract. A case is described by Moeschlin (40) where a workman experienced severe vomiting 6 hr after work; however, the symptoms subsided rapidly without aftereffects. Acute, high exposure may cause dyspnea inebriation with euphoria and tinnitus, which rapidly leads to typical deep anesthesia. If the victim is not treated at this stage, respiratory arrest rapidly ensues, often associated with muscular twitching and terminal convulsions (40). When inhaled by man, benzene has no effect at 25 ppm, but at 50 to 150 ppm produces headache, lassitude, and weariness, and at 500 ppm causes more exaggerated symptoms; 3000 ppm may be tolerated for 0.5 to 1.0 hr, 7500 ppm may result in toxic signs in 0.5 to 1.0 hr, and 20,000 ppm may be fatal if inhaled for 5 or 10 min (38). The latter facts have been substantiated by a number of accidental exposures with unknown air concentrations. However, in most cases mixtures of benzene and other hydrocarbons, mainly toluene, were involved. A case of sudden death has been described by Tauber (108), where a tank started to overflow in a light oil loading area. A combination of high air exposure concentrations of benzene and toluene, excitement due to the mishap, and running through the tank farm appeared to have contributed to the death of the worker. The benzene content was determined as 0.38 mg percent in the blood, 1.38 percent in the brain, and 0.26 percent in the liver. A sudden death occurred in a 16-year-old boy as a result of "sniffing" rubber cement containing benzene as a solvent (109, 110). A blood sample revealed 94 μ g of benzene per 100 ml, and kidney tissue 0.55 mg/100 g. Similar "glue-sniffing" cases have been recorded from other U.S. sources and several European countries. In addition, a young laborer installing plastic tiles with liquid adhesives was found dead in the basement at the workplace and another worker unconscious nearby. The causes of the intoxication and death were attributed to fumes of benzene and toluene (111). Cause of deaths

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to 27.8 percent was
percent excreted with
nt to urinary phenol,
hydroquinone (106),
ates (106). Sato et al.
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ceeded to loss of con-
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benzene may produce
and sometimes tran-
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vomiting 6 hr after
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and tinnitus, which
treated at this stage,
ascular twitching and
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to 1.0 hr, and 20,000
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other hydrocarbons,
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may have been a result of ventricular fibrillation, probably a result of myocardial sensitization to endogenous epinephrine (102). It is believed that the metabolism of benzene in man proceeds similarly to that observed in the rabbit, but at a more rapid rate than in the dog (38).

Cellular Effects. Acute erythromyclosis was diagnosed in a worker following his exposure to moderate benzene concentrations over a period of 30 years (62). Other effects of precursor cell hematopoiesis (98), peripheral blood and bone marrow changes (112), and autopsy reports with signs of hemorrhaging in the brain, pericardium, urinary tract, mucous membranes, and skin (102) have been described.

Acute Animal Experiments. Limited acute experiments with benzene have verified some of the above human findings.

Oral Administration. Oral LD₅₀ values in the rat varied from 3.4 to 5.6 ml/kg, depending on the age or strain of the rat (41); see Table 47.3. Parke and Williams (43) investigated the fate of orally administered, radioactive benzene to rabbits. A dose of 150 to 350 mg/kg containing radioactive benzene at 2.5 to 11.0 μ l was partially reexhaled and excreted, as shown in Table 47.3. Secondary to ingestion, aspiration into the lungs may occur. Gerarde (16) observed that direct instillation into the lungs of rats caused pulmonary edema and hemorrhaging at the site of contact. Cardiac arrest was observed when 0.25 ml of benzene was instilled into the rat lungs (16).

Eye Toxicology. Benzene in the rabbit eye is a moderate irritant, causes conjunctival irritation, and causes transient slight corneal injury (42).

Skin Effects and Permeability. When applied to the guinea pig skin, benzene elicits increased dermal permeability (113). Injected intracutaneously, it caused immediate local pallor and early ulceration (113). When benzene was injected subcutaneously, of 0.8 mg/kg in the adult rabbit, 25 percent was converted to urinary phenol, compared to only 12.5 percent in the young animal (114). The mouse epidermis, following 1 ml of benzene by subcutaneous injection, exhibited cellular injuries, such as nucleoplasmic vacuolation, condensation of nuclear chromatin, flattening of basal cells, and showed fewer organelles 24 hr postinjection. Recovery occurred within 2 weeks (115). Single subcutaneous doses of benzene decreased the iron (⁵⁹Fe) incorporation into circulating erythrocytes of the mouse; 440 mg/kg produced a 27 percent and 2200 mg/kg a 50 percent inhibition, but 88 mg/kg produced no effect (45).

Intraperitoneal Injection. The acute lethal doses for the rat, mouse, and guinea pig, when injected intraperitoneally (48, 50), are in the range of 0.5 to 1.0 g/kg;

see Table 47.3. Subsequently, 23 percent of 0.88 g/kg intraperitoneally administered benzene to the rat was observed to be excreted as phenol metabolites, 74 percent as sulfates, 9 percent as glucuronides, and 7 percent as free phenols (116). Intraperitoneally administered benzene induced reversible enzyme changes in the mouse liver within 6 hr (117). In the rat, benzene, gasoline, and related petroleum products elevated the liver but decreased the kidney alkaline phosphatase by 50 to 60 percent (118). Electronic measurements in the guinea pig revealed that 0.6 ml intraperitoneally injected benzene produced initial CNS excitation, then depression with muscle spasms and convulsions accompanying the hemolytic effect (119). In coho salmon, intraperitoneally injected benzene accumulated in the gallbladder, liver, brain, muscle, and in the carcass (120).

Inhalation of Benzene. In acute animal experimentation, adult rats and mice were more resistant to the effects of benzene than were young animals (121). The effects depend partly on the respiration rate and retention. The retention was 63 to 81 percent for the dog (122) and was independent of ventilation rates. The highest retention occurred at the lowest ventilatory rate. A LC_{50} value of 13,700 ppm has been recorded for the rat (123); see Table 47.3. Benzene inhaled by rabbits was absorbed at 37 and 54.5 percent (38). Rabbits exposed to concentrations of 35,000 to 45,000 ppm showed slight anesthesia in about 4 min and deaths in 22 to 71 min (55). Variations in time ranges until death were attributed to differences in tolerance and immunologic response of the laboratory animals (55). The inhalation of air saturated with benzene vapor resulted in ventricular extrasystole in the cat and the primate, with periods of ventricular tachycardia that occasionally terminated in ventricular fibrillation. In the mouse, 7 mg/liter produced narcosis, 15 mg/liter in 51 min, and 38 mg/liter in 8 min, with recovery. At 38 mg/liter, death occurred in 38 to 295 min, and at 77 mg/liter in about 50 min (38). In the rabbit, 4000 ppm produced narcosis, and more than 10,000 ppm proved fatal (54). At 35,000 to 45,000 ppm the effects were mainly on the central nervous system; anesthesia occurred in 3.7 min, with excitation and tremors in 5.0 min, and loss of pupil reflexes in 6.5 min (55). In the rabbit, sudden death from ventricular fibrillation has also been observed. Pharmacokinetic events include rapid release of adrenal hormones, epinephrine and norepinephrine, which then may sensitize the cardiac system, especially the myocardium, to the action of benzene (124). In acute inhalation by male rats, benzene-induced respiratory paralysis occurred, followed by ventricular fibrillation (125). In an elegant study, Peronnet (56) determined the benzene levels in blood of the guinea pig after an acute inhalation exposure. The author observed that different absorption and clearance kinetics prevailed for 20 and 50 mg/liter exposure levels. In 20 min, the blood level increased to 3.2 and 8.0 mg benzene/100 ml, respectively, and cleared to 1.9 and 5.1 mg/100 ml 5 min post-exposure. During the 20 min of

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the clearing period tested, the blood phenol level increased from 1.6 and 1.7 to 1.8 mg/100 ml, where it plateaued for both concentrations, practically independent of exposure concentration. However, these data also confirm that the CNS effects are due to benzene, rather than phenol. Histochemical studies revealed increased alkaline phosphatase in the spinal cord of the mouse, indicating disturbed neuronal transport characteristics (126). It appears that the central nervous system is more susceptible than kidney tissue to effects of benzene (127). The cellular action in acute exposures is overshadowed by CNS effects. However, Uyeki et al. (98) have demonstrated that precursor renewal systems are sensitive to inhaled benzene. An 8-hr inhalation experiment with mice at 4680 ppm and an in vitro cell culture showed significant depletion of bone marrow colony-forming cells.

Other Routes of Entry or Effects. Benzene injected intravenously into rabbits at 0.1 ml/kg caused rapid CNS effects, intense hemolysis, and death (128). Das et al. (129), with subcutaneously injected benzene to guinea pigs, demonstrated the development of hematological changes, of leukocytosis, followed by leukopenia and granulocytopenia in 6 to 8 days after exposure. Benzene, when inhaled or by other routes, can act as a potent sensitizer, skin or cardiac, even without epinephrine (130). Overshadowed by the intensive CNS effects, acute benzene intoxication does not appear to cause immediate cellular injury, except for early changes in liver endoplasmic reticulum (131).

Single Dose Metabolism. In man and animals, 25 to 80 percent benzene from an acute exposure is partially reemitted in unchanged form through exhalation, depending on the species and the route of entry. Another portion is oxidized mainly to phenol, and in minor quantities to 1,2-di-, 1,4-di-, or 1,2,4-trihydroxy-benzenes, cresols, mercapturic, or muconic acids, and conjugated to the sulfate or glucuronide, depending on the nutritional state (116), route of entry (38), dose, or general pathway overload involved (132-134). Experimental young rabbits were shown to excrete mainly nonconjugated compounds (114). The conjugation appears to take place mainly in the liver and lung (51) but depends on the supply or induction of microsomal P-450 cytochrome and enzymes. Conversely, excessive phenol accumulation can inhibit microsomal activity by feedback inhibition (135). Since various hydrocarbons can induce microsomal enzymes (136), determinations such as serum or ornithine carbamyl transferase may represent an indicator for solvent exposure (137). Fasted animals show different pathways of metabolism, hydroxylation, and type of conjugations (114). High benzene concentrations increased the serotonin level in the rat brain (138). Another biologic indicator represents iron citrate, since its incorporation into red blood cells is blocked by benzene (139). The combination of benzene and lead also inhibits both intact reticulocyte heme and protein synthesis (140). Some drugs, such as phenobarbital, stimulate microsomal

enzyme production (141, 142), whereas toluene inhibits this activity and thus affects benzene oxidation (143). Subcutaneously injected into mice, 880 mg/kg toluene reversed the benzene-induced depression of iron uptake (144). Protection from intraperitoneal benzene toxicity to the rat occurred only when phenobarbital and benzene were injected simultaneously (123), but not with phenobarbital pretreatment for 3 days (123). This was confirmed by Timbrell and Mitchell (145), who found that phenobarbital pretreatment of rats increased the urinary metabolism but decreased respiratory benzene. Ascorbic acid appears to protect from intoxication by the facilitation of metabolic reactions (38).

Aquatic Studies. Some TLM and LC₅₀ studies listed in Table 47.3 reveal similar values for benzene and crude oil (57). Benzene was lethal the first and second day after exposure to a tidepool copepod *Tigriopus californicus* (58).

Human Chronic Benzene Exposure. General. In the early 1930s, benzene was used extensively as a fast-drying solvent in a variety of trades. In the late 1930s, the hematopoietic potential effects of benzene were realized and benzene was controlled more strictly or subsequently replaced wherever feasible. Yet until recently its potential high inhalation exposure has been very common, also stemming from consumer product use where the exposure could not be controlled. Thus, in general, benzene intoxication stems from its use in improperly ventilated establishments. A summary in Table 47.4 illustrates some past high exposures with the chemical effects observed. However, diagnoses do not always agree, owing to variations in definitions and translations from assorted European languages, as, for example, the term leukemia. In addition, clinical determination procedures, such as chromosomal analyses, may represent 50 percent and more false positive results.

Inhalation. Human chronic inhalation of benzene on a lower-order repeated basis appears to be related to a number of pathological conditions. The levels to produce effects vary widely with the individuals. Contributing factors are poor nutrition, certain immunologic tendencies, and consumption of alcohol or drugs. Some symptoms include headaches, dizziness, fatigue, anorexia, dyspnea, visual disturbances, and vague symptoms not connected usually with benzene poisoning. Signs also include fatigue, vertigo, pallor, visual disturbances, and loss of consciousness (106). Findings documented from occupational exposures have been recorded, such as membrane effects (59), hyperbilirubinemia (63), spleno- and adrenomegaly (146), blood dyscrasias with hemolytic effects (59), anemia (67), aplastic anemia (66, 68), hemocytoblastic and lymphatic involvement (61, 147) and reticulocytosis (63), leukopenia, pancytopenia, eosinophilia, basophilia and thrombocytopenia (65), monocytosis (69), and hyperplastic bone marrow effects (63). Also, an increase in chromosomal aberrations has been

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observed (66). There are three stages of involvement apparent: low, intermittent exposure resulting in very subtle hematopoietic changes; moderate to high exposures affecting enzyme synthesis, causing sensitizing effects and anemias; and high exposures producing irreversible blood dyscrasias. Further details have been presented in a summary and discussion by Goldstein (60).

Human Metabolism under Chronic Exposure. Benzene poisoning stems almost exclusively from its atmospheric occurrence, from where it is inhaled, absorbed, and translocated into the bloodstream if not emitted again with the exhaled air. It appears that with benzene in repeated administration metabolic reactions occur different from those observed in acute single exposure. It is apparent that repeated exposures to selected solvents such as benzene deplete a variety of protective enzyme systems. These include those active in iron incorporation or membrane transport, whereby cessation of action causes red blood cell hemolysis. This, in turn, leads to anemia. Another type concerns agglutination mechanisms and causes macrocell formation. Another influences cell mitosis and tubular aggregation, which also results in macromolecule formation or unlimited cell formation as, for example, with leukocytes. Benzene eventually also permeates bone marrow and depletes its red blood cell supply. Benzene per se is soluble in the body fluids to a very small degree only, but is rapidly deposited in any type of fatty tissue or absorbed through membranes, owing to its high lipid solubility. For example, the blood saturation equilibrium is reached at 2.1 mg/liter for each 100 ppm of benzene in the inhaled air (148). This appears to be a function of the fluid-air partition coefficient, which is 11.7 for blood and 5.5 for plasma, and the tissue-blood partition coefficient, which is 16.2 for bone marrow and 58.5 for fat (54). Ingested or inhaled benzene in part is reeliminated in unchanged form in the exhaled air or in the urine. However, the main portion in the human system is metabolized through a variety of major and minor pathways. The primary site of action is the liver, where benzene is oxidized to phenol (hydroxybenzene), catechol (1,2-dihydroxybenzene), or quinol (1,4-dihydroxybenzene). Phenol is subsequently conjugated with inorganic sulfate to phenylsulfate, the other hydroxybenzenes to a lesser extent, and all excreted in the urine (133). Minor pathways include further oxidation of catechol to hydroxyhydroquinol (1,2,4-trihydroxybenzene) or catabolism to *cis,cis*- or *trans,trans*-muconic acids, and phenol conjugation with glucuronic acid to form glucuronides, or with cysteine to produce 2-phenylmercapturic acid (133). Some Russian investigators determined that phenylsulfate peak excretion occurs about 4 to 8 hrs after exposure to benzene (149, 150). This has been substantiated with experiments using mice (151). If the metabolism is overwhelmed from excessive exposure concentrations, the excretion pathways via the glucuronides are used primarily (152). It appears that excessive benzene or phenol accelerates, but also inhibits, a variety of enzyme systems. Indeed, chronic exposure of various animal species to benzene has

substantiated this point, as detailed in the next section. Pawelski et al. (153) observed that human granulocytic alkaline phosphatase was slightly reduced by benzene exposure. Benzene, owing to its affinity to lipids, may be stored and accumulated in human adipose tissue. According to Sato et al. (154), benzene from lipid storage is released much more slowly in the female than in the male.

Other Physiologic Effects. The main effect, even in chronic benzene intoxication, appears to be the CNS involvement, which, however, may not be recognized owing to other visible effects. At low chronic exposure workers have exhibited signs of CNS lesions, abnormal caloric labyrinth irritability, and impairment of hearing (155). In combination with other solvents, workers revealed neurological syndromes of asthenoneurotic or astheno-vegetative polyneuritis with occasional neuronal progression, even after cessation of the exposure (156).

The possibility exists that benzene is a dermal sensitizer (157). Continued skin contact with benzene results in defatting of the skin which leads to erythema, dry scaling, and in some cases the formation of vesicular papules. Prolonged exposure may produce lesions resembling first- or second-degree burns. Systemic intoxication by cutaneous absorption is unlikely, owing to the overwhelming possibility of absorption by inhalation (106).

Intoxication at extremely high levels produces cardiac sensitization (158), a property not unique to benzene. Benzene has been noted to cause dyspnea and tachycardia (159). A later study revealed that benzene decreased arterial pressure and peripheral resistance, and caused diffuse-dystrophic myocardial alterations, apparently functional, which are reversible following termination of the exposure. The increased cardiac output, accelerated circulation rate, and greater potency of the precapillary bed are apparently of a compensatory-adaptive nature, to promote tissue oxygenation (160).

Benzene has appeared on "suspect carcinogen" lists; however, to date no confirming evidence has been found. Benzene is a suspected teratogen; however, only scant confirmative evidence is available. One study has revealed that benzene was determined at about the same concentration in maternal and cord blood (161), indicating that placental transfer does occur. For further details of metabolic events, see Reference 152.

Hematologic Effects. Benzene's effect on the hematopoietic system and its unique myelotoxicity have been known for many years. In most cases the effects are not uniform: symptoms and laboratory findings are not parallel. However, generally, three stages can be diagnosed. Initially, there may occur blood-clotting defects, caused by functional, morphological, and quantitative platelet alteration (thrombocytosis), as well as generally reduced occurrence and production of all blood components (pancytopenia and aplastic anemia). At this stage, if diagnosed and treated, the effects are readily reversible. At a more advanced stage, the bone marrow becomes hyperplastic, then hypoplastic, the

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iron metabolism is disturbed, and internal hemorrhaging occurs. Sometimes the effects are associated with monocytosis or absolute lymphocytosis (69). At this stage, diagnosis and treatment should be prompt and intense, and all future exposure to benzene avoided. Indicative clinical findings are erythrocyte counts below 3.5 million, leukocytes below 4500, decreased platelet numbers, increased iron, and decreased transferrin. Unless treated, a third phase may be entered where bone marrow aplasia may become progressive (40). It is possible that bone marrow regeneration is excessively stressed through augmented destruction of peripheral cells, leading to final regenerative exhaustion, although individual variations in hematologic absolute values and continuous changes are extreme. For example, leukocyte counts may vary more during a life cycle than can be achieved through exposure to benzene (54). Over the years attempts have been made to correlate aplastic anemia and leukemia possibly caused by benzene or other solvent exposure. According to some Italian investigators, inhalation of benzene and other chemical agents may have caused observable hyporegenerative anemias or pancytopenias which, in some cases, may have evolved into myeloblastic (162, 163), granulocytic (164), or hemocytoblastic leukemia (68, 165). In France, Goguel et al. (166) in 1967 contemplated that benzene exposure, possibly in combination with other solvents, might have been the cause of 50 cases of leukemia, 13 of the myelocytic, and eight of the chronic lymphocytic diagnosed between 1950 and 1955.

Hematopoietic effects bordering on leukemia have been observed by Aksoy et al. in a number of cases in Turkey. Four shoemakers developed acute leukemia (167) from exposures to very high quantities of benzene and other adhesive components and solvents; one case of lymphoblastic and one of myeloblastic leukemia were observed (168). In the time frame 1967-1973, of 28,500 shoe workers, 31 were diagnosed with acute leukemia or preleukemia (169, 170). These incidents represent 13 cases per 100,000 workers, more than six cases of leukemia for 100,000 of the normal population (169, 170). According to Aksoy, however, the number of cases decreased in 1974-1975 and no new cases were reported in 1976 following the gradual substitution of benzene by gasoline (171). Gasoline in southeastern Europe, however, still contained a relatively high percentage of benzene. Aksoy believes that genetic and other extraneous factors may play an etiologic role, as observed in two families with the occurrence of leukemia in pairs of identical twins (172). This is substantiated by a case of familial chronic lymphocytic leukemia that occurred in a family that had worked in the dry-cleaning business for 20 to 30 years (173). Conversely, among 38,000 workers in the petroleum and petrochemical industries, the number of leukemia cases did not exceed the normal statistical occurrence (174).

Subcellular Effects. Chromosomal aberrations were found in peripheral blood lymphocytes of benzene workers (175) but not in personnel handling toluene (66). Khan and Khan (176) observed some chromosomal aberrations in bone

marrow preparations. The aberrations were classified by chromatid and chromosome type, whereby both types were found to increase with years of exposure. Aberrations in some subjects were detected even several years after cessation of the benzene exposure (177). One female, following benzene exposure during pregnancy, showed an increase in chromosomal aberrations, particularly in group Gp-, but then delivered a normal, healthy boy and later a girl (177). In one case of granulocytic leukemia, after benzene and solvent exposure intermittently over 7 years, bone marrow showed 16 cells lacking one or more G-group chromosomes and 12 with one or two supernumerary C-group chromosomes (164). Another case with erythroleukemia showed inconsistent chromosomal changes (178).

Current analyses are complicated by preexisting familial chromosomal deviations and the natural changes that occur during normal life cycles (179). Therefore, at this time studies reveal some consistent but also inconsistent changes. Also, the direct mechanism involved in the formation of hematopathologically related chromosomal aberrations is unknown. However, according to Freedman (180), benzene interference on the molecular level appears to affect DNA replication, inhibition of hepatic polyribosomes, and inhibition of protein synthesis. More specifically, experiments with rabbits have demonstrated that benzene on the subcellular level interferes with the thymidine incorporation into bone marrow DNA (181). There exists the possibility that a very short-lived benzene metabolite could produce the subcellular effects, a benzene epoxide or so-called arene oxide (181). Such a compound may have the noted DNA and enzyme inhibitory properties, but is too unstable to be isolated and positively identified.

Immunologic Response. It has long been suspected that benzene exposure may alter the immune response and general functions (54). Bone marrow functions appear to be closely related to immune mechanisms. Workers that had been exposed to benzene, isopropylbenzene, hydroperoxide, and phenol exhibited decreased phagocytic activity, diminished bactericidal activity of the skin, and lowered lysozyme and blood cholinesterase activity. Physiologically, they showed a tendency to hypotension, decreased pulse rate, and CNS changes (182). This was confirmed by the finding of decreased serum complement level in 62 of 79 workers exposed to benzene, toluene, and xylene (183).

Clinical Determinations. Elevated urinary phenol levels normally indicate that benzene exposure occurred in the preceding 8 to 10 hr. Extensive salicylate, other drug intake, or certain foods may elevate the normal 30 mg/liter up to 75 mg/liter (184). A phenol excretion above 20 mg/liter following some benzene exposure is excessive, but not specific. An exposure of 25 to 30 ppm may raise the phenol excretion up to 100 or 200 mg/liter (39, 185). Conversely, with up to 5 ppm, no changes may occur. Ratio changes of urinary inorganic to organic

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sulfate excretion represents a further indicator. The normal value of inorganic total sulfate is about 86 percent (17), as determined by differential precipitation with barium salts (17). However, foods such as bananas, coffee, fish, and smoked meats can also alter the sulfate ratio (17). Exposures of 10 to 40 ppm benzene may lower the ratio to 72 percent, 40 to 75 ppm to 61 percent, 75 to 100 ppm to 43 percent, and 100 to 700 ppm to 38 percent (17). Early signs of benzene intoxication, but not diagnostic tools, are from phenol, sulfate, and direct blood changes. Better indicators are hematologic and cytological determinations on a continuous basis in order to detect progressive changes. Additionally, determination of Heinz bodies (186) and Feulgen's reaction in response to peripheral blood lymphocytes may be of value (187), as well as of serum replacement levels (183), exogenous colony formation (188), and bone marrow activity (80).

Unfortunately, clinical manifestations tend to be insidious at the time of onset. The earlier recorded cases were well advanced at the time of diagnosis and most complaints and visual signs did not correlate too well with the clinical picture, the characteristics, the exposure, or the prognosis. There is a possibility that benzene exposure can facilitate the onset of genetic leukemia. However, more epidemiologic, experimental, and statistical evidence is required to substantiate this point. Also, other chemical agents or drugs may induce similar signs and symptoms, as, for example, drug-induced thrombocytopenia (189) or agranulocytosis (190).

Prophylaxis and Follow-up. It appears that a variety of factors may predispose some individuals' sensitivity towards benzene effects. The young are more vulnerable; nutrition, genetics, and immunoresponse play a role. Vitamins aid in prophylaxis and treatment, such as vitamins of the B group, B₄, B₆, and B₁₂, and vitamin C (191). Recovery from moderate benzene exposure may take 1 to 4 weeks, whereby unsteady gait, nervous irritability, and breathlessness may persist for 2 weeks, and cardiac distress and the peculiar yellow color of the skin as long as a month, although evidence of chronic benzene intoxication still may be encountered later (106). Recovery from chronic high exposure may take from 2 months to several years (106). A Russian author comments on the hygienic effectiveness of the benzene and toluene levels in an industrial plant strictly controlled for 5 years. In 186 workers the nervous system changes returned to normal and urinary phenol levels were found at the base lines for 76 percent of the employees (192).

Benzene in Combination with Other Chemical Agents. Benzene, cyclohexane, and other chemical components used in the synthetic fiber manufacture exhibited early signs of nonspecific involuntary dysfunction in workers (193). Mixing benzene with toluene and gasoline appears to have additive effects as shown in young children exposed 2.5 to 10 years (194). The erythrocytes and

thrombocytes were less affected than the leukocytes and circulating neutrophils. Similarly, glue sniffers were affected more severely with benzene, toluene, and gasoline mixtures, so that aside from asphyxiation, a number of deaths occurred (195). Definite immunologic effects were recognized in workers exposed to benzene, toluene, and xylene (196), but also periodontal problems (197). The effects of benzene and lead appear to exhibit additive inhibitory action on endogenous heme synthesis (180).

Chronic Experiments with Animals. Oral Administration. Animal experiments with oral dosing showed a no-effect level of 1 mg/kg in the rat and leukocyte changes with higher quantities, see Table 47.5. Simultaneous oral administration and inhalation exposure resulted in additive effects. Very high doses affected rat liver and protein fraction weights and decreased hepatic aminopyrine, *N*-demethylase, but increased acetanilide hydroxylase activity. Simultaneous administration of 9.0 mg/kg and an inhalation exposure of 6.63 mg/m³ demonstrated sensitizing effects in the rat (75). This concentration might translate approximately into a 70-kg human exposure of 1500 ppm for 8 hr. In experiments with rabbits, Frash (86) has presented a model of "exhausted" bone marrow, with initial increases, then compensatory decreases of eosinophils, neutrophils, and myelocytes.

Dermal Application. Skin treatment of laboratory animals demonstrated that benzene can be absorbed to a moderate degree: see Table 47.5. Some papillomas and systemic hematopoietic effects were noted.

Subcutaneous Injections. Rats injected subcutaneously with benzene exhibited various hematopoietic and bone marrow effects, as shown in Table 47.5. The rat was more susceptible to benzene toxicity than the mouse and the rabbit, and the hamster least (198). Leukopenia has been recorded for the rat and the rabbit, increased DNA binding in the mouse, pancytopenia, and inhibition of DNA synthesis in the rabbit; also a significant decrease in granulocytes occurred, whereas toluene produced only transient granulocytopenia (199).

Experimental Inhalation. Young rats of 1.5 months when subjected to 1.6 ppm (5 mg/m³) benzene exhibited a lower resistance than 4.5-month-old animals. At 9.5 ppm (0.03 mg/liter) the type of resistance was also independent of age (76). In general, continuous inhalation exposures exerted a more pronounced effect on the animals than did intermittent trials (95). The enzymatic activity of liver microsomes, and thus the metabolism of benzene, increased in rats exposed to benzene vapor for several weeks (200). Continuous inhalation of 4.8 ppm of benzene caused changes in the rat endoplasmic reticulum, associated with inflammation, as shown in Table 47.5. Histopathological, hematologic, cytological, and biochemical changes were induced. A distribution experiment in the

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Metabolism. Absorption, distribution, and metabolism of repeated benzene exposures proceed similarly in laboratory animals as observed for man, except the relative absorption appears higher in man as, for example, in the rabbit of 80 to 85 percent versus 37 to 55 percent at a concentration of 10 to 16 mg/liter (38). In a kinetic study (154) in the female and male rats with a large body fat content, benzene was eliminated more slowly and stored longer than in lean animals. Also, a decrease in white blood cells was observed only in rats with large body fat contents (154). Nutritional fats and paraffin oils stimulated the intestinal benzene absorption in the rat (201). Benzene distribution in the rabbit was highest in the adipose tissue, high for bone marrow, and lower for brain, heart, kidney, lung, and muscle (202), although direct binding was higher in the liver than in bone marrow (203). In the mouse, subcutaneously injected benzene formed mainly free phenol and phenylsulfate (203). Other metabolic changes from subcutaneous injections of benzene into the rabbit have been observed in the brain cortex and erythrocytes, in σ -aminolevulinic acid, porphobilinogen, and protoporphyrin (204). For hepatic benzene oxidation to phenol, involvement of a microsomal cytochrome P-450 iron complex is proposed (205). Hepatic microsomes are the main tools for benzene detoxification (136). A 4-hr benzene exposure at a concentration of 11.9 mg/liter enhanced hepatic microsomal cytochrome P-450 and NADPH oxidase activities in rats, while 35 mg/liter (the LC_{10}) inhibited the enzymes studied (206). This was accompanied by a decreased rate of hepatic hydroxylations, a drop of free SH groups, and reduced tissue oxygen tension (206). Pretreatment with phenobarbital in some cases protected the organism; in others it did not influence the rate of benzene detoxification (123, 207-209). So far, only benzene (80), but not its metabolites, has been demonstrated to interfere with myelogenic mechanisms (181). In mouse blood, benzene inhibits iron uptake into red cells (181).

Cytotoxic Effects. Experimental work with cats has demonstrated that the primary action of benzene may be its capability to change vessel membrane permeability, and secondarily to affect the nutritional state of the cell and subcell, which also includes hydration and dehydration (210). Effects on the skin of the hairless mouse were signs of hyperplasia of the regenerative epithelium (78). Histochemical determinations indicated that benzene affected neuron enzymes of the mouse spinal cord (126) and pathomorphologically changed rat organ structures and the rabbit liver following injection of 0.05 g or 1 g/kg for 20 to 70 days (211). Degenerative muscle fibers were detected in the heart, bronchi, blood vessels, and bile duct in the rat, and degenerative and dystrophic changes in the central nervous system, cortex, thalamus, brain, and

brainstem in the rabbit (211). Liver injury is restricted mainly to the endoplasmic reticulum (77, 131), as is true also in the renal cell, where it in addition decreased the number of mitochondria or caused their swelling (212).

Hematologic and Subcellular Effects. Hematologic effects appear in female rats more rapidly than in the male (213). It induced the biosynthesis of porphyrins in rabbit erythrocytes (89). In the guinea pig, benzene initiated the destruction of erythrocytes in the spleen and lymph nodes, Kupffer cells in the liver, and enhanced phagocytic and hemolytic action (38). Chronic exposure of rats to benzene at 1 g/m³, 4 hr daily for 6 months, caused an inhibition of the phagocytic activity of the leukocytes (214).

Subtle effects of decreased mitochondrial and lysosomal enzyme activities were observed in rabbits at 16 mg/liter, 6 hr/day for 14 days (215). No effect was detected at 10 mg/liter (215). The myelotoxic effect caused by chronic benzene exposure resembles that of ionizing radiation or exposure to radiomimetic substances (216). Both erythropoiesis and leukopoiesis of different cell lines are influenced (216). Various actions include hematopoiesis in the stem cells of the bone marrow and spleen and hypoplasia, which is a reduction of the hematopoietic stem cells (217). This is parallel to the reduction of erythrocytes, thrombocytes, and leukocytes in peripheral blood (217). Subcutaneous injection of 0.3 ml/kg to rabbits for 1 to 9 weeks was accompanied by pancytopenia and, in a portion of the animals, hypoplasia of the bone marrow (87). Rabbits subcutaneously injected with 0.2 ml/kg per day for 18 weeks exhibited induced peripheral pancytopenia and a high incidence of chromosomal aberrations, which persisted long past the hematologic recovery (218).

In vitro incubation of rabbit or human reticulocytes with iron transferrin at nonhemolytic concentrations of 56 to 113 mM resulted in cellular protein inhibition (219).

Sensitization. Benzene appears to be a dermal sensitizer according to tests carried out with the female guinea pig (157).

Immunologic Response. Experimental work with the rabbit indicates that benzene affects autoimmune processes leading to hemopathy (220).

Sex Differences, Teratology, and Reproduction. In general, females or species with increased adipose tissue weight are more highly disposed to the effects of benzene intoxication. Benzene alone exerted no effect on fertility, conception, or embryonic development in rats exposed until days 17 to 19 of gestation. However, benzene, in combination with dichloroethane, for example, resulted in increased embryonic deaths and lower implantation rates (221). Mice, when injected subcutaneously with 3 ml/kg on days 11 to 15 of gestation, developed cleft palate, agnathia, or micrognathia (222).

mainly to the endoplasmic reticulum, where it in addition causes swelling (212).

It appears in female rats that the synthesis of porphyrins initiated the destruction of liver cells in the liver, and chronic exposure of rats to benzene led to an inhibition of the

somal enzyme activities after 4 days (215). No effect was observed caused by chronic exposure to radiomimetic agents. The maturation of different cell populations in the stem cell compartment is a reduction of the production of erythrocytes, leukocytes, and platelets. Subcutaneous injection of benzene led by pancytopenia and bone marrow (87). Rabbits after 4 weeks exhibited induced chromosomal aberrations, and a reduction of the

with iron transferrin at the level of cellular protein

tizer according to tests

Abbit indicates that benzene (220).

eral, females or species exposed to the effects of benzene on fertility, conception, and 17 to 19, of gestation. For example, resulted in stillbirths (221). Mice, when exposed to benzene during gestation, developed

Mutagenicity. Benzene injected subcutaneously into rats at 200 mg/kg once a day for 12 days produced some chromosomal changes in bone marrow. In the rat, some chromosomal aberrations, breaks, and gaps were noted (223). In the hairless mouse, reticulum cell neoplasms mounted to about 38 percent in the benzene-painted and the control animals (77). In the host-mediated assay, mutagenic reactions of benzene were negative (224).

Carcinogenicity. Benzene, when injected subcutaneously at 0.05 to 0.2 ml twice a week for 44 weeks, then once a week for 10 weeks, into weanling male C57B1/6N mice, produced no carcinogenic effects during the 50-week observation period (85). Hairless mice painted twice weekly on the back skin up to 20 months yielded the same percentage of reticulum cell neoplasm as the control animals (77). Skin of white mice painted with benzene for 6 weeks showed ultrastructural changes in the dermis and epidermis, indicating nonspecific irritation of these tissues (225). However, these findings differed substantially from those produced by the carcinogen methylcholanthrene used as a positive control (225, 226). Conversely, when benzene is used as a solvent or carrier, the action of carcinogens such as epoxides is potentiated (227).

Metabolic Interactions. Some chemical or nutritional agents administered preceding, following, or in combination with benzene can have synergistic or antagonistic effects.

The primary antagonistic mechanism of the mammalian's system is the increase in hepatic microsomal production for the induction or promotion of hydroxylase activity in order to accelerate detoxification reactions. Phenobarbital does not affect the benzene LC_{50} in the female CD rat. It increases the hepatic phenol production from 0.84 to 4.43 nM/min per milligram of protein, but does not alter the lung microsomal phenol yield (123). Barbiturates partially reverse benzene-initiated decreased weight gains and lymphopenia in the rat; they also increase the rate of benzene metabolism up to tenfold. Intraperitoneally injected phenobarbital and, 4 days later, benzene, stimulated morphological, cytoenzymatic, and hepatic changes in the Wistar rat (207).

Ikedo and Ohtsui found that sodium phenobarbital pretreatment increased hepatic hydroxylase activity three- to fourfold and increased tolerance to the leukopenic action of benzene (142). Toluene inhaled by rats several times a week increased the liver microsomal enzyme activity and the metabolism of benzene in vitro (200). Subcutaneous administration of 1720 mg/kg toluene reversed the benzene-induced depression of in vitro iron uptake into red cells and metabolic conversion in bone marrow (144). Toluene generally is a competitive inhibitor of the benzene metabolism in the rat (189), but also reduces the cytochrome C reductase activity (228). Human solvent intoxication usually concerns mixtures such as one case recorded by Moeschlin (40) where a black naphtha paint containing benzene, toluene, and xylene was used. After

the paint was used for several weeks in nonventilated quarters, symptoms included headache, giddiness, loss of appetite, nausea, and vomiting. Clinical signs were hemoglobin, erythrocyte, leukocyte, lymphocyte, and thrombocyte changes, typical of benzene poisoning, which reversed after 2 years of treatment.

The action of benzene and lead for hematopoietic effects appears additive (140).

In addition, physical stress, such as vibration, can amplify the action of benzene or toxicants in general (229). Conversely, ascorbic acid, anhydrovitamin A, and retrovitamin A can counteract the toxic effects of benzene (230-232). Steroids, such as adrenocortical hormones, can favorably influence the action of benzene vapors (233). Iron sorbitol counteracted benzene toxicity by reversing DNA synthesis inhibitors in the rat and the rabbit (234), and protected against benzene when subcutaneously administered as sodium selenate by stomach tube to rats (235). A slow decrease of the blood benzene content was noted with the ingestion of 5 g cysteine HCl prior to benzene ingestion (236). When followed by ingestion of choline chloride and betaine aspartate, benzene levels in the blood were further reduced but toluene could be detected (236).

Toxicity to Marine and Plant Life. When tested with a tide pool copepod, *Tigriopus californicus*, of a series of crude oil fractions, benzene was most lethal between days 1 and 2 (58).

Benzene is taken up by plants such as the avocado fruit (237) and grapes (238) and metabolizes it to carbon dioxide.

Biodegradation. Various microorganisms, such as the *Pseudomonas* strains, can use benzene as their sole carbon source. The oxidative degradation is proposed to involve either the epoxide, with subsequent dehydrogenation, phenol, or peroxides as intermediates for the formation of catechol, which has been chromatographically identified (239). Benzene can also undergo ring opening with the aid of *Pseudomonas* and *Moraxella* species (240). In addition, benzene can be phytochemically degraded in reactions with ozone (24).

1.2.6 Industrial Hygiene

Threshold Air Concentrations. In 1974, NIOSH (241) recommended lowering the previous threshold limit value (TLV) of 25 ppm to 10 ppm with an action level of 5 ppm. An emergency temporary new standard was published for a time-weighted average (TWA) of 1 ppm for an 8-hr period with a 5-ppm maximum peak for 15 min, proposed to be effective May 21, 1977 (241). NIOSH (242) has also published a monitoring method, S-311, for benzene, using charcoal tubes for absorption, carbon disulfide for desorption, sample separation, and quantitation by flame ionization gas chromatography. Earlier

methods included benzene determination by a nitration procedure (100). The interferometer, the benzene indicator, and the aromatic hydrocarbon detector were used as field equipment for air analyses.

Benzene can be detected in the air at concentrations of 1.5 to 5 ppm (15, 39). Therefore, odor properties are inadequate for reliable benzene detection in air.

Biologic Monitoring. In biologic monitoring, quantitation is most important for benzene and other hydrocarbon solvent levels. Secondly, urinary phenol determinations are a valuable guide. Further, benzene in exhaled air presents an indication of blood benzene concentration and thus constitutes approximate exposure. Urinary phenol levels of 100 mg/liter represent an exposure of 200 ppm-hr or 8 hr of 25 ppm. Less than 20 to 25 mg/liter indicates normal phenol excretion values. Frequent determination of sulfate is advised; see the NIOSH criteria document (243). The current required regular physical examination should include blood pressure check, lung functions, blood chemistry, hematology, urinalysis, and skin examination (243). A variety of procedures are available for the determination of benzene and related compounds in the blood. The major method relies on extraction and gas chromatographic methods.

Customers or personnel occupationally exposed to benzene should be fully informed of the types and levels of hazards connected with the use of benzene-containing materials.

Precautionary Measures. A variety of procedures and devices are available for the removal of benzene from circulating air. Hands and face protection is required and respiratory equipment for levels above the threshold limits should be available. For a further review, see a discussion of criteria documents by Utidjian (244).

1.3 Toluene

1.3.1 Properties, Sources, and Use

Toluene is the lowest molecular weight member of the alkylbenzenes. Alkylbenzenes, in general, possess properties similar to those described for benzene, except they lack the benzene's hematopoietic characteristics. Alkyl substitution changes physical characteristics and, in turn, the absorption, partition, and metabolic properties.

The time required for hydroxylation, excretion, and sulfate ratio normalization increases with increasing doses and molecular weight of the side chain. The possibility of ring hydroxylation rises with increasing side chain size and probably utilization of alternate catabolic pathways (132).