

2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of styrene and a depiction of significant exposure levels associated with various adverse health effects. It contains descriptions and evaluations of studies and presents levels of significant exposure for styrene based on toxicological studies and epidemiological investigations.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure--inhalation, oral, and dermal--and then by health effect--death, systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods--acute (less than 15 days), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in this chapter in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. These distinctions are intended to help the users of the document to identify the levels of exposure at which adverse health effects start to appear. They should also help to determine whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the tables and figures may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons may be interested in levels of exposure associated with "serious" effects. Public health officials and project managers concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAEL) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels, MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with the carcinogenic effects of styrene in animals are indicated in Figures 2-1 and 2-2.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made, where data were believed reliable, for the most sensitive noncancer effect for each exposure duration. MRLs include adjustments to reflect human variability from laboratory animal data to humans.

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Although methods have been established to derive these levels (Barnes et al. 1988; EPA 1989d), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

2.2.1 Inhalation Exposure

Most information on the effects of inhalation exposure to styrene in humans comes from studies of workers exposed to styrene vapors in the production and use of plastics and resins, especially polystyrene resins. In most cases, the studies involve workplace exposures such as fiberglass boat building factories where the actual levels of styrene are reported as a range of styrene air concentrations. However, there are a few human clinical studies in which exposures are better quantified. Provided below are descriptions of the known effects of inhalation exposure of humans and animals to styrene.

2.2.1.1 Death

There have been no reports of deaths in humans directly associated with exposure to styrene in the workplace (EPA 1988b; Gosselin et al. 1984; NIOSH 1983).

In animals, inhalation studies indicate that the acute toxicity of styrene is low to moderate. An LC_{50} of 2,770 ppm after 2 hours of exposure was reported in rats, and the LC_{50} for mice after exposure for 4 hours was 4,940 ppm (Shugaev 1969). All rats and guinea pigs survived after exposure to 1,300 ppm styrene for 30 hours and 16 hours, respectively (Spencer et al. 1942). However, all animals died after 40 hours of exposure.

All reliable LOAEL values and LC_{50} values for lethality in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.2 Systemic Effects

No studies were located regarding cardiovascular and musculoskeletal effects in humans or animals after inhalation exposure to styrene.

For the following systemic effects resulting from inhalation exposure to styrene, the highest NOAEL values and all reliable LOAEL values in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

TABLE 2-1. Levels of Significant Exposure to Styrene - Inhalation

Key to figure ^a	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
ACUTE EXPOSURE							
Death							
1	Rat	3-40+ hr				1300 (LC100)	Spencer et al. 1942
2	Rat	4 hr				2770 (LC50)	Shugaev 1969
3	Gn pig	16-40 hr				1300 (LC100)	Spencer et al. 1942
4	Mouse	2 hr				4940 (LC50)	Shugaev 1969
Systemic							
5	Human	1 d (occup)	Renal		212 (increased urinary levels of alanine aminopeptidase)		Aliberti and Severini 1987
6	Human	15-45 min	Resp Derm/oc	216	216 (nasal irritation) 376 (eye and skin irritation)		Stewart et al. 1968
7	Mouse	3 min	Resp		156 (irritation of upper respiratory tract)		Alarie 1973
Neurological							
8	Human	1 d (occup)			55 (slowed reaction time)		Gamberale et al. 1976
9	Human	1 hr			87 (inhibition of vestibular-oculomotor system)		Odkvist et al. 1982
10	Human	7 hr			99 (impaired balance)		Stewart et al. 1968

TABLE 2-1 (Continued)

Key to figure ^a	Species	Exposure frequency/duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
11	Human	1 d (occup)			92 (tiredness, slow reaction times, mood changes)		Cherry et al. 1980
12	Mouse	4 hr			413 (behavioral changes)		DeGaurriz et al. 1983
Developmental							
13	Rat	10 d 7hr/d		600			Murray et al. 1978
14	Rabbit	13 d 7hr/d		600			Murray et al. 1978
15	Hamster	12 d 6hr/d		750		1000 (fetal deaths or resorptions)	Kankaanpaa et al. 1980
Reproductive							
16	Mouse	5 d 6hr/d		300			Salomaa et al. 1985
INTERMEDIATE EXPOSURE							
Systemic							
17	Rat	21 d 5d/wk 4hr/d	Resp	150	1000 (nasal mucosa effects)		Ohashi et al. 1986
18	Rat	11 wk 5d/wk 6hr/d	Hepatic		300 (enzyme alterations, liver morphology)		Vainio et al. 1979
			Renal	300			
19	Rat	13 wk 5d/wk 7hr/d	Renal	133			Viau et al. 1987

TABLE 2-1 (Continued)

Key to figure ^a	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
20	Gn pig	26 wk 5d/wk 7-8hr/d	Derm/oc			1300 (nasal irritation, severe lung irritation after a few exposures)	Spencer et al. 1942
21	Pig	3 wk 5d/wk 6hr/d	Hemato	360			Johnston et al. 1983
Neurological							
22	Rat	3 mo (cont)		90	320 (astroglial alterations)		Rosengren and Haglid 1989
23	Rat	18 wk 5d/wk 16hr/d		1400			Kulig 1988
24	Rat	21 d 14hr/d			800 (ototoxicity)		Pryor et al. 1987
CHRONIC EXPOSURE							
Systemic							
25	Human	5.1 yr (occup)	Hepatic	120			Harkonen et al. 1984
26	Human	ND (occup)	Hemato		44 (lowered red blood cell count)		Checkoway and Williams 1982
27	Human	1-20 yr (occup)	Hepatic		100 (increased serum levels of OCT and ALAT)		Hotz et al. 1980
28	Human	6 yr (mean)	Renal	24			Viau et al. 1987

TABLE 2-1 (Continued)

Key to figure ^a	Species	Exposure frequency/duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
29	Human	11 yr (mean) (occup)	Renal	53			Vyskocil et al. 1989
30	Human	>1 yr (occup)	Hepatic		100 (elevated serum amino-transferase levels)		Axelsson and Gustavson 1978
31	Rat	18-21 mo 5d/wk 6hr/d	Hepatic		600 (increased liver weight)		Jersey et al. 1978
Neurological							
32	Human	5.1 yr (mean) (occup)			31 (EEG abnormalities)	55 (visuomotor accuracy and psychomotor performance decline)	Harkonen et al. 1978
33	Human	8.6 yr (mean) (occup)			25 ^b (decreased verbal learning skills)		Mutti et al. 1984a
34	Human	6.2 yr (mean) (occup)			130 (neuroendocrine effects)		Mutti et al. 1984b
Cancer							
35	Rat	52 wk 5d/wk 4hr/d				100 ^c CEL (mammary tumors)	Conti et al. 1988

TABLE 2-1 (Continued)

Key to figure ^a	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
36	Rat	18-21 mo 5d/wk 6hr/d				600 ^c CEL (mammary adenocarcinomas - females)	Jersey et al. 1978

^aThe number corresponds to entries in Figure 2-1.

^bUsed to derive a chronic inhalation MRL of 0.06 ppm: exposure level adjusted by 5/7 and 8/24 to account for intermittent exposure, and divided by an uncertainty factor of 100 (10 for use of a LOAEL, and 10 for human variability).

^cThere is significant uncertainty in this CEL value for styrene; see text for discussion of study limitations.

ALAT = alanine aminotransferase; CEL = cancer effect level; cont = continuous; d = day(s); Derm/oc = dermal/ocular; EEG = electroencephalograph; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); LC50 = lethal concentration, 50% kill; LC100 = lethal concentration, 100% kill; LOAEL = lowest-observed-adverse-effect level; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; ND = no data; NOAEL = no-observed-adverse-effect level; occup = occupational (typically 5 d/wk, 8 hr/d); OCT = ornithine carbamyl transferase; Resp = respiratory; wk = week(s); yr = year(s)

FIGURE 2-1. Levels of Significant Exposure to Styrene – Inhalation

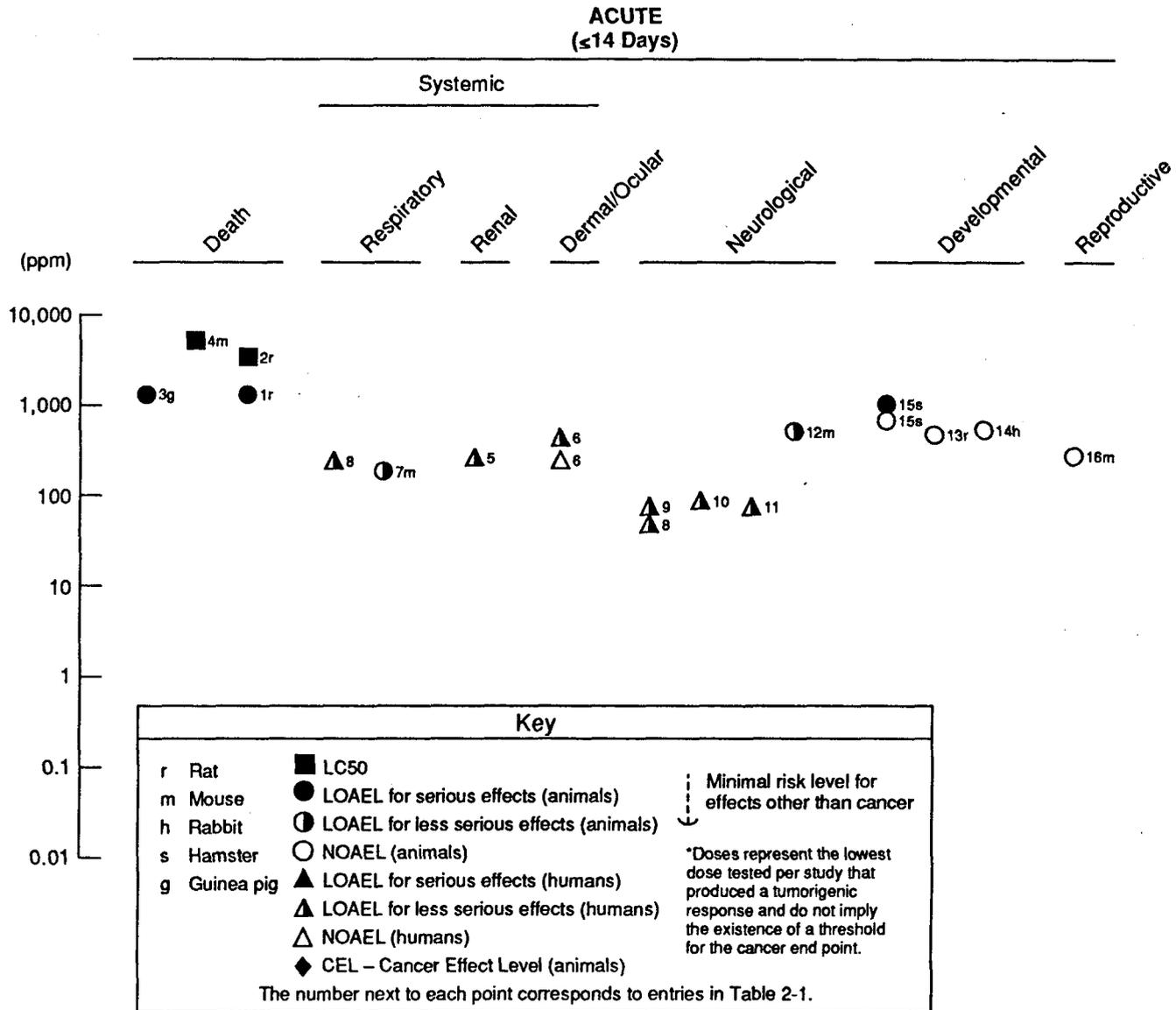
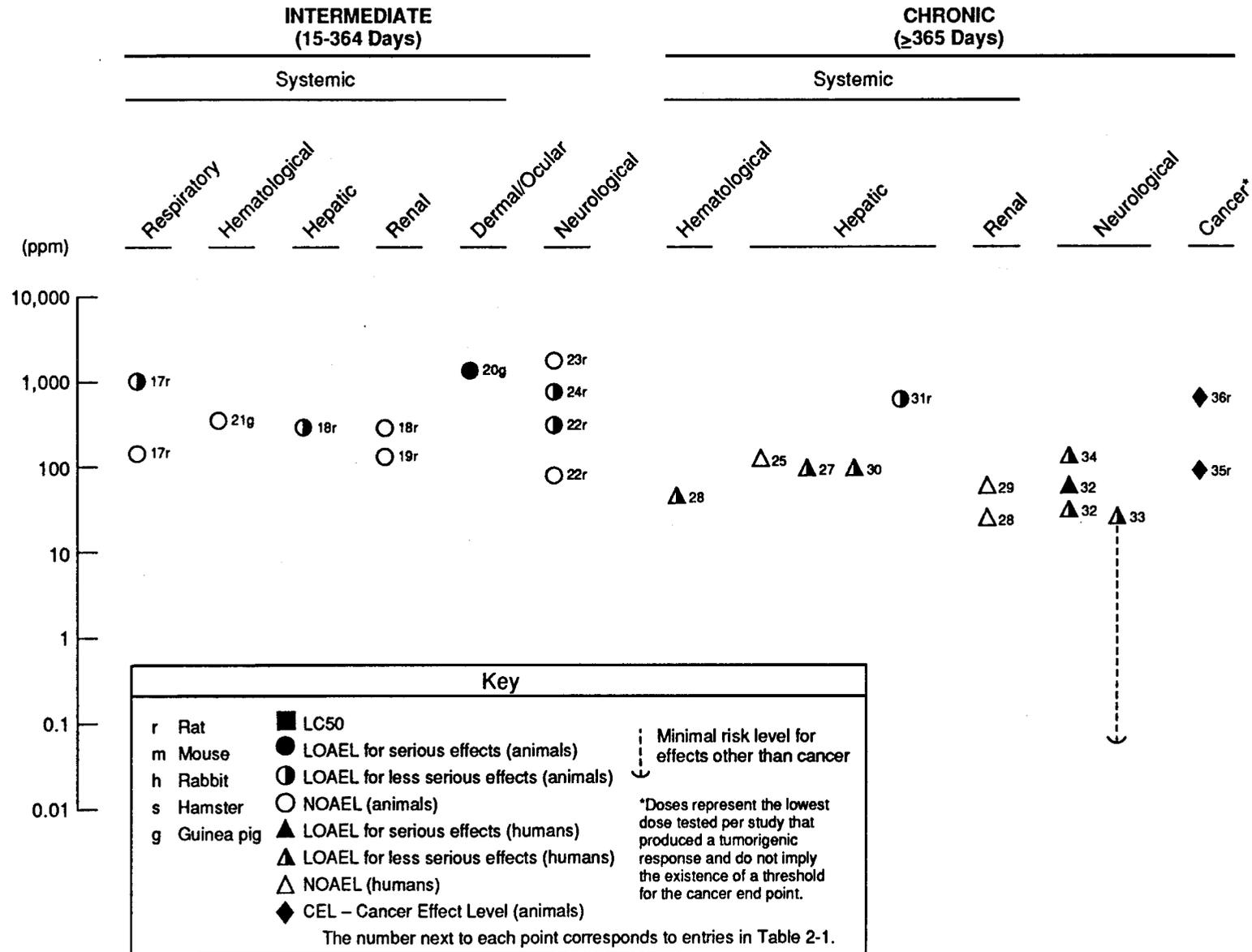


FIGURE 2-1 (Continued)



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Respiratory Effects. Several human studies have examined the respiratory effects caused by inhalation exposure to styrene. The most commonly reported general symptom is mucous membrane irritation. Irritation of the upper respiratory tract (i.e., nose and throat) has occurred in human volunteers (Carpenter et al. 1944; Stewart et al. 1968) and workers (NIOSH 1983). Throat irritation and increased nasal secretion occurred following exposure of two male subjects to 800 ppm for 4 hours (Carpenter et al. 1944). Nasal irritation was observed in all volunteers after exposure to 376 ppm styrene for 60 minutes (Stewart et al. 1968). Obstructive lung changes were observed in 4 of 21 workers exposed to styrene for about 10 years (Chmielewski and Renke 1975; Chmielewski et al. 1977). However, exposure levels were not defined.

Similar effects have been reported in animals following exposure to styrene vapor. In rats, pathological changes in the respiratory mucosa were found following exposure to 1,000 ppm styrene for 4 hours/day, 5 days/week for 3 weeks. The upper nasal mucosa demonstrated decreased ciliary activity and abnormal morphology. Also, vacuolization and increased numbers of dense bodies of respiratory epithelial cells and sporadic rupture of the cytoplasmic membrane were observed in the rats exposed to 1,000 ppm for 21 days (Ohashi et al. 1986).

Rats and guinea pigs exposed to styrene at a level of 1,300 ppm for 7-8 hours/day, 5 days/week for 6 months showed nasal irritation, but rabbits and monkeys did not (Spencer et al. 1942). Histopathological examinations revealed no changes between test and control rats, but pronounced lung irritation was seen in guinea pigs that died after a few exposures. The irritation, which included congestion, hemorrhages, edema, exudation, and a general acute inflammatory reaction, was not seen in the guinea pigs, rabbits, and monkeys that survived the 6-month exposure period (Spencer et al. 1942). In another study, sensory irritation of the upper respiratory tract in mice was observed following exposure to a styrene aerosol of 666 mg/m³ (156 ppm) (Alarie 1973). This effect was determined by measuring the decrease in respiratory rate during the exposure of the mice to the aerosol. β -Nitrostyrene, which was also tested in this study, exhibited much greater activity than styrene. No pathological data were available.

These well conducted human and animal studies (see Table 2-1 and Figure 2-1) demonstrate the characteristic irritant properties of styrene on the upper respiratory tract.

Gastrointestinal Effects. Nausea was observed in humans exposed to 376 ppm styrene after 1 hour exposure (Stewart et al. 1968). This effect is probably secondary to effect on the central nervous system, although mucociliary transport of styrene aerosol droplets from the upper respiratory tract to the gastrointestinal tract might also contribute to gastrointestinal irritation. A Russian study (Basirov 1975) reviewed by the World Health Organization (WHO 1983) investigated the effects of styrene on digestive function by testing the secretory, excretory, motor, and pepsinogen-generating functions of the stomach in 20 unexposed and 80 exposed workers. The authors

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reported that some workers in the styrene-butadiene synthetic rubber manufacture exposed to 60-130 mg/m³ (14-31 ppm) styrene for less than 5 to more than 10 years had decreased digestive function and decreased stomach acidity.

No studies were located regarding gastrointestinal effects in animals after inhalation exposure to styrene.

Hematological Effects. Several studies indicate that inhalation exposure of humans to styrene cause mild or no effects on the blood. In one study, the incidence of abnormal values for hematological parameters including erythrocyte, leukocyte, and platelet counts, and hemoglobin levels for 84 styrene workers generally exposed to less than 1 ppm styrene for 1-36 years was investigated. However, these workers were also exposed to intermittent high levels of styrene as well as to other chemicals. The percentages of the exposed group with abnormally low hemoglobin and erythrocyte values or abnormally high leukocyte values were less than those percentages in the 62 person control group. There were no abnormal thrombocyte values reported in either the exposed or control groups (Thiess and Friedheim 1978). Findings from a group of 93 workers engaged in the manufacture of styrene polymers and exposed to generally less than 1 ppm styrene for 1-38 years were also presented in this study; only the incidence of abnormally low erythrocyte counts (in the group exposed to styrene) was found to be statistically significant ($p \leq 0.05$). However, because exposures could not be determined accurately and because there were concomitant exposures to other chemicals, the results of these studies are difficult to interpret.

Lowered erythrocyte counts, hemoglobin, platelets, and neutrophils and slightly higher mean corpuscular red cell volumes and neutrophil band counts were observed in workers in a styrene-butadiene rubber manufacturing plant (Checkoway and Williams 1982). The highest mean styrene level was 13.67 ppm. However, interpretation of this study is limited because multiple-chemical exposures were involved and exposure and clinical signs were measured at the same time and only once. An earlier study of styrene workers showed no definite pattern of hematological changes (Lorimer et al. 1978). In these studies, exposure levels were uncertain and multiple chemicals were involved.

In an animal inhalation study, no adverse hematological effects were noted in Jersey pigs exposed to 72 or 360 ppm styrene for 3 weeks (Johnston et al. 1983). In rats exposed to 49 ppm styrene, erythrocyte-aminolevulinate dehydratase (ALA-D) was depressed markedly. The decrease in enzyme activity was accompanied by a decrease in the enzyme content in bone marrow cells (Fujita et al. 1987). The author's suggestion that the changes may have been a result of styrene oxide reducing the enzyme protein is based on in vitro data.

The well-conducted Thiess and Friedheim (1978) study as well as the more limited studies indicate that few adverse hematological effects occurred in styrene-exposed workers. However, the full meaning of the findings is not clear because of poor characterization of the exposure level and concurrent

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exposures to other chemicals. The animal data do not permit a characterization of hematological effects in animals after inhalation exposure to styrene. However, oral animal data support the suggestive evidence in humans that styrene may affect hematological parameters (Section 2.2.2.2).

Hepatic Effects. Human studies on the hepatic effects of styrene inhalation frequently used serum levels of enzymes as indicators of liver dysfunction. In general, human studies have resulted in negative or equivocal results (Harkonen et al. 1984; Hotz et al. 1980; Lorimer et al. 1978; Thiess and Friedheim 1978). The effects of styrene, at exposure levels generally less than 1 ppm, were evaluated in 84 styrene workers exposed for 1-36 years (Thiess and Friedheim 1978). In this study, serum glutamate-oxalacetate transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT) and gammaglutamyl transferase (GGT) were measured in exposed workers and a reference population. Within the styrene-exposed group the incidence of high SGOT or SGPT values was less than the reference group. The number in the exposed group having abnormally high GGT values was twice as high as the control group. However, only a small number of exposed workers and controls were included in this study and the results were not statistically significant. In another study, workers exposed to styrene in the range of 100-300 ppm as an 8-hour time-weighted average in small manufacturing operations showed elevated levels of liver amino transferase (Axelson and Gustavson 1978). However, the cause of the increased levels and effects on liver function are uncertain. In another exposed population of 93 workers exposed to less than 1 ppm styrene for 1-38 years, hepatic enzyme levels (SGOT, SGPT, and GGT) were measured (Thiess and Friedheim 1978). There were no statistically significant differences in the hepatic enzyme levels of the exposed and reference population.

Liver enzyme levels were also measured in 57 workers exposed to styrene in the polyester industry for 1-20 years (Hotz et al. 1980). The styrene exposure concentrations were from 1 to 100 ppm. The hepatic enzymes, ornithine carbamyl transferase (OCT), aspartate amino transferase (ASAT), alanine amino transferase (ALAT), and GGT were measured and showed increased activity compared to control values. The OCT and ALAT levels correlated better than the GGT and ASAT levels with the degree of styrene exposure. These study results suggest hepatic injury at 100 ppm and less. In another study, the effect of styrene exposure in 34 styrene-exposed and 34 control female workers was evaluated (Harkonen et al. 1984). These workers were exposed to approximately 50-120 ppm styrene for a mean duration of 5.1 years in the breathing zone with the highest levels temporarily exceeding 200 ppm. Liver function was assessed by measurement of ASAT, ALAT, and GGT. Bile acid concentrations were also measured. The styrene-exposed group did not have higher activity levels of either liver enzymes or bile acids when compared to the control group values. A few abnormal values in both the exposed and control groups were associated with the use of drugs or alcohol and the study results indicated no hepatic effects associated with exposure to styrene. In

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a different study, workers exposed to approximately 5-20 ppm styrene for up to 20 years or more were found to have high GGT values even when the use of alcohol was taken into consideration (Lorimer et al. 1978). However, no other hepatic parameters were affected.

In animal studies, rats were exposed to 300 ppm styrene intermittently for 11 weeks (Vainio et al. 1979). Free glutathione in rat liver significantly decreased (approximately 59%) after an exposure period of 2 weeks. The glutathione depression continued throughout the intermittent 11-week exposure. The cytochrome P-450 content of hepatic microsomes was doubled during the first 2 weeks of exposure to 300 ppm. The epoxide hydratase and UDP glucuronosyltransferase activity in rat liver increased upon exposure to 300 ppm over 11 weeks of intermittent exposure. Degenerative morphologic alterations were also observed in the parenchymal cells of the liver 2 weeks after exposure to 300 ppm (Vainio et al. 1979). In another study by the same author, a 4-day exposure at even lower styrene levels (less than 200 ppm) resulted in decreased free glutathione in rat liver. In this case the decrease was reversible as evidenced by a slight elevation of hepatic glutathione concentrations in styrene exposed animals 18 hours after exposure.

In a 24 month study of rats exposed to 600 or 1,000 ppm styrene, increased absolute and relative liver weights were observed in females at both exposure levels at 6 months (Jersey et al. 1978). At 12 months and terminal necropsy, these effects were inconsistent and not dose-related. Histopathological findings were similar for exposed and control females at the 6 and 12 month intervals. For males, there was evidence of a nutritional state associated with decreased body weight gain. At termination, alveolar histiocytosis was observed in the lungs of females exposed to 1,000 ppm only. Excessive mortality and chronic mucosa pneumonia prevented an appropriate evaluation of the male rat histopathology.

Although the well-conducted studies on workers generally gave negative results, the animal studies involving higher exposures suggest that styrene inhalation may affect liver function.

Renal Effects. Human studies generally confirm the importance of urinary enzymes as indicators of kidney damage due to exposure to styrene (Aliberti and Severini 1987; Viau et al. 1987; Vyskocil et al. 1989) and other chemicals. The urine of 15 subjects exposed to styrene (900 mg/m³ or 212 ppm) for an 8-hour workshift and 20 unexposed control subjects was evaluated for urinary enzyme effects (Aliberti and Severini 1987). The subjects exposed to styrene demonstrated higher levels of alanine-aminopeptidase (AAP) at the end of the workshift compared to unexposed controls (p<0.001). The N-acetylglucosaminidase (NAG) was increased much less than AAP but the increase over the control group was statistically significant (p<0.01). These results are considered to represent an early biochemical indication of adverse renal effects. Viau et al. (1987) measured urinary excretion of β -microglobulin, retinol-binding protein and albumin in 65 workers exposed to styrene (24 ppm) for a mean duration of 6 years. No significant difference was observed in the urinary excretion of proteins when compared to controls. No significant

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difference was found by Vyskocil et al. (1989) in the urinary excretion of albumin β -microglobulin, retinol-binding protein, total protein, glucose, lysozyme, lactate dehydrogenase, and β -N-acetyl-D-glucosaminidase in workers exposed to an average of 53 ppm styrene when compared to a control group. The minor kidney effects noted in these well-conducted human studies indicate that styrene may exert a minor effect on some kidney enzyme functions.

Minor changes in renal enzyme activities and no effects on morphology (Vainio et al. 1979; Viau et al. 1987) have also been observed in animals after exposure to styrene. Intermittent 11-week exposure to styrene by inhalation (300 ppm) induced the activities of the drug hydroxylating enzymes ethoxycoumarin O-deethylase, and cytochrome P-450. Activities of the conjugating enzymes, epoxide hydratase, and UDP glucuronosyltransferase were also induced in the exposed rats (Vainio et al. 1979). Since there were no degenerative morphologic alterations observed in the kidney, this is not a clear adverse effect. In another study, no functional or morphological renal changes could be detected in rats exposed to 133 ppm styrene (5 days/week) for 13 weeks (Viau et al. 1987).

Dermal/Ocular Effects. Eye irritation in humans has been reported at high styrene concentrations (Carpenter et al. 1944; Stewart et al. 1968). Immediate eye irritation was reported in two human subjects exposed to 800 ppm styrene for 4 hours (Carpenter et al. 1944). Eye irritation was also noted by Stewart et al. (1968) in two of five volunteers exposed to 376 ppm styrene for 1 hour. Also, 345 styrene-exposed workers (98% male) were evaluated for ocular toxicity due to exposure to styrene (5-200 ppm) for 7-20 years. No evidence of optic neuritis, central retinal vein occlusion, or retrobulbar neuritis was found. Conjunctival irritation was a complaint of 22% of the 345 workers exposed to styrene levels above 50 ppm (Kohn 1978). Eye and nasal irritation was observed in rats and guinea pigs exposed to 1,300 or 2,000 ppm styrene, 7-8 hours/day, from 21 to 30 weeks (Wolf et al. 1956). 5 days/week for durations ranging Rabbits and monkeys were exposed for up to 360 days with no effects.

Other Effects. Exposure to styrene vapors has been found to increase the levels of several pituitary hormones (prolactin, growth hormone) in female workers (Mutti et al. 1984b). This effect is probably mediated through the nervous system, and so is discussed in Section 2.2.1.4.

2.2.1.3 Immunological Effects

No dose-related differences in the concentrations of serum alpha, beta and gamma globulins were found in workers exposed to different concentrations of styrene (Chmielewski et al. 1977). However, exposure levels and durations were not specified. In patch-testing studies of cross-reactors to styrene, styrene epoxide was more sensitizing than styrene itself (Sjoberg et al. 1984). The authors interpreted this as evidence that styrene requires metabolism by skin aryl hydrocarbon hydroxylase to styrene epoxide for its sensitizing activity.

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No other studies were located regarding immunological effects in humans or animals after inhalation exposure to styrene.

2.2.1.4 Neurological Effects

Several epidemiological and clinical studies have shown that styrene exposure causes alterations of central nervous system functions in humans. Men exposed to levels of 52-117 ppm (mean = 92 ppm) in a boat-building factory were more subject to mood changes, were more likely to report feeling tired, and had slower reaction times than unexposed workers (Cherry et al. 1980). A similar decrease in reaction time was observed in four groups of workers exposed to styrene at levels of 17-101 ppm (mean = 55 ppm) (Gamberale et al. 1976). Workers exposed to styrene in several industries at mean concentrations of 5-125 ppm had mild sensory neuropathy characterized by decreased sensory conduction amplitude and increased duration, but there were too few people to define no-adverse-effect levels (Rosen et al. 1978). An increased occurrence of fast activity in central and precentral areas of the brain was also noted in styrene workers. These effects were clearly visible in workers exposed to an average of 47 ppm or more. In a study by Stewart et al. (1968), no toxicity was noted following 1- or 2-hour exposure to 51 ppm (3 subjects) or 117 ppm (1 subject) styrene, respectively. In the same study, 6 subjects were exposed to 99 ppm styrene vapor for 7 hours. Three of these subjects reported that they were having difficulty performing the modified Romberg test which measures balance and coordination. Also, 1-hour exposure to 376 ppm styrene vapor resulted in abnormal neurological findings and complaints of nausea and inebriation. In another study, immediate muscular weakness, listlessness, drowsiness and impaired balance was observed in two human subjects exposed to 800 ppm styrene (Carpenter et al. 1944). Odkvist et al. (1982) investigated the inhibition of the vestibular-oculomotor system in ten human subjects experimentally exposed to 87-139 ppm styrene for 1 hour. Visual suppression was disturbed and the authors concluded that styrene acts, along with other organic solvents, to block cerebellar inhibition of the vestibula-oculomotor system.

Chronic exposure of workers to styrene results in increased incidence of abnormal electroencephalograms (EEGs) (Harkonen et al. 1978). In this study, exposure levels were estimated from an empirical relationship that was established between workplace air concentrations and urinary levels of mandelic acid (MA) in exposed workers. This relationship is expressed as:

$$\ln(\text{styrene air concentration, ppm}) = -3.4915 + 1.0568 \cdot \ln(\text{urinary MA concentration, mg/L})$$

The authors had previously observed a strong correlation ($r^2 = 0.86$, $p < 0.001$) between the time weighted average styrene concentrations in air and urinary mandelic acid concentrations. Thus, exposure estimates derived from urinary metabolite levels are considered to be sufficiently reliable to establish meaningful dose-response relationships. Exposure to an 8 hour time-weighted average of 31 ppm or above resulted in a 30% incidence of altered EEGs, compared to 10% in workers exposed to less than 31 ppm. The abnormalities

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included local slow wave activity (14/23), diffuse theta activity (8/23), and bilateral discharges (2/23). Exposure to 55 ppm or higher resulted in decreased visuomotor accuracy and impaired psychomotor performance. As part of the same study, 9 out of 40 workers who displayed subjective symptoms of styrene exposure were found to have abnormal nerve conduction velocities. However, no clear relationship between the altered conduction velocity and styrene exposure could be established. Mutti et al. (1984a) reported verbal learning skills were significantly impaired in workers exposed to mean daily concentrations of styrene greater than 25 ppm (this value is also estimated from levels of urinary metabolites). This exposure level (25 ppm) is the lowest concentration known to have caused significant neurological effects in humans, and so has been selected to calculate a chronic inhalation MRL of 0.06 ppm, as described in the footnote to Table 2-1. Logical memory and visuo-constructive abilities were also significantly affected in workers exposed to greater than 50 ppm styrene.

Inhalation exposure to styrene may also cause peripheral neuropathy. A man exposed to an undetermined concentration of styrene vapor for 5 years (4-10 hours/day, 7 days/week) developed burning sensations in the feet and moderate slowing of nerve conduction velocity in the lower limbs (Behari et al. 1986). Histologic examination revealed demyelination of sural nerve fibers. However, this individual also had a history of prescription drug use and may have been exposed to chemicals other than styrene. Therefore, this report does not contain adequate information to establish an unequivocal cause-effect relationship.

The effects of styrene on the neuroendocrine activity of the tuberoinfundibular dopaminergic system (TIDA) in humans was indirectly investigated with measurements of adenohipophysial hormones, including serum prolactin (PRL), human growth hormone (HGH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in 30 female workers exposed to approximately 130 ppm styrene and 30 age-matched controls (Mutti et al. 1984b). The exposed subjects' serum levels of PRL were more than double the reference values and correlated with exposure to styrene. The serum levels of HGH in exposed women were also higher than the reference group. The investigators concluded that the styrene-induced neuroendocrine effects were mostly due to acute exposure and were not influenced by the duration of exposure after control for age and concentration of styrene.

The effect of occupational exposure to styrene on high-frequency hearing loss was evaluated in workers exposed to 35-165 ppm styrene. The studies did not demonstrate an increased age-dependent decrease in hearing high frequencies when compared to controls (Muijser et al. 1988). However, a comparison within the exposed group indicated a statistically significant difference in hearing thresholds for high frequencies in the workers exposed to the highest concentration of styrene (up to 700 mg/m³).

Limited neurobehavioral and neurotoxic effects of styrene have been investigated in animal studies. In rabbits, exposure to 750 or 1,500 ppm of styrene resulted in dose-dependent decreases in striatal and tubero-

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infundibular dopamine and homovanillic acid levels. Noradrenalin levels were not significantly changed (Mutti et al. 1984c). Mice exposed to 413-851 ppm styrene for 4 hours exhibited decreased immobility in a confined area swimming test (DeCeaurriz et al. 1983). In another study, groups of rats were exposed to 350, 700, and 1,400 ppm of styrene for 18 weeks (Kulig 1988). Compared to controls, styrene-treated rats exhibited an initial reduction in activity and grip strength, but this effect tended to diminish during the study period. This suggests that some level of tolerance might develop during continuous exposure. Coordinated movement and nerve conduction time were not affected. Initially there were some styrene-related neurobehavioral effects. However, at the end of the exposure period the performance of styrene-treated rats was not significantly different from the control group and there were no deficits in performance during the post-exposure period. In another animal study, Rosengren and Haglid (1989) demonstrated that constant styrene exposure of rats to 320 ppm (24 hours/day) for 3 months induced increases in glial fibrillary acidic proteins 4 months after the exposure period. Using this glial cell marker, this study suggests that styrene may induce abnormalities in the central nervous system of rats. Pryor et al. (1987) reported that 800 ppm styrene caused an elevation in behavioral auditory response thresholds (12kHz) in rats exposed for a 3 week period (14 hours/day). No differences were found between exposure groups in the acquisition of multisensory conditioned avoidance response tasks.

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.5 Developmental Effects

Limited information concerning developmental effects of styrene in humans is available from studies of delivery outcome of women employed in the plastics industry (processing styrene or polyurethane plastics). Case-control studies performed in Sweden and Norway did not detect an increase in the odds ratio for developmental effects (stillbirth, infant death, malformations, low birth weight) in women who worked in the plastic industry (Ahlborg et al. 1987). However, actual levels of styrene exposure were not known for either group of workers. In another study, the birth weights of infants whose mothers worked during pregnancy in the reinforced plastics industry were analyzed by Lemasters et al. (1989). Women who worked in areas with elevated levels of styrene (estimated from industrial hygiene data to average about 82 ppm) had offspring with adjusted birthweights that were 4% less than the offspring of unexposed women. However, this decrease was not statistically significant ($p=0.08$). These studies suggest that developmental effects in exposed workers are not of major concern, but the data are not adequate to exclude this effect. Moreover, interpretation of the results is complicated due to exposure of the workers to other chemicals in the workplace such as toluene, xylene, acetone, methylene chloride, and methyl ketone (Lemasters et al. 1989), as well as thermal degradation products of styrene polymers (Ahlborg et al. 1987). Workers may also be exposed to aerosols containing aldehydes, ketones, alcohols, esters, acids, and anhydrides.

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Evaluations of developmental effects of styrene in rats, rabbits, mice, and hamsters have been reported (Kankaanpaa et al. 1980; Murray et al. 1978). Rats were exposed to 0 or 300 ppm styrene on days 6 to 15 of gestation and additional rats were subsequently exposed to 0 or 600 ppm styrene (Murray et al. 1978). The average fetal crown-rump length was significantly reduced in the 300 ppm group, but not in the 600 ppm group. The authors concluded this effect was not treatment-related. The number of live fetuses or resorptions/litter was not affected by exposure to styrene. A few skeletal variants such as lumbar spurs and delayed ossification of sternebrae occurred in the styrene-exposed litters at a higher incidence than the control litters; however, the occurrence of this effect was similar to historical controls. Additionally, it was reported that there were no fetotoxic or teratogenic effects in rabbits exposed to 300 or 600 ppm styrene on days 6-18 of gestation (Murray et al. 1978). Although there was a significant increase in the incidence of unossified sternebrae in the 600 ppm group, it did not exceed that found in historical control data. Embryotoxicity was observed following exposure of pregnant mice to 250 ppm styrene on days 6-16 of gestation (Kankaanpaa et al. 1980). The incidence of dead or resorbed fetuses was higher in the exposed group but was not statistically significant ($p < 0.10$). Some minor skeletal malformations (rib fusions, extra ribs) were observed in the mice. In the same study, hamsters were exposed to 300, 500, 750, and 1,000 ppm of styrene on days 6-18 of gestation. Fetotoxicity (dead or resorbed fetuses) was observed only at 1,000 ppm and teratogenicity was not reported at any of the exposure levels. The incidence of dead or resorbed fetuses was 26% in the control group versus 60% in the exposed group. No skeletal malformations were noted in the hamsters.

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.6 Reproductive Effects

Information on the reproductive effects of styrene in humans is available from epidemiological studies of the reproductive outcomes of females employed in the various industrial operations in which styrene is used. However, exposures to styrene were not adequately quantified in any of the studies cited. In one study, spontaneous abortions among 9,000 Finnish chemical workers from 1973 to 1976 were analyzed (Hemminki et al. 1980). The risk of spontaneous abortion (expressed as number of abortions per 100 pregnancies) was significantly higher in women employed in styrene production compared to all women in Finland (15.0 vs. 5.5). However, this increase was not detected in a follow-up study of the same workers (Hemminki et al. 1984). The possible embryotoxic effects of styrene on 67 female lamination workers compared to 67 age-matched controls were evaluated in a second study (Harkonen and Holmberg 1982). The number of births was significantly lower among the workers exposed to styrene. This result was explained in part by a greater number of induced abortions in the styrene-exposed group. The number of spontaneous abortions was not elevated in the exposed women. No increased risk of spontaneous abortions among workers processing polymerized plastics or

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heated plastics made of vinyl chloride or styrene was reported (Lindbohm et al. 1985). The authors reported that the statistical power of the study was low due to the small study population. These studies are not conclusive since the workers were exposed to chemicals other than styrene in the workplace and the concentrations of styrene were not adequately reported.

Mice exposed to 150 or 300 ppm styrene by inhalation for 5 days (6 hours/day) did not have a statistically significant increase in the frequency of abnormal sperm heads 3-5 weeks after exposure (Salomaa et al. 1985). These findings suggest that reproductive effects, if they exist, may not be produced through a genotoxic mechanism.

2.2.1.7 Genotoxic Effects

Chromosomal damage in peripheral lymphocytes and other cellular effects have frequently been studied in workers exposed to styrene in the production of reinforced plastic products and styrene/polystyrene production. In general, these studies are limited by the fact that workers in these industries are often exposed to chemicals other than styrene such as methylene chloride and epoxide resins. Confounding factors such as age, sex, and smoking status must also be considered. Studies of this nature may also be limited by small sample sizes and differing cell culture methodologies.

Chromosomal aberrations have been reported in several studies of workers exposed to styrene for 1 to 15 years in reinforced plastic operations (Andersson et al. 1980; Hogstedt et al. 1979; Meretoja et al. 1977, 1978). For example, in an evaluation of lymphocytes from 16 men exposed to styrene (1-140 ppm) and 5 controls, styrene-exposed men showed 11% - 26% aberrant cells versus 3% or less in the control subjects (Meretoja et al. 1977). The aberrations were almost totally chromosome breaks. The frequency of micronuclei and cells connected with nuclear bridges were also increased in exposed workers. In another study, 10 styrene-exposed workers (1-140 ppm) and 5 controls were reexamined. It was reported that the frequencies of aberrant lymphocytes varied from 10% to 26% in the exposed group and from 1% to 4% in the referents (Meretoja et al. 1978). The most frequent class of aberrations was again chromosome breaks or gaps. The incidence of sister chromatid exchange (SCE) among styrene-exposed workers in this study was not significantly higher in the controls. In another study of 36 exposed workers and 37 controls, Andersson et al. (1980) reported an increase in the incidence of chromosome breaks/gaps and SCEs (21 exposed subjects (2.8-154 ppm) versus 20 control subjects for the SCE subset). Chromosome aberrations in lymphocytes from peripheral blood were more frequent in 6 workers when compared to 6 age- and sex-matched controls (Hogstedt et al. 1979). In this study workroom concentrations were a little lower (12-74 ppm) than in previously reported studies. In another study, negative results were reported for chromosomal aberrations and SCEs in reinforced plastics workers (16 exposed versus 13 controls) in workroom exposures from 33 to less than 70 ppm styrene (Watanabe et al. 1981).

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Styrene exposures are generally lower (1-58 ppm) in styrene and polystyrene manufacturing facilities than in reinforced plastic operations, and studies of chromosome damage and other cellular effects in these facilities have been generally negative (Hansteen et al. 1984; Nordenson and Beckman 1984; Thiess et al. 1980). For example, Thiess et al. (1980) reported that 24 employees exposed to styrene in the laboratory (6.0 ppm) and a polyester processing plant (58.1 ppm) showed a nonstatistically significant increase in chromosomal aberration rates compared to controls. In another study, chromosomal aberrations were studied in lymphocytes of 15 workers and 13 controls employed in the manufacture of polyester reinforced windowglass fiber where workroom exposure was 24 ppm styrene. No increased frequency of chromosome breaks/gaps was observed, but the number of micronuclei was significantly increased (Nordenson and Beckman 1984). The authors concluded that the mitotic spindle membranes may be more sensitive to styrene and its metabolites than DNA. Eighteen workers exposed to less than 50 ppm styrene were found to have a significant increase in chromosome gaps (Hansteen et al. 1984). No increase in the number of chromosome breaks and SCE's was found compared to controls. In another study, increased frequency of lymphocyte micronuclei in workers exposed to a mean 13 ppm styrene was reported (Hogstedt et al. 1983). An increase in chromosomal aberrations was observed in workers exposed to a mixture of phenol, styrene, and formaldehyde (levels not specified) (Mierauskiene and Lekevicius 1985). In an evaluation of cytogenetic monitoring of industrial populations potentially exposed to genotoxic chemicals including styrene in the Netherlands, DeJong et al. (1988) concluded that the results of chromosome analyses are difficult to interpret due to variable and high background levels of chromosome aberration in control populations.

Male and female rats exposed to styrene vapor (600 and 1,000 ppm) for 1 year did not show an increased incidence of chromosome abnormalities in bone marrow cells collected at the end of the last exposure (Sinha et al. 1983), although detectable chromosomal abnormalities are not likely to endure for such a long period.

In summary, the mutagenicity data from studies of workers exposed to styrene suggest that styrene exposure can produce an increased incidence of chromosomal aberrations (primarily gaps and breaks). However, interpretation of these data are complicated by the possible involvement of concomitant exposures to other chemicals. The data are insufficient to show styrene exposure produces an increased incidence of sister chromatid exchanges or micronuclei formation.

Other genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

Although there are several epidemiologic studies which suggest there may be an association between styrene exposure and an increased risk of leukemia and lymphoma, the evidence is generally inconclusive due to multiple chemical exposures and inadequate documentation of the levels and durations of exposure

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to styrene. In a study of employees who worked in the development or production of styrene-based products, deaths due to malignant neoplasia were fewer than expected in the total study group (2,904 subjects) (Ott et al. 1980). An increase in lymphatic leukemia (4 observed deaths versus 0.5 expected) was observed in a group of employees exposed to polymer extrusion fumes, solvents, and colorants but was not found to be related to duration or level of exposure. A retrospective cohort mortality study was conducted for two styrene-butadiene rubber plants, designated as Plant A and Plant B (Meinhardt et al. 1982). Occupational history records were available from 1943 at Plant A and from 1950 at Plant B to the study end-date of March 31, 1976. No statistically significant excess in total or cause-specific mortality rates was observed for the overall worker population at either plant. Plant A workers had a statistically nonsignificant increase in leukemia and aleukemia. The mean concentrations of styrene, butadiene and benzene in Plant A were 0.94, 1.24, and 0.1 ppm, respectively, and in Plant B styrene and butadiene levels were 1.99 and 13.5 ppm, respectively. The presence of a known leukemogenic agent, benzene, obviously further confounded the study results with regard to styrene carcinogenicity. The authors concluded that the study findings suggested that the production and manufacture of styrene-butadiene rubber may be associated with an excess of lymphatic and hematopoietic neoplasms. In a study of 560 male employees of a styrene-polystyrene manufacturing plant who had at least 5 years of exposure, there were no significant increases in cause-specific mortality (Nicholson et al. 1978). The reported leukemia incidence suggested the need for further study. In another study, a statistically significant excess of lymphoma deaths in an exposed population (662 subjects) was reported, and 2 of the 3 deaths occurred in men less than 40 years of age who had been exposed for at least a year (Hodgson and Jones 1985). However, the lack of association with actual exposure levels or specific durations and the small number of observed deaths requires cautious interpretation. In a very large epidemiological study of nearly 16,000 workers in the styrene plastic industry, the death rate from leukemia was twice as high in areas of high exposure as in areas of low exposure (Wong 1990). However, there were too few cases for this to be statistically significant. Several other studies in humans have not detected any evidence of leukemia, lymphoma, or other cancers (Coggon et al. 1987; Okun et al. 1985; Matanoski and Schwartz 1987). The International Agency for Research on Cancer (IARC) has concluded that the evidence for carcinogenicity in humans from epidemiological studies is inadequate and classifies styrene in Group 2B, possibly carcinogenic to humans (IARC 1987). EPA agreed that results of epidemiological studies were confounded by multiple chemical exposures and considered the epidemiological evidence inadequate to determine potential human carcinogenicity of styrene (EPA 1988b).

Three chronic animal inhalation studies (Conti et al. 1988; Jersey et al. 1978; Maltoni et al. 1982) have been conducted to evaluate the carcinogenicity of styrene. These studies have produced variable results. Groups of 85 male and 85 female rats were exposed to 600 or 1,200 ppm styrene (99.5% purity) for 6 hours/day, 5 days/week for 18-20 months (Jersey et al. 1978). The concentration in the high-dose group was decreased to 1,000 ppm due to decreased weight gains in male rats. The incidence of mammary

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adenocarcinomas in the 600 ppm female group was significantly higher than in controls. However, there was no significant response evident at 1,000 ppm, and the control rats had an unusually low mammary adenocarcinoma incidence when compared with historical controls. In the same study, the incidence of lymphosarcomas and leukemia in females was identical for both exposed groups at 5.27% and 1.04%, respectively. These values were not statistically higher than the respective values of concurrent control animals, but were significant when compared to historical control data. However, there was an absence of dose response. A high incidence of chronic murine pneumonia in these rats makes a complete evaluation of the data difficult and the results uncertain.

In a second study, designed to determine if styrene would induce brain tumors, groups of 40 male and 40 female rats were exposed to 0, 25, 50, 100, 200, and 300 ppm styrene for 52 weeks (Maltoni et al. 1982). There was no increased incidence of brain tumors in any of the exposed groups of rats. In a third study (performed by the same group as the second study), 30 male and 30 female rats were exposed to 25, 50, 100, 200, or 300 ppm styrene for 52 weeks (Conti et al. 1988). A higher incidence of total malignant tumors in the group exposed to 100 ppm styrene was observed. The increased incidence was not due to any specific type of tumor. However, the higher incidence of total malignant tumors in the 100 ppm styrene-exposed group was not dose related since the 200 and 300 ppm groups did not have significantly higher total malignant tumor incidences. A higher incidence of malignant and total (benign plus malignant) mammary tumors was observed in females of all the groups exposed to styrene. Although the authors did not report tests for statistical significance or levels of significance, the data provided in tabular form clearly indicate a dose trend of increased incidence of combined benign and malignant mammary tumors. Although statistical methods are not provided, the data reported indicate that statistical significance may be marginal at the two lower doses (25 and 50 ppm) and significant at 100 ppm and above.

2.2.2 Oral Exposure

No studies were located regarding health effects in humans after oral ingestion of styrene. Based on the animal data that follow, the oral toxicity of styrene in humans would be expected to be low to moderate.

2.2.2.1 Death

No deaths in humans from ingesting styrene have been reported in the evaluations of case studies (EPA 1989c; Gosselin et al. 1984; NIOSH 1983).

The approximate reported oral LD₅₀ for male and female rats was 5,000 mg/kg (Wolf et al. 1956). A 100% survival rate and 100% mortality rate were reported in rats exposed to single oral doses of styrene (observation period 2 weeks) at 1,600 and 8,000 mg/kg, respectively (Spencer et al. 1942). Death in this study was mainly due to pronounced irritation of the esophagus and stomach. In another study, female mice were given a single oral dose of 1,350 mg/kg styrene on the 17th day of pregnancy (Ponomarkov and Tomatis

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1978). After weaning, the progeny received the same dose once per week. The treatment was suspended after 16 weeks due to high mortality among the progeny (including both males and females). Fifty percent of the males and 20% of the females had died after 20 weeks, despite the suspension of treatment at week 16. The cause of death was liver necrosis and lung congestion. A high mortality rate (number not specified) was reported in 40 female rats exposed to 250 mg/kg/day styrene for 52 weeks (Conti et al. 1988). Mortality was significantly elevated in male and female rats administered styrene by gavage at a dosage level of 2,000 mg/kg/day for 78 weeks (NC1 1979b). In this study, mortality was unaffected at dosage levels of 500 and 1,000 mg/kg/day in male and female rats. Male mice administered styrene at doses of 150 or 300 mg/kg/day for 78 weeks showed increased mortality; however, the female mice did not.

The highest reliable LOAEL values and LD_{50's} values in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, musculoskeletal, or dermal/ocular effects in humans or animals after oral exposure to styrene.

For the following systemic effects resulting from oral exposure to styrene, the highest NOAEL values and all reliable LOAEL values for each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to styrene.

Severe lung congestion was observed in mice that were the offspring of dams given a single oral dose of styrene at 1,350 mg/kg on the 17th day of gestation and that continued to receive the same dose once per week after weaning (Ponomarkov and Tomatis 1978). The lung congestion was noted following continuous administration of the styrene for 16 weeks.

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to styrene.

Male and female purebred beagle dogs were exposed to 0, 200, 400, or 600 mg/kg/day styrene by gavage for up to 561 days (Quast et al. 1979). Treatment with styrene was stopped on day 316 in the 600 mg/kg/day group and resumed on day 470 for 90 additional days to investigate the reversibility of any effects. There were only minimal toxicological changes. Intraerythrocytic Heinz bodies were regularly detected in a dose-related manner in males and females in the 400 and 600 mg/kg/day groups and sporadically in females in the 200 mg/kg/day group. There were occasional decreased red blood

TABLE 2-2. Levels of Significant Exposure to Styrene - Oral

Key to figure ^a	Species	Route	Exposure frequency/ duration	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
						Less serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE								
Death								
1	Rat	(GO)	1 d		1600		8000 (100% lethality)	Spencer et al. 1942
2	Rat	(GO)	1 d				5000 (LD50)	Wolf et al. 1956
Systemic								
3	Rat	(GO)	7 d 1x/d	Renal		900 (decreased glutathione content)		Das et al. 1983
4	Rat	(GO)	7 d 1x/d	Hepatic		900 (decreased glutathione content)		Das et al. 1981
Neurological								
5	Rat	(GO)	7 d 1x/d		270	450 (inhibition of glutathione-S-transferase, glutathione depletion)		Dixit et al. 1982
6	Rat	(GO)	14 d 1x/d		100	200 (increased serotonin levels in several brain sections)		Husain et al. 1985
Developmental								
7	Rat	(GW)	10 d 1x/d		300			Murray et al. 1978

TABLE 2-2 (Continued)

Key to figure ^a	Species	Route	Exposure frequency/ duration	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
						Less serious (mg/kg/day)	Serious (mg/kg/day)	
Reproductive								
8	Rat	(GW)	10 d 1x/d		300	(35% decreased weight gain on days 6-9 of gestation, reduced food consumption)		Murray et al. 1978
INTERMEDIATE EXPOSURE								
Death								
9	Mouse	(GO)	16 wk 1x/wk				1350 (50% of males and 20% of females dead 4 weeks after treatment suspended)	Ponomarkov and Tomatis 1978
Systemic								
10	Rat	(GO)	100 d 6d/wk 1x/d	Hepatic	200 ^b	(changes in mitochondrial and microsomal enzymes)	400 (small areas of necrosis with degenerated hepatocytes and inflammatory cells)	Srivastava et al. 1982
11	Rat		6 mo 5d/wk	Hepatic	133	400 (increased liver weight)		Wolf et al. 1956
				Renal	133	400 (increased kidney weight)		
12	Mouse	(GO)	16 wk 1d/wk	Resp			1350 (severe lung congestion)	Ponomarkov and Tomatis 1978
Neurological								
13	Rat	(GO)	90 d 1x/d		200	(increased spiroperidol binding to brain membranes)		Agrawal et al. 1982

TABLE 2-2 (Continued)

Key to figure ^a	Species	Route	Exposure frequency/ duration	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
						Less serious (mg/kg/day)	Serious (mg/kg/day)	
Reproductive								
14	Rat	(GO)	60 d 6d/wk 1x/d		200		400 (decreased spermatozoa, tubular degeneration)	Srivastava et al. 1989
15	Rat	(W)	90 d (cont)		35			Beliles et al. 1985
Cancer								
16	Mouse	(GO)	16 wk 1d/wk				1350 CEL (lung tumors)	Ponomarkov and Tomatis 1978
CHRONIC EXPOSURE								
Death								
17	Rat	(GO)	78 wk 5d/wk 1x/d				2000 (decreased survival in males and females)	NCI 1979b
Systemic								
18	Rat	(W)	105 wk 7d/wk	Resp Cardio Gastro Hemato Musc/skel Hepatic Renal Derm/oc Other	21 21 21 21 21 21 21 21			Beliles 1985
19	Dog	(GO)	561 d 1x/d	Hemato	200	400 (Heinz body formation)		Quast et al. 1979

TABLE 2-2 (Continued)

Key to figure*	Species	Route	Exposure frequency/ duration	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
						Less serious (mg/kg/day)	Serious (mg/kg/day)	
Cancer								
20	Mouse	(GO)	78 wk 5d/wk 1x/d				300 CEL (lung tumors)	NCI 1979b

*The number corresponds to entries in Figure 2-2.

^bUsed to derive an intermediate oral minimal risk level (MRL) of 0.2 mg/kg/day; dose divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, 10 for human variability and 6/7 for less than continuous exposure).

Cardio = cardiovascular; CEL = cancer effect level; cont = continuous; d = day(s); Derm/oc = dermal/ocular;
 Gastro = gastrointestinal; (GO) = gavage - oil; (GW) = gavage - water; Hemato = hematological; LD50 = lethal dose, 50% kill;
 LOAEL = lowest-observed-adverse-effect level; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect
 level; Resp = respiratory; (W) = drinking water; wk = week(s); x = time(s)

FIGURE 2-2. Levels of Significant Exposure to Styrene – Oral

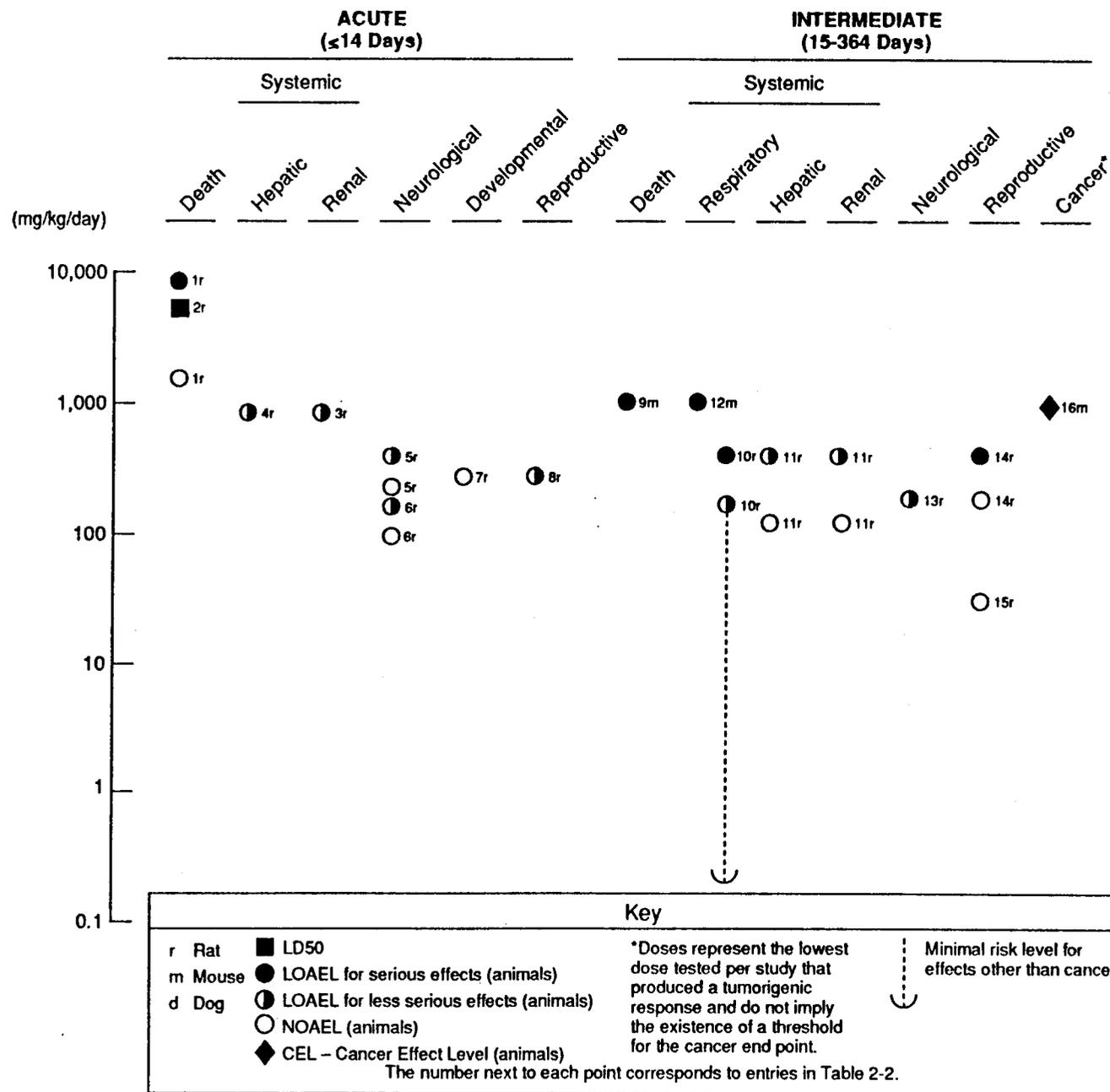
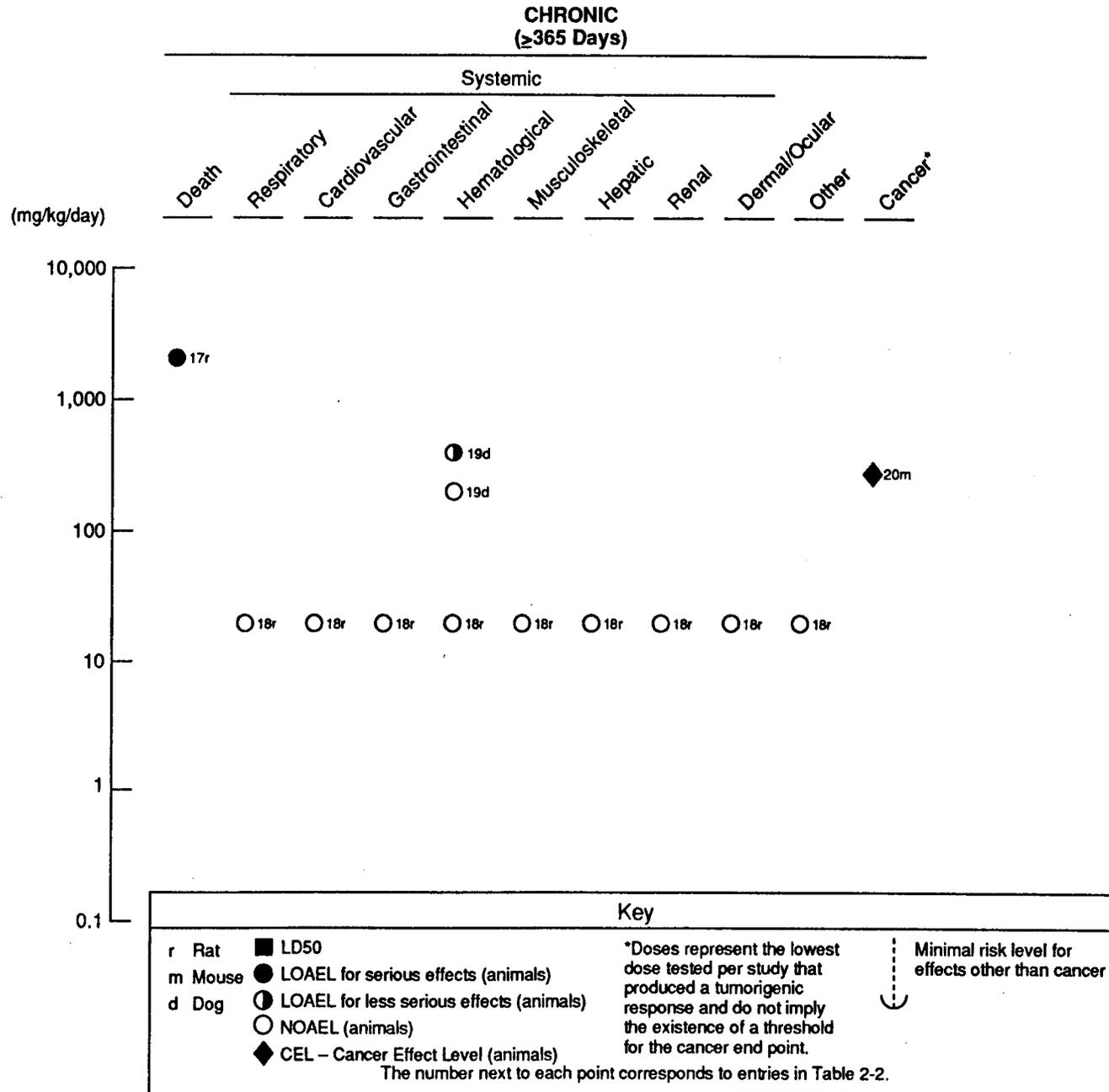


FIGURE 2-2. (Continued)



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cell counts, hemoglobin levels and erythrocyte sedimentation rates in males and females in the 600 mg/kg/day groups. Increased hemosiderin deposits and intranuclear inclusions in liver were noted in animals dosed with 600 mg/kg/day. This was probably secondary to the effects on the red blood cells. The formation of intra-erythrocytic Heinz bodies was readily reversible upon discontinuing the administration of styrene in the 600 mg/kg/day group.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to styrene.

Hepatic glutathione content was reduced in rats orally administered 900 mg/kg styrene for 7 consecutive days (Das et al. 1981). In male rats that received 200 or 400 mg/kg/day styrene by gavage for 100 days, changes in mitochondrial and microsomal enzymes were observed at both doses. In addition to elevated enzyme activity, small areas of focal necrosis were noted in rats administered 400 mg/kg/day indicating a dose-response trend. In another study, growth depression and increased liver weight (general indicators of toxicity) were noted in rats orally administered 400 and 667 mg/kg/day styrene for 6 months (Wolf et al. 1956). As noted above, increased numbers of hemosiderin deposits and intranuclear crystalline inclusions were reported in the hepatocytes of dogs orally administered 600 mg/kg/day of styrene by gavage for 316 days (Quast et al. 1979). This was presumably secondary to Heinz body formation, and no other hepatic histological effects were in this study. The LOEL of 200 mg/kg/day for enzyme level changes in rats (Srivastava et al. 1982) was selected as the basis for an oral intermediate MRL of 0.2 mg/kg/day. This MRL value is supported by the study by Wolf et al. (1956) in which a LOEL of 400 mg/kg/day and a NOAEL of 133 mg/kg/day were reported. Additional support comes from other intermediate oral studies in which renal, neurological, and reproductive effects have been observed at or near the critical LOEL (Agrawal et al. 1982; Srivastava et al. 1989; Wolf et al. 1956).

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to styrene.

A decrease in renal glutathione content and decreased glutathione-transferase activity was noted in rats orally administered 900 mg/kg styrene for 7 days (Das et al. 1983). This was similar to a reduction in the activity of these enzymes that was seen in hepatic tissue (Srivastava et al. 1982). Growth depression and increased kidney weight were reported in female rats orally administered 400 and 667 mg/kg/day of styrene for 6 months (Wolf et al. 1956). Histopathological examination of kidney tissue showed no abnormalities. Elevated levels of serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were observed at 667 mg/kg/day. Dose-dependent increases of the cytochrome 450-dependent enzymes, benzo[a]pyrene hydroxylase and aminopyrine-N-demethylase were also observed. In another study, female rats and **mice** were exposed to 1,350 mg/kg/day of styrene on the 17th day of gestation. The offspring were also administered styrene, by gavage, at the following doses: rats, 500 mg/kg/day; O₂₀ mice, 1,350 mg/kg/day; and C57B1

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mice, 300 mg/kg/day. Treatment was weekly for 120 weeks. Hyperplasia of the kidney pelvis epithelium was frequently reported in the offspring of the rats but not in the mice (Ponomarkov and Tomatis 1978).

2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in humans after oral exposure to styrene.

The World Health Organization (WHO 1983) reviewed a Russian study (Sinitskij 1969) in which styrene was fed to 36 rabbits at doses of 250 mg/kg for 58 days, 5 mg/kg for 216 days, and 0.5 mg/kg for 202 days. Impairment of the immunological defense system was indicated by a nearly total suppression of leukocyte phagocytic activity. Although no statistical analysis was provided, the data showed a dose-response relationship for both the severity of the effect and the time of onset.

2.2.2.4 Neurological Effects

Although it is not clear if oral administration of styrene causes neurological effects in humans, the following animal studies support, in part, a biochemical basis for neurological effects of inhaled styrene.

It has been suggested that exposure of rats to styrene alters the biotransformation capacity of the brain dependent on glutathione content (Dixit et al. 1982). Significant inhibition of aryl hydrocarbon hydroxylase and glutathione-S-transferase activity followed by glutathione depletion was observed in rats exposed to styrene at 450 and 900 mg/kg/day for 7 days (Dixit et al. 1982). Oral intubation of male rats with styrene (94 mg/kg/day) for 15 days resulted in increased serotonin and noradrenalin levels in brain tissue (Husain et al. 1980).

Behavioral effects were observed by Husain et al. (1985) in rats exposed to styrene at 100 or 200 mg/kg/day for 14 days. Styrene significantly increased the mean percent avoidance response (learning) but no definite doseresponse relationship was evident. Conditioned stimuli, consisting of the sound of a buzzer and turning on light in the test chamber were used during an induced pole climbing task. The unconditioned stimulus was electric shock. Serotonin levels in hippocampus, hypothalamus, and mid-brain were raised at the 200 mg/kg/day styrene exposure. The study results indicate that elevated serotonin levels may account for the increased avoidance response.

In another study, styrene was administered to rats at doses of 200 or 400 mg/kg/day for up to 90 days. A significant increase in specific binding of ³H-spiroperidol to the striatal membranes of the brain 24 hours after the last dose was observed (Agrawal et al. 1982). The data suggested that styrene altered the sensitivity of the dopamine receptors. Other neurotoxic chemicals such as acrylamide and manganese also are known to involve the dopaminergic system (Ali et al. 1983; ATSDR 1990).

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The highest NOAEL and LOAEL values for neurological effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.5 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to styrene.

No teratological effects were observed in the offspring of rats given oral doses of either 180 or 300 mg/kg/day styrene during gestation (Murray et al. 1978).

The highest NOAEL value for developmental effects is recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to styrene.

Exposure of pregnant rats to 180 or 300 mg/kg/day of styrene on days 6-15 of gestation resulted in no significant effects on maternal mortality or percent pregnancy (Murray et al. 1978). However, a 35% decrease in maternal weight gain was observed in the 300 mg/kg/day group during days 6-9 of gestation.

A three-generation reproduction study (Beliles et al. 1985) was conducted in which rats were maintained on styrene-treated drinking water for 2 years (7.7-21 mg/kg/day). The styrene-treated rats had no treatment-related changes, including mortality patterns. There was no evidence of adverse reproductive performance related to exposure to styrene. The only finding was that styrene-treated rats exhibited reduced water consumption due to poor palatability. In another study, styrene was administered by gavage to adult male rats for 60 days (Srivastava et al. 1989). At the high dose of 400 mg/kg/day, the activities of some marker enzymes for testicular function were significantly altered and there was a decrease in spermatozoa count. Histopathological examination revealed degeneration of seminiferous tubules and lumina devoid of sperm. The study results indicate that the male reproductive system may be sensitive to styrene exposure.

The highest NOAEL values and all reliable LOAEL values for reproductive effects in rats in the acute and intermediate duration categories are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after oral exposure to styrene.

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The capacity for orally administered styrene to induce chromosomal aberrations in animals was studied by Sbrana et al. (1983). No mouse bone marrow cell chromosomal aberrations were detected after a 0-day treatment with 500 mg/kg/day or a 70-day exposure to 200 mg/kg/day. In a 3-generation reproduction study, no cytogenetic effects were noted in the bone marrow of pups born to rats that received styrene in their drinking water at doses of 125 or 250 ppm for approximately 90 days before mating (Beliles et al. 1985).

Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer effects in humans after oral exposure to styrene.

Investigations of the carcinogenic potential of styrene in animals after oral exposure have yielded variable results. Studies were conducted in which 29 female O₂₀ mice (1,350 mg/kg), 15 female C57Bl mice (300 mg/kg), and 21 female BDIV rats (1,350 mg/kg) received styrene, by gavage administration, on day 17 of gestation (Ponomarkov and Tomatis 1978). The offspring of the C57Bl mice and BDIV rats were treated with styrene for life. The O₂₀ mice offspring were only treated for 16 weeks due to excessive mortality from toxic effects. The weekly doses used for offspring were 1,350 mg/kg for O₂₀ mice, 300 mg/kg for C57Bl mice, and 500 mg/kg for BDIV rats. After 100 weeks, the oral administration of styrene resulted in an increased incidence of lung tumors in male and female O₂₀ mice compared to olive oil controls. An increased incidence of liver tumors was reported in styrene-treated C57Bl mice (12%) as compared to controls (3%), although this was not a statistically significant increase. There were no statistically significant increases in tumor incidences in the styrene-exposed BDIV rats. However, a few rare tumors were observed in styrene-exposed rats including stomach tumors and neurinomas of the heart and intestine. These results provide only weak evidence of the carcinogenicity of styrene in the O₂₀ and C57Bl mice.

The carcinogenic potential of styrene was evaluated in male and female Fischer 344 rats (500, 1,000, and 2,000 mg/kg/day) and B6C3F1 mice (300 and 150 mg/kg/day) (NC1 1979b). In male mice, there was a significant positive association between styrene dosage and the combined incidence of adenomas and carcinomas of the lung. However, the statistical significance of this result may have been due to an unusually low tumor incidence in the concurrent controls, since the results were not statistically significant when compared to historical controls from the same laboratory. No association was detected between styrene exposure and tumor incidence in female mice or in rats. The NC1 concluded that, while there was suggestive evidence for carcinogenicity in male mice, overall the results were not convincing for carcinogenicity in either rats or mice.

Styrene was also evaluated for its chronic toxicity and carcinogenic potential in male and female Sprague-Dawley and Wistar rats administered the chemical in the drinking water for 105 weeks (Beliles et al. 1985). The doses

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were 17.5 and 35 mg/kg/day. There was some difficulty in maintaining the styrene level in the drinking water; the levels averaged 89.8% for the low level and 88.5% for the high level. Although there were incidental findings (e.g. ocular opacity) in both test and control rats, there were no gross pathological findings at the 52-week interim kill or terminal necropsy which were associated with the chronic administration of styrene. Likewise, there were no histopathological findings. The authors concluded that there was no evidence of carcinogenicity under the conditions of the study.

The potential for styrene to induce brain tumors was evaluated in Sprague-Dawley rats exposed for 52 weeks to 50 or 250 mg/kg/day styrene (Maltoni et al. 1982). The tumor incidence was not significantly different in exposed rats versus vehicle or historical controls. Similarly, in a 2-year study, no brain tumors were observed in male or female rats administered styrene at levels of 125 and 250 ppm in their drinking water (Beliles et al. 1985). In another study, the carcinogenic potential of styrene was investigated in Sprague-Dawley rats exposed to 50 or 250 mg/kg styrene for 52 weeks (Conti et al. 1988). The results of this study were also negative. However, due to a higher mortality rate, a lower incidence of total benign and malignant tumors and total mammary tumors was observed in rats at the highest (250 mg/kg/day) dose.

The International Agency for Research on Cancer (IARC) has concluded that evidence for styrene carcinogenicity in humans is inadequate, while evidence for carcinogenicity of styrene in animals is limited (IARC 1987). Using the lung tumor incidence of B6C3F1 mice (NC1 1979b) and appropriate dose conversions, the EPA (1988b) calculated a slope factor (potency factor or q_1^*) of $0.03 \text{ (mg/kg/day)}^{-1}$. EPA has requested public comment on the EPA group cancer classification of styrene for regulation under the Safe Drinking Water Act (EPA 1989b). The EPA has proposed the possibility of classification in Group B2 (Probable Human Carcinogen) or Group C (Possible Human Carcinogen).

2.2.3 Dermal Exposure

No studies were located regarding health effects in humans after dermal exposure to styrene.

2.2.3.1 Death

No studies were located regarding lethality in humans or animals after dermal exposure to styrene.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, and renal effects in humans or animals after dermal exposure to styrene.

Dermal/Ocular Effects. Marked irritation with denaturation of the skin was noted when styrene was applied in small amounts over a 4 week period to

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the shaved abdomen of rabbits at 20,000 mg/kg (total dose) (Spencer et al. 1942). This study is summarized in Table 2-3. In another study, moderate conjunctival irritation and transient corneal injury of the eyes were observed when undiluted styrene was tested in rabbit eyes (Wolf et al. 1956). The effects were produced immediately (within 3 minutes) by a single administration of two drops (about 0.1 mL) and persisted throughout the 7-day observation period.

No studies were located regarding the following health effects in humans or animals after dermal exposure to styrene:

2.2.3.3 Immunological Effects

2.2.3.4 Neurological Effects

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located regarding cancer affects in humans or animals after dermal exposure to styrene.

2.3 TOXICOKINETICS

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

The uptake of styrene following inhalation exposure in humans and animals is rapid (Ramsey and Anderson 1984; Ramsey and Young 1978; Ramsey et al. 1980; Withey and Collins 1979; Withey and Karpinski 1985). Pulmonary retention of inhaled styrene in humans is approximately 2/3 of the administered concentrations (Engstrom et al. 1978a, 1978b). For example, male human subjects were exposed to styrene in inspired air during 30 minute rest and three 30-minute work periods on a bicycle ergometer. The mean uptake was approximately 63% (range was 59%-70%) of the amount of inspired styrene. Exposures of rats to styrene concentrations of from 50 to 2,000 ppm for 5 hours yielded blood uptakes which showed a continued and increasing rapid absorption, proportional to the styrene air level (Withey and Collins 1979). Plateau levels of styrene in rats' blood were reached within 6-8 hours during exposures ranging from 80 to 1,200 ppm styrene for up to 24 hours (Ramsey and Young 1978). Physiologically-based inhalation pharmacokinetic models indicate that styrene metabolism becomes saturated at inhaled levels above 200 ppm in mice, rats, and humans (Ramsey and Andersen 1984). When inhaled concentrations are below 200 ppm, the ratio of styrene concentration in the blood to inhaled air is moderated by perfusion-limited metabolism rather than blood:air partition coefficients.

TABLE 2-3. Levels of Significant Exposure to Styrene - Dermal

Species	Exposure frequency/ duration	System	NOAEL	LOAEL (effect)		Reference
				Less serious	Serious	
ACUTE EXPOSURE						
Systemic						
Rabbit	1 d	Derm/oc		0.1 mL (moderate conjunctival irritation, transient corneal injury)		Wolf et al. 1956
INTERMEDIATE EXPOSURE						
Systemic						
Rabbit	4 wk	Derm/oc		20 g/kg (total) irritation with blistering, hair loss)		Spencer et al. 1942

d = day(s); Derm/oc = dermal/ocular; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; wk = week(s)

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2.3.1.2 Oral Exposure

No studies were located regarding absorption in humans after oral exposure to styrene.

The absorption of styrene from the gastrointestinal tract was rapid and complete in rats deprived of food overnight and given styrene by gavage at a total dose of 3.147 mg styrene in 10 mL aqueous solution. A peak blood level of 6 µg/mL was reached in a few minutes. There was a much slower uptake of the styrene administered in vegetable oil (Withey 1976). Styrene administered in vegetable oil at a total dose of 32.61 mg produced a peak level of 12 µg/mL. This was reached at about 100 minutes (Withey 1976).

2.3.1.3 Dermal Exposure

Limited data indicate that absorption of styrene via the dermal route is probably low compared to absorption via other routes. When liquid styrene was applied to the forearms of male subjects, the absorption rate was estimated to be 9-15 mg/cm²/hour (Dutkiewicz and Tyras 1968). By contrast, the rate of absorption through human skin was very low (1±0.5 µg/cm²/min) in subjects who dipped one hand into liquid styrene (Berode et al. 1985). It is believed that the higher absorption rate reported by Dutkiewicz and Tyras (1968) also included the disappearance rate of the solvent from the surface of the skin (Guillemin and Berode 1988). Riihimaki and Pfaffli (1978) demonstrated that in humans, dermal exposure to moderate concentrations of styrene vapor (300 and 600 ppm) resulted in percutaneous penetration corresponding to approximately 0.1%-2% of the amount estimated to be absorbed from the respiratory tract.

Although absorption of styrene applied to the abdomen of rabbits was reported, there was no information on absorption rates (Spencer et al. 1942).

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

Inhalation studies in both humans and animals resulted in the widespread distribution of styrene with the highest concentration in adipose tissue.

Three humans were exposed to 8-20 ppm styrene which resulted in a mean daily uptake of 193-558 mg styrene (Engstrom et al. 1978b). The concentration of styrene in adipose tissue was 2.8-8.1 mg/kg at the beginning of the week and 4.7-11.6 mg/kg at the end of the week. The authors estimated the half-life of styrene in the subcutaneous fat of man to be about 72 hours. Subsequent studies by this author confirmed this estimate and reported the half-life of styrene in adipose tissue to be 24-96 hours (Engstrom et al. 1978a).

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Fiberglass factory workers exposed to greater than 215 mg/m³ of styrene for 8-hour work shifts had blood styrene levels which ranged from 120-684 µg /L at the end of the shift (Apostoli et al. 1983). The concentrations of urinary MA and phenylglyoxilic acid (PGA) were 133-2,100 and 107-685 mg/L, respectively. These levels were also determined at the end of the work-shift. Distribution of styrene was also studied in adult men exposed to about 300 mg/m³ of styrene for 2 hours during light physical exercise (Wigaeus et al. 1983). Blood styrene reached a level of approximately 20 µmol/l after 75 minutes. The concentrations of styrene in adipose tissue was about 50 µmol/kg after 30-90 minutes of exposure.

Rats were exposed for 5 hours to styrene at concentrations ranging from 50 to 2,000 ppm (Withey and Collins 1979). Tissue concentrations of styrene in the heart, liver, lung, kidney, spleen, brain, and perirenal fat demonstrated different patterns of distribution as the dose increased. The styrene concentration in perirenal fat was 10 times greater than in other organs. The largest amounts of styrene were found in the subcutaneous fat of male rats exposed to about 45 ppm of radioactively labeled styrene in the inspired air for 1-8 hours (Carlsson 1981). The concentration increased steadily during the first 4 hours of exposure. Styrene concentrations in brain tissue and muscles were about 70% of the arterial blood value. Other investigators (Ramsey and Anderson 1984; Ramsey and Young 1978; Savolainen and Pfaffli 1978; Withey 1976) demonstrated that higher levels of styrene in adipose tissue increase with higher exposures to styrene. Styrene was found to distribute to the fetuses of pregnant rats after inhalation exposure, but at concentrations much lower than those measured in maternal organs and tissues (Withey and Karpinski 1985).

2.3.2.2 Oral Exposure

No studies were located regarding distribution in humans after oral exposure to styrene.

An oral dose of 20 mg/kg of ¹⁴C styrene was administered to male and female rats (Plotnick and Weigel 1979). Tissue levels peaked at 4 hours or earlier after dosing. Less than 10% of the administered dose was found in the stomach, small intestine, and large intestine 8 hours after dosing. The kidney had the highest concentration of radioactivity at all time intervals, with decreasing amounts in the liver and pancreas. Fat tissue showed increased levels after 2 hours. All tissue levels were below 1 µg /g at 24 hours and at 48 and 72 hours were below the limit of detection. Excretion data from the Plotnick and Weigel (1979) study are presented in Section 2.3.4.2.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution in humans after dermal exposure to styrene.

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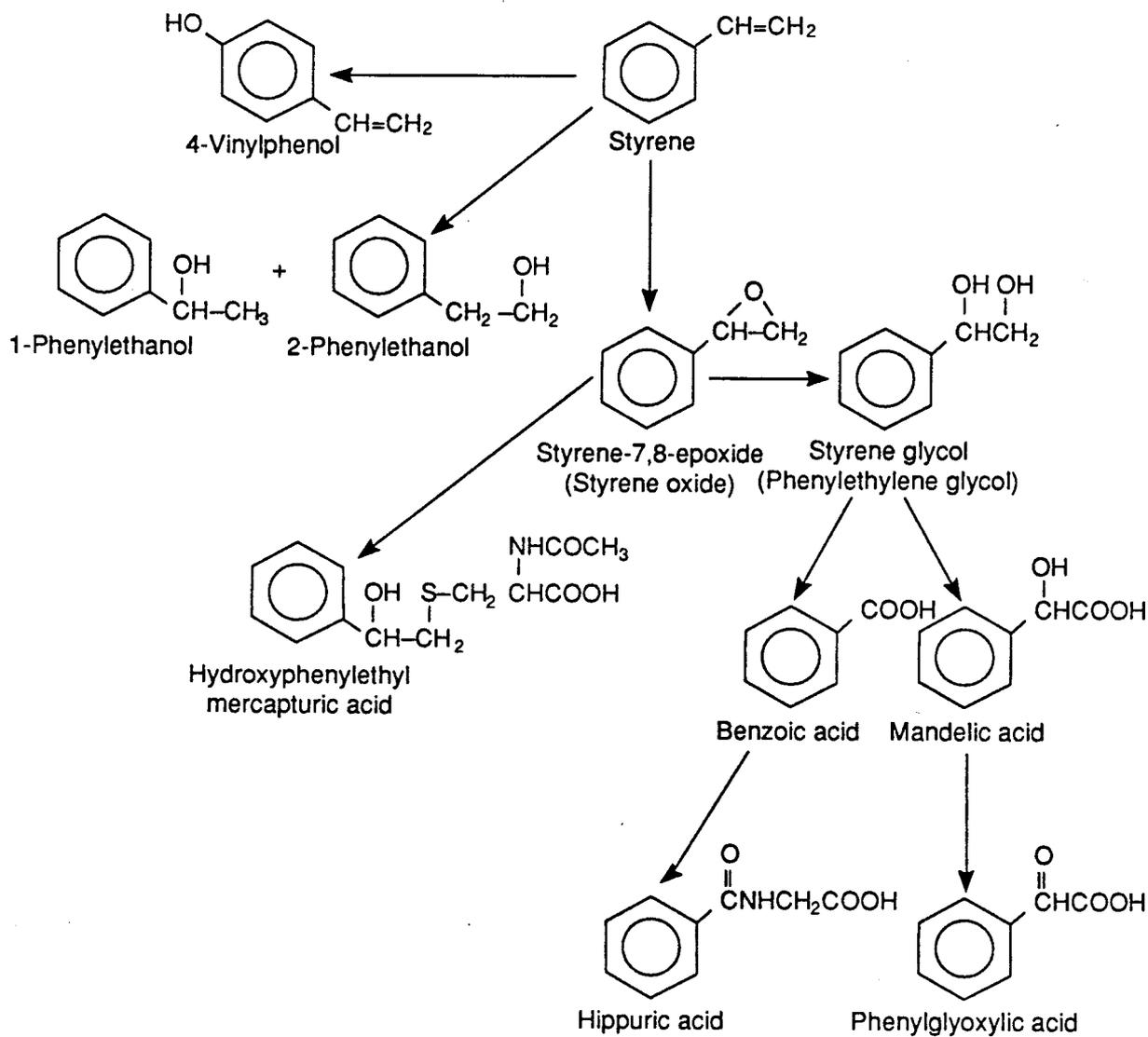
One animal study involved immersion of rats' tails in pure liquid styrene for 1 hour (Shugaev 1969). This procedure resulted in styrene levels in the liver and brain that were estimated to be between 50% and 70% of the concentrations found in the same organs after 4-hour inhalation exposure to a vapor concentration of 11.8 g/m³.

2.3.3 Metabolism

There have been numerous studies, conducted primarily via inhalation, that address the metabolism of styrene in humans and animals (Drummond et al. 1989; Engstrom et al. 1976; Korn et al. 1984; Korn et al. 1987; Leibman 1975; Lof et al. 1983; Withey and Collins 1979; Young et al. 1979). The proposed pathways of styrene metabolism are shown in Figure 2-3. Styrene is metabolized by the microsomal NADPH-cytochrome P-450 dependent mono-oxygenase to styrene oxide. The styrene oxide is then hydrated to phenylethylene glycol (styrene glycol). This transformation is catalyzed by microsomal epoxide hydratase. The styrene glycol is then metabolized directly to MA or to benzoic acid and then hippuric acid. Mandelic acid is also metabolized to PGA. The MA, hippuric acid and PGA are excreted in the urine. In another pathway, styrene oxide is metabolized by cystolic glutathione-S-transferase to mercapturic acids appearing in the urine as hydroxyphenylethyl mercapturic acid. A minor metabolic pathway of styrene in rats involves the formation of 1- and 2-phenylethanol and ring hydroxylation to form vinyl phenol as urinary metabolites. The presence of 4-vinylphenol has been reported in the urine of workers exposed to styrene, but this may have been due to the contamination of the styrene to which the subjects were exposed (Pfaffli et al. 1981). The urinary metabolites that predominate in humans are MA and PGA. In rats, the predominant urinary metabolites are MA, PGA, hippuric acid and glucuronide. Metabolic conversion to styrene-7,8-epoxide (styrene oxide) by the microsomal mixed function oxidase and epoxide hydratase from the liver and spleen of several rodent species has been demonstrated (Belvedere and Tursi 1981; Cantoni et al. 1978; Leibman 1975; Lof et al. 1984; Vainio et al. 1979). However, styrene oxide has only been found at low concentration, close to detection levels (0.02 $\mu\text{mol/L}$), in the blood of workers exposed to styrene (Lof et al. 1986a). Mendrala et al. (1991) investigated the species differences in the in vitro hepatic metabolism of styrene. The results indicated that mice had the greatest capacity to produce styrene oxide, (highest styrene epoxidase activity), followed by rats and then humans. In addition, humans may have the highest capacity to metabolize styrene oxide to styrene glycol, since the human form of styrene oxide hydratase had the highest affinity (lowest K_m) for styrene oxide. Assuming that styrene oxide is the metabolite responsible for styrene-induced toxicity (see, below), the results of this study indicate that care must be taken in extrapolation of data from animal studies to humans for risk assessment.

The formation of styrene oxide may be a key step in the carcinogenicity of styrene. Several studies indicate that styrene oxide is genotoxic (DeMeester et al. 1981; Vainio et al. 1976), and three studies in animals indicate that ingestion of styrene oxide leads to hyperplasia and neoplasia of

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FIGURE 2-3. Metabolic Pathways of Styrene*

*Adapted from Bond 1989; EPA 1988b; Leibman 1975

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the stomach (Conti et al. 1988; Lijinsky 1986; Ponomarkov et al. 1984). The fact that tumors occurred in the stomach and were not detected in other tissues suggests that styrene oxide acts mainly at the point of contact. Thus, tissues most active in metabolizing styrene to styrene oxide might be most susceptible to the carcinogenic potential of styrene. Direct dermal application of styrene oxide did not cause a statistically significant increase in skin tumors in mice (Van Duuren et al. 1983).

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

Several studies have demonstrated that styrene is almost totally excreted as urinary metabolites in humans, and at higher doses, the elimination profile indicates saturation of metabolic excretion or processes (Ramsey and Young 1978; Ramsey et al. 1980). Most of the inhaled styrene is excreted in urine as MA and PGA. In a study of the excretion of styrene and its metabolites resulting from a 100-ppm/8-hour inhalation exposure, 2.6% of the total uptake was excreted as unchanged styrene in exhaled air (Guillemin and Berode 1988). The metabolites MA, PGA, and hippuric acid were excreted in the urine at 56.9%, 33% and 7.5% of the absorbed dose, respectively. In human volunteers exposed to 80 ppm styrene, it is cleared from the blood in a bi-phasic manner, indicating a two-compartment pharmacokinetic model. The half-lives for the rapid and slow clearance phases are 0.58 and 13.0 hours, respectively. The half-life of styrene in subcutaneous adipose tissue of humans is 2-4 days (Engstrom et al. 1978a). The quantities of the major metabolites of styrene in urine compared with the quantity of styrene eliminated unchanged in expired air indicated that approximately 97% is cleared by the metabolic route (Ramsey et al. 1980).

Another human inhalation study determined that between 59%-66% of inhaled styrene (50-200 ppm) was retained after a 4-8 hour exposure (Guillemin and Bauer 1979). Urinary elimination of MA was biphasic with a half-life for the first phase of 4 hours and for the second phase, 25 hours. These findings were comparable to those reported by Engstrom et al. (1976). The half-life of urinary elimination of PGA was determined to be 11 hours. This was regarded by the authors as being the first phase of elimination since MA is a precursor of PGA.

Styrene is almost totally excreted as urinary metabolites in animals. The blood elimination curve for rats is biphasic exponential at 80 and 200 ppm styrene over 6 hours. For exposures greater than 600 ppm exposure levels for (6 hours duration), a nonlinear blood elimination curve following Michaelis-Menten kinetics was observed. In going from 80 to 1,200 ppm (a 15-fold increase) the area under the blood concentration curves increases by 112-fold (Ramsey and Young 1978; Young et al. 1979). Rats exposed to 50-2,000 ppm styrene by inhalation for 5 hours exhibited a dose dependent biphasic pattern of elimination (Withey and Collins 1979).

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2.3.4.2 Oral Exposure

No studies were located regarding excretion in humans after oral exposure to styrene.

Excretion of styrene was studied in the same rats for which there was good distribution data (Plotnick and Weigel 1979). Styrene was rapidly excreted in the urine with 90% of the dose detected in the urine within 24 hours of administration, Less than 2% of the dose was found in the feces. Detectable tissue levels were not found 48 and 72 hours after administration.

2.3.4.3 Dermal Exposure

In a study of the absorption of liquid styrene applied to the forearms of male volunteers, about 13% of the absorbed dose was excreted as MA (Dutkiewicz and Tyras 1968).

No studies were located regarding excretion in animals after dermal exposure to styrene.

2.4 RELEVANCE TO PUBLIC HEALTH

Illness or injury from exposure to styrene is most commonly reported among workers using styrene in the production of polystyrene plastics, protective coatings, polyester resins, and other products. Exposure of the general public to high levels of styrene in air from the home, in the urban environment, or around hazardous waste sites is unlikely, based on occurrence data. The most commonly reported adverse health effects from exposure to styrene include subjective symptoms of central nervous system depression and irritation of the eyes and upper respiratory tract. Oral or dermal exposure to styrene in significant amounts has not been commonly reported in the workplace or the general environment.

Studies in workers exposed to styrene in workplace air suggest that neurological effects are probably the most sensitive indicator of styrene toxicity. Available data do not provide a clear picture of the NOAEL for neurological effects following acute- or intermediate-duration inhalation exposure, but two chronic studies in workers identify LOAELs of 25 ppm (Mutti et al. 1984a) and 31 ppm (Harkonen et al. 1978). Based on the lower LOAEL (25 ppm), a chronic inhalation MRL of 0.06 ppm has been derived using factors of 8/24 and 5/7 to account for exposure 8 hours/day, 5 days/week, and an uncertainty factor of 100 (10 to account for human variability, and 10 to account for use of a LOAEL). Oral studies in animals suggest that hepatic effects may be the most sensitive indicator of toxicity (Srivastava et al. 1982). Based on a LOAEL of 200 mg/kg/day, an intermediate oral MRL of 0.2 mg/kg/day was derived. The LOAEL was multiplied by 6/7 to account for exposure occurring 6 days/week, and was then divided by an uncertainty factor of 1,000 (100 to account for intraspecies and interspecies extrapolation, and 10 to account for use of a LOAEL). Available data do not permit for the derivation of acute or chronic oral MRLs. Dermal MRLs were not derived for

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styrene due to lack of dose-response data and lack of an appropriate methodology for the development of dermal MRLs.

Death. No deaths of humans have been reported after inhalation, oral or dermal exposure to styrene (EPA 1989c; NIOSH 1983). Although no deaths were reported in humans exposed to styrene by inhalation at concentrations in workplace air that exceeded 1,000 ppm (NIOSH 1983) or in laboratory studies at 800 ppm (Carpenter et al. 1944), these levels are severely irritating to the eyes, nose, and throat. Animal studies confirmed the relatively low to moderate acute toxicity following inhalation (Jaeger et al. 1974; Shugaev 1969; Spencer et al. 1942), oral (Ponomarkov and Tomatis 1978; Spencer et al. 1942; Wolf et al. 1956), and dermal (Spencer et al. 1942) exposures. Levels of styrene at hazardous waste sites (1-6 mg/m³) are orders of magnitude below the workplace levels, and oral exposure to significant levels from food or water is unlikely. However, the potential effects on human longevity of longterm inhalation or dermal exposure of humans to low levels of styrene at waste sites, in industrial neighborhoods, or in the environment have not been evaluated.

Systemic Effects.

Respiratory Effects. Occupational and laboratory studies of humans have demonstrated that the most commonly reported immediate symptom after exposure to styrene is irritation of the mucous membranes of the nose and throat. The symptoms appeared when workers were exposed to styrene below 300 ppm for short-time periods (NIOSH 1983). Similarly, human volunteers exposed to 216 ppm for 20 minutes or 376 ppm for 1 hour developed the characteristic nasal irritation (Stewart et al. 1968). The effect has been confirmed in animal studies in which the styrene exposures were high (1,000 ppm) over a period of 3 weeks (Ohashi et al. 1986). Pathological changes of the respiratory mucosa of the rats were also observed. No respiratory effects have been seen in humans after oral exposure to styrene. Mice that were administered styrene one time per week at a dosage of 1,350 mg/kg/week for 6 weeks developed severe lung congestion. This single observation is difficult to correlate with other aspects of styrene toxicity. There are no reports of respiratory effects associated with dermal exposure of humans or animals to styrene. It appears that the respiratory system and the central nervous system are important target organs of styrene. However, the potential effects of long-term human exposure to low levels of styrene at waste sites, in industrial neighborhoods, or in the environment have not been evaluated.

Hematological Effects. No significant hematological effects have been reported in humans exposed to styrene. However, effects on erythrocytes were reported in dogs exposed to high doses of styrene (Quast et al. 1979). Increased numbers of Heinz bodies in the erythrocytes, decreased packed cell values and sporadic decreases in hemoglobin and erythrocyte counts were seen in dogs that received oral doses of 400 or 600 mg/kg/day by gavage for 560 days. No such effects were seen in dogs administered 200 mg/kg/day. The EPA has used the NOAEL of 200 mg/kg/day identified in this study to derive a chronic oral RfD of 0.2 mg/kg/day (IRIS 1991). However, the NOAEL for this

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effect (200 mg/kg/day) is quite close to a chronic LOAEL for increased mortality (Conti et al. 1988), and is also close to doses that caused biochemical changes in the brain in several acute oral exposure studies in animals (Agrawal et al. 1982; Husain et al. 1985; Srivastava et al. 1982). Therefore, the true chronic oral NOAEL is judged to be sufficiently uncertain and no chronic oral MRL is derived. No studies were located that relate dermal exposure of humans or animals and hematological effects. However, based on experimental data that demonstrated eye, nose, and throat irritation are principal effects of styrene, it is unlikely that sufficient concentrations would be tolerated on the skin for a long enough time to accumulate and cause hematological effects. However, the potential effects on the blood and blood-forming organs of the body from long-term exposure of humans to low levels of styrene at waste sites, in industrial neighborhoods, or in the environment have not been evaluated.

Hepatic Effects. Evaluation of the potential adverse effects of styrene on the liver has resulted in mixed results in human and animal studies. The studies either involved occupational exposures of workers or inhalation and oral studies in animals. There are no studies relating dermal exposure and hepatic effects in humans or animals. Measurements to identify elevated serum enzyme levels in styrene-exposed workers have been inconclusive in determining if styrene causes a decrement in liver function (Harkonen et al. 1984; Hotz et al. 1980; Thiess and Friedheim 1978). Rodent inhalation studies have demonstrated that exposure to 300 ppm styrene for 11 weeks results in depletion of glutathione levels and an increase in the cytochrome P-450 content of liver cells (Vainio et al. 1979). A reduction in glutathione content was also seen in rats orally administered 900 mg/kg/day styrene for 7 days (Das et al. 1981). Similarly, increases in liver weights were noted in rats orally administered 400 and 667 mg/kg/day for 6 months (Wolf et al. 1956). Although the results of both human and animal studies are difficult to interpret, and their findings may be nonspecific, the enzyme and organ weight changes reported in these studies suggest that the liver must be regarded as an end point for the inhalation and oral routes of exposure. No changes in microsomal and mitochondrial liver enzymes were observed in rats receiving 200 or 400 mg/kg/day styrene by oral gavage (Srivastava et al. 1982). In addition, areas of focal necrosis were noted in animals administered the high dose, indicating a dose-response trend. Based on the LOAEL of 200 mg/kg/day (Srivastava et al. 1982) an intermediate oral MRL of 0.2 mg/kg/day has been derived. This MRL value is supported by the study by Wolfe et al. (1956) in which a LOAEL of 400 mg/kg/day and a NOAEL of 133 mg/kg/day were identified for hepatic effects. In addition, inhalation studies serve to indicate the liver as a target of styrene toxicity (Axelson and Gustavson 1978; Hotz et al. 1980; Jersey et al. 1978; Vaino et al. 1979). Other studies in the intermediate oral data base have reported renal, neurological, and reproductive effects at doses at or near the critical LOAEL (Agrawal et al. 1982; Srivastava et al. 1989; Wolf et al. 1956). The potential hepatic effects of long-term human exposure to low levels of styrene at waste sites, in industrial neighborhoods, or in the environment, have not been evaluated.

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Renal Effects. The potential for styrene to cause kidney toxicity has been evaluated in both humans and animals following inhalation exposures. There are no studies relating oral or dermal exposure and renal effects in humans or animals. Human studies generally confirm the importance of urinary enzymes as indicators of kidney damage due to occupational exposure to styrene (Aliberti and Severini 1987; Viau et al. 1987; Vyskocil et al. 1989). However, findings indicate only minor effects of styrene on some kidney enzyme functions. Similar evidence for styrene causing minor effects on the kidney is provided by animal studies involving both inhalation (Vainio et al. 1979; Viau et al. 1987) and oral (Das et al. 1983) exposures. The induction reported in the activity of these enzymes is similar to that seen in hepatic tissue. This may mean that the kidney is a potential end point for evaluation of the inhalation and oral routes of exposure. As with the liver, histopathological findings are lacking. The potential renal effects of long-term human exposure to low levels of styrene at waste sites, in industrial neighborhoods, or in the environment have not been evaluated.

Immunological Effects. Systemic immunological studies of styrene have not been conducted in humans or animals by the inhalation, oral, or dermal routes of exposure. However, there is limited information that suggests immunotoxicological concerns. For example, styrene has produced sensitizing reactions in humans. It has also been shown that styrene is apparently metabolized in the skin by aryl hydrocarbon hydroxylase to the more sensitizing epoxide (Sjoborg et al. 1984). Effects of styrene on lymphocyte chromosomes are discussed under "Genotoxic Effects" (below). Because of the association of dermal exposure and immunological reactions, the lack of data on styrene or its metabolites makes it difficult to reach conclusions about potential immunotoxicological concerns. Also, potential immunological effects of long-term human exposure to low levels of styrene at waste sites, in industrial neighborhoods, or in the environment have not been evaluated.

Neurological Effects. Epidemiological and clinical studies on workers have demonstrated that inhalation exposure to styrene may cause alterations of central nervous system function. The symptoms are typical of central nervous system depression, and appear to be the most sensitive end point for styrene exposure via the inhalation route (Kulig 1988; Pryor et al. 1987). High levels (800 ppm) produced immediate muscular weakness, listlessness, drowsiness, and impaired balance within minutes of exposure (Carpenter et al. 1944). Exposures to levels in the range of 50-200 ppm have resulted in a number of signs and symptoms, including impairment of balance and coordination, altered reaction times, sensory neuropathy, impaired manual dexterity, headaches, nausea, mood swings, malaise, and decrement in concentration (Cherry et al. 1980; Lindstrom et al. 1976; Mutti et al. 1984a; Rosen et al. 1978; Stewart et al. 1968). Exposure levels above 50 ppm were frequently encountered in the workplace in the past, but current regulations restrict workplace concentrations to less than 50 ppm (see Chapter 7). However, the study of Harkonen et al. (1978) indicates that some neurological effects, as evidenced by altered EEGs, occur at exposure levels as low as 31 ppm, and the study of Mutti et al. (1984a) indicates effects may occur at a

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level of 25 ppm. Based on a LOAEL of 25 ppm from the Mutti et al. study, a chronic inhalation MEL of 0.06 ppm has been derived.

Less information is available on neurological effects following oral exposure to styrene. No data were located for humans, but studies in animals reveal that repeated oral exposure can lead to altered enzyme levels in brain (Dixit et al. 1982), increased brain levels of several neurotransmitters (serotonin, norepinephrine) (Husain et al. 1980), increased binding of spiroperidal to brain membranes (Agrawal et al. 1982), and increased avoidance response learning (Husain et al. 1985). Some of these responses are similar to those seen following inhalation exposure. For example, the increased spiroperidal binding noted in the oral study of Agrawal et al. (1982) might be due to the decreased dopamine levels noted in the inhalation study by Mutti et al. (1984). However, other effects do not always agree between oral and inhalation studies. For example, the increased levels of norepinephrine in brain reported by Husain et al. (1980) following oral exposure were not observed in rabbits following inhalation exposure (Mutti et al. 1984c). Similarly, the increased conditional response reported by Husain et al. (1985) following oral exposure was not observed in a study of rats exposed by inhalation (Pryor et al. 1987). These apparent differences in effect between routes might be the result of toxicokinetic differences between exposure routes, or might simply be the result of differing experimental designs (different species, doses, durations, end points).

The potential neurological effects of long-term human exposure to low levels of styrene at waste sites, in industrial neighborhoods, or in the environment have not been evaluated. No data are available concerning the neurological effects following dermal exposure to styrene in either humans or animals.

Developmental Effects. Evaluation of the potential developmental effects in occupationally exposed styrene workers suggests that styrene is not teratogenic in humans (Ahlborg et al. 1987). However, a single study (Lemasters et al. 1989) reported that women who work in high styrene exposure settings, such as laminators in fiberglass boat manufacturing companies, had offspring with a 4% lower birthweight than unexposed women. However, this effect was not statistically significant. The actual levels of styrene exposure were not adequately determined for the workers in either the Ahlborg or Lemasters studies. Further, interpretation of the data is complicated by the fact that the women were exposed to other chemicals in the workplace including thermal degradation products of styrene polymers. There are no studies of developmental effects in humans after oral or dermal exposure. Animal studies have produced mixed results. No fetotoxicity or teratogenicity was observed in rats or rabbits exposed via inhalation to either 300 or 600 ppm styrene (Murray et al. 1978). However, an increase in dead or resorbed fetuses was observed in mice exposed to 250 ppm and hamsters exposed to 1,000 ppm (Kankaanpaa et al. 1980). No teratogenic effects were observed in the mice and hamsters. Limited oral studies in rats and rabbits revealed no fetotoxicity or teratogenicity (Murray et al. 1978). There are no reports

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of developmental effects after dermal exposure of animals to styrene. Based on the human and animal data and the low concentrations of styrene in air (1-6 $\mu\text{g}/\text{m}^3$) that have been reported at hazardous wastes sites, developmental effects should not be expected. However, potential developmental effects associated with long-term human exposure to the low levels of styrene at waste sites, in industrial neighborhoods, or in the environment have not been evaluated.

Reproductive Effects. The results of some studies of occupationally exposed female workers in the plastics industry in which styrene is used have suggested an increased risk of spontaneous abortion (Hemminki et al. 1980). Other studies suggest no such increased risk (Lindbohm et al. 1985). Interpretation of these studies is difficult because small study populations were used and there were multiple chemical exposures in the workplace environment. There are no studies of reproductive effects in humans after oral or dermal exposure. Animal studies, either by the inhalation (Salomaa et al. 1985) or the oral route (Beliles et al. 1985; Murray et al. 1978), indicate that styrene is not a reproductive toxicant. However, male rats administered styrene by gavage for 60 days had altered testicular function at the high dose of 400 mg/kg/day (Srivastava et al. 1989). There are no reports of reproductive effects after dermal exposure of animals to styrene. The potential reproductive effects associated with long-term human exposure to low levels of styrene at waste sites, in industrial neighborhoods, or in the environment have not been evaluated.

Genotoxic Effects. Styrene has been tested for genotoxic potential in a variety of systems, and the results have been mixed. Bacterial assays for gene mutation in the absence of activation have been negative, while studies with activation were positive in two out of six cases (see Table 2-4). The lack of evidence for styrene genotoxicity in bacteria may be due in part to volatilization of styrene from the test systems, or possibly to metabolism of styrene to nongenotoxic forms (Dunkel et al. 1985; Yoshikawa et al. 1980). Styrene has induced genotoxic effects in animals following intraperitoneal injection. Male mice were injected with single doses of styrene at levels of 250, 500, 1,000 or 1,500 mg/kg b.w. Significant increases in micronuclei of polychromatic erythrocytes were observed only at the 250 and 1,000 mg/kg levels, and nonsignificant increases were noted at the other two doses. In another study, styrene administered intraperitoneally to male mice at single doses of 177-1,051 mg/kg b.w. induced increases in single-strand breaks in DNA. This genotoxic effect observed in the kidney, liver, lung, testes, and brain 1 hour after administration and in all organs except the liver after 24 hours (Wallis and Orsen 1983).

Styrene production of chromosomal aberrations (breaks and gaps) in peripheral lymphocytes of workers in the styrene industry has been reported (Andersson et al. 1980; Hogstedt et al. 1979; Meretoja et al. 1977, 1978) (see Table 2-5). However, positive findings are limited by the fact that workers are often exposed to other chemicals besides styrene, and that aberrations also depend upon parameters such as age and smoking. On the other hand, negative studies (e.g., Thiess et al. 1980; Watanabe et al. 1981) may also be

TABLE 2-4. Genotoxicity of Styrene In Vitro

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
<u>Salmonella typhimurium</u> (2 strains, plate incorporation method)	Gene mutation	+	-	Vainio et al. 1976
<u>S. typhimurium</u> (3 strains, plate incorporation method)	Gene mutation	-	-	Vainio et al. 1976
<u>S. typhimurium</u> (3 strains, vapor exposure - desiccator test)	Gene mutation	+	-	DeMeester et al. 1981
<u>S. typhimurium</u> (4 strains, vapor exposure - desiccator test)	Gene mutation	-	-	DeMeester et al. 1981
<u>S. typhimurium</u> (5 strains, preincubation method)	Gene mutation	-	-	Dunkel et al. 1985
<u>Escherichia coli</u> (1 strain, preincubation method)	Gene mutation	-	-	Dunkel et al. 1985
Mammalian cells:				
Human lymphocytes	Sister chromatid exchange	No data	+	Norppa et al. 1983
Human lymphocytes	Chromosomal aberrations	No data	+	Jantunen et al. 1986

+ = positive result; - = negative result

TABLE 2-5. Genotoxicity of Styrene In Vivo

Species (test system)	End point	Results	Reference
Mammalian cells:			
Human lymphocytes	Chromosomal aberrations	+	Meretoja et al. 1977
Human lymphocytes	Chromosomal aberrations	+	Meretoja et al. 1978
Human lymphocytes	Chromosomal aberrations	+	Hogstedt et al. 1979
Human lymphocytes	Chromosomal aberrations	-	Thiess et al. 1980
Mouse bone marrow, liver cells, and alveolar macrophages	Sister chromatid exchange	+	Conner et al. 1980
Human lymphocytes	Chromosomal aberrations	-	Andersson et al. 1980
	Sister chromatid exchange	+	
Human lymphocytes	Chromosomal aberrations	-	Watanabe et al. 1981
	Sister chromatid exchange	-	
Mouse bone marrow, polychromatic erythrocytes	Micronuclei	±	Norppa 1981
Mouse kidney, liver, lung, testes, and brain	DNA	+	Walles and Orsen 1983
Human lymphocytes	Unscheduled DNA synthesis	+	Pero et al. 1982
Mouse bone marrow	Chromosomal aberrations	-	Sbrana et al. 1983
Rat bone marrow	Chromosomal aberrations	-	Sinha et al. 1983
Human lymphocytes	Chromosomal aberrations	-	Nordenson and Beckman 1984
	Micronuclei	+	
Human lymphocytes	Chromosomal aberrations	-	Hansteen et al. 1984
	Sister chromatid exchange	-	
Human lymphocytes	Micronuclei	+	Hogstedt et al. 1983
Human lymphocytes	Chromosomal aberrations	-	Maki-Paakkanen 1987
	Sister chromatid exchange	-	
	Micronuclei	-	
Human lymphocytes	Chromosomal aberrations	-	Jablonicka et al. 1988

+ = positive result; - = negative result; DNA = deoxyribonucleic acid

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due to the wide variability in aberration levels. Thus, evidence for styrene-induced chromosomal aberrations in humans is suggestive, but not conclusive. One inhalation study has been conducted in order to evaluate chromosome changes, and this was negative (Sinha et al. 1983). The results were also negative in two studies in which styrene was orally administered to mice (Sbrana et al. 1983) and rats (Beliles et al. 1985). Levels of styrene in air at hazardous waste sites ($1-6 \mu\text{g} / \text{m}^3$) are not likely to cause genotoxicity regardless of the route or duration of exposure. However, potential genotoxic effects of long-term human exposure to low levels of styrene at waste sites, in industrial neighborhoods, or in the environment have not been evaluated.

Cancer. There are several epidemiologic studies of styrene workers that suggest an association between occupational exposure and an increased incidence of leukemia (Meinhardt et al. 1982; Nicholson et al. 1978; Ott et al. 1980) and lymphoma (Hodgson and Jones 1985). However, the reported studies are inconclusive due to exposure to multiple chemicals (including benzene) and the small size of the cohorts. Other studies have reported negative results (Coggon et al. 1987; Matanoski and Schwartz 1987; Okun et al. 1985). There are no reports of cancer resulting from styrene exposure by the oral or dermal routes in humans. It is, therefore, unknown if styrene causes cancer in humans.

Although animal evidence is limited, the results suggest that styrene is weakly carcinogenic in some strains of rats and mice. Overall, human and animal studies suggest that styrene may be a weak human carcinogen. This conclusion is supported by the presence of small amounts of the metabolite styrene oxide (a carcinogen and mutagen) in the blood of styrene industry workers (Lof et al. 1986a).

Three chronic inhalation studies have been conducted in which rats were exposed to styrene vapor (Conti et al. 1988; Jersey et al. 1978; Maltoni et al. 1982). In one study, there was an increased incidence of mammary adenocarcinomas in female rats at the low dose (600 ppm), but a significant response was not evident at the higher dose (1,200 ppm reduced to 1,000 ppm due to growth retardation) (Jersey et al. 1978). In another rat study, designed to detect an elevated incidence of brain tumors, the results were negative (Maltoni et al. 1982). The third inhalation study in rats reported a higher incidence of total (benign and malignant) mammary tumors and malignant mammary tumors in females (Conti et al. 1988).

Other studies have investigated the effects of styrene in animals after long-term oral exposure. In one study, styrene was administered to BD IV rats (500 mg/kg/day) and mice (1,350 mg/kg/day for O₂₀ strain and 300 mg/kg/day for C57Bl strain) for 100 days. There was an increased incidence of lung tumors in the O₂₀ strain of mice (Ponomarkov and Tomatis 1978). In another study (NC1 1979b), rats and mice were given styrene for a lifetime. In male mice, there was a significant increase in the combined incidences of adenomas and carcinomas of the lung. However, this was significant only when compared to concurrent controls but not compared to historical controls from the same laboratory. Another study (Maltoni et al. 1982) did not detect an increased

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incidence of brain tumors in rats. A more recent study to evaluate the carcinogenicity of styrene via the oral route in rats was negative (Conti et al. 1988).

There are no reports of cancer effects associated with dermal exposure of animals to styrene.

Based on the information obtained from human and animal studies, it is not known if styrene causes cancer in humans, and the EPA has not yet assigned styrene to a cancer weight of evidence category (IRIS 1991). The IARC has assigned styrene to Group 2B, possibly carcinogenic to humans (IARC 1988). However, cancer effects associated with long-term human exposure to low levels of styrene at waste sites, in industrial neighborhoods, or in the environment have not been evaluated.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time biologic samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to styrene are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are often not substance specific. They also may not be directly adverse, but can indicate potential health impairment

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(e.g., DNA adducts). Biomarkers of effects caused by styrene are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE."

2.5.1 Biomarkers Used to Identify and/or Quantify Exposure to Styrene

The elimination of styrene via expired air may be used to identify exposure to styrene (Guillemin and Berode 1988; Stewart et al. 1968). Only a small percentage of unchanged styrene is expired after cessation of exposure. There are no adequate studies correlating post-exposure exhaled styrene with previous exposure levels. Assessment of occupational exposure involving measurement of unchanged styrene in urine has been reported (Dolara et al. 1984). In this study of workers, the styrene air concentrations were 16-61 mg/m³ and the urinary concentrations of styrene were 0-7-4.1 µg/L. Urinary mutagenic activity was also evaluated in this study and was not a good indication of exposure to styrene. Only a small fraction of unchanged styrene is recovered in the urine. However, measurement of styrene in urine is a reliable indicator of styrene exposure if the exposure is recent (Dolara et al. 1984; Guillemin and Berode 1988; Pezzagno et al. 1985).

Analysis of unchanged styrene in blood may be used as a qualitative indicator of styrene exposure (Antoine et al. 1986). In one study, styrene was detected in the blood of humans exposed to 80 ppm (Ramsey et al. 1980). The maximum blood concentration at the end of exposure was 0.92±0.26 µg/mL. The half-life values for rapid and slow clearance curves were 0.58 and 13 hours, respectively. In another study, the concentration of styrene in blood (0.2-3.7 mg/L) increased with the level and duration of styrene exposure (Baselt 1988a).

The presence of styrene in adipose tissue is also an indicator of exposure. The concentration of styrene in the adipose tissue of two workers exposed to 32-85 mg/m³ of styrene during a work week suggested a half-life of 5.2 days for one worker and 2.8 days for the other worker. The elimination time was estimated to be 5 weeks (Engstrom et al. 1978b).

Levels of occupational exposure to styrene may also be estimated by measurement of styrene metabolites such as MA and PGA in urine (Bartolucci et al. 1986; Elia et al. 1980; Engstrom et al. 1976; Sedivec et al. 1984; Sollenberg et al. 1988). It should be noted that large intra-individual differences in MA and PGA urinary concentrations have been reported. Some studies found a good correlation between the time-weighted styrene exposure and urinary MA concentrations (Engstrom et al. 1976; Harkonen et al. 1979), while other studies found a better correlation with the sum of urinary MA and

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PGA at the end of the work period (Elia et al. 1980; Sollenberg et al. 1988). Total MA and PGA measured the morning after exposure may be a more reliable biological indicator of styrene exposure in factories where there is high variability in the environmental styrene concentration (Bartolucci et al. 1986).

Reference levels of styrene likely to be observed in workers exposed to the TWA concentrations by inhalation have been reported. These are called biological exposure indices (ACGIH 1988-1989). Values recommended are: MA in urine, 1 g/L; PGA in urine, 250 µg/L, styrene in mixed-exhaled air (before shift) 40 ppb; styrene in mixed-exhaled air (during shift), 18 ppm; styrene in blood (before shift), 0.02 mg/L; and styrene in blood (end of shift), 1 mg/L.

2.5.2 Biomarkers Used to Characterize Effects Caused by Styrene

The most common symptom of styrene exposure is depression of the central nervous system. Other organic solvent vapors cause similar effects. However, neurological symptoms can be used with caution to estimate styrene exposure and adverse effects. Central nervous system depression induced by styrene has been correlated with a urinary MA concentration in excess of 800 mg/L. A measured decrement in psychomotor performance has been associated with urinary MA concentrations of greater than 1,200 mg/L (Harkonen et al. 1978).

Logic, memory, and visuo-constructive abilities were significantly affected in 50 workers with MA and PGA levels corresponding to greater than 50 ppm of styrene in air (Mutti et al. 1984a). Reaction time to a sequence of light stimuli in two female workers resulted in marked impairment in workers with the highest MA excretion. The correlation coefficient for reaction time versus urinary MA (measured as mmol/mmol creatinine) was 0.86 (Mackay and Kelman 1986).

Cytogenetic monitoring of peripheral lymphocytes as a biomarker of effect has been proposed (DeJong et al. 1988; Pero et al. 1982). Future biomarkers may include hemoglobin adducts. Using unscheduled DNA synthesis (UDS) as an indicator of DNA damage, the lymphocytes of 38 individuals occupationally exposed to styrene were evaluated. The induced UDS was significantly increased for the group exposed to 1-40 ppm styrene (Pero et al. 1982). Measurement of chromosome aberration in peripheral blood lymphocyte has been used for many years to monitor the biologic effects of genotoxic chemicals. However, due to high background levels of chromosomal aberration and exposures to other genotoxic workplace chemicals, the sensitivity of this biomarker for the effects of styrene is probably not adequate (DeJong et al. 1988). The role of hepatic glutathione in the toxicity of styrene has been proposed as inhibiting the covalent binding of styrene. This has been confirmed in animal studies by decreased glutathione in styrene-exposed animals (Parkki 1978). However, its use as a biomarker of effect in humans remains to be demonstrated since data on the adverse effects of styrene on the human liver are insufficient.

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Levels of styrene oxide may also be a useful biomarker of effect, since this metabolic intermediate may be responsible for many of styrene's toxic effects. However, no data were located regarding a correlation between styrene oxide and any adverse health effect.

2.6 INTERACTIONS WITH OTHER CHEMICALS

Styrene metabolism is known to be inhibited by the presence of other chemicals such as toluene, trichloromethylene, and ethyl benzene. The biotransformation of styrene in rats to PGA, MA, and hippuric acid was suppressed by coadministration of toluene (Ikeda et al. 1972). This may be due to competitive inhibition of oxidative mechanisms. Similar results were reported by Ikeda and Hirayama (1978) in rats when styrene metabolism was inhibited by the administration of trichloroethylene. Urinary metabolites of styrene may be markedly reduced when humans or animals are concurrently exposed to organic solvents that inhibit styrene metabolism.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

Styrene is a hazardous substance found in the workplace with much lower levels found in the environment. Therefore, the populations at risk are workers in industries making polystyrene plastics, coating, polyester resins, and other products'. Persons with pre-existing respiratory or neurological problems would be at risk for the irritant action and central nervous system depressant effects of styrene, respectively. Individuals deficient in glucose-6-phosphate dehydrogenase (G6PD) may be at increased risk since Heinz bodies, which are readily formed in the blood of G6PD-deficient humans (Wintrobe et al. 1970), have been found in the red blood cells of dogs orally exposed to styrene. Women from families pre-disposed to mammary gland tumors may also be at increased risk since this type of tumor was observed in some animal studies. Some studies suggest that the incidence of adverse reproductive outcome (low birth weight, spontaneous abortions) may be elevated in styrene-exposed female workers (see Section 2.2.1.6), therefore, pregnant women may be susceptible. Individuals with liver dysfunction might also be somewhat more susceptible, since this liver is affected by styrene.

2.8 MITIGATION OF EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to styrene. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to styrene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

Human exposure to styrene may occur by inhalation, ingestion, or by dermal contact. General recommendations for reducing absorption of styrene following exposure include removing the exposed individual from the contaminated area and removing the contaminated clothing. If the eyes and skin were exposed, they are flushed with water. There are some disagreements

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regarding appropriate procedures for mitigation of absorption of styrene following oral exposure. Since aspiration of styrene into the lung can cause pulmonary edema and hemorrhage, some authors advise against the use of emetics, but recommend administration of water for dilution of gastric lavage (Bronstein and Currance 1988; Haddad and Winchester 1990). Others suggest administration of syrup of ipecac to induce vomiting, but consider the usefulness of activated charcoal to bind the styrene and cathartics to speed fecal excretion as questionable (Ellenhorn and Barceloux 1988). Following acute inhalation exposure, administration of oxygen and use of mechanical ventilation to support respiration have been suggested (Bronstein and Currance 1988; Ellenhorn and Barceloux 1988; Haddad and Winchester 1990). Administration of aminophylline and inhaled bronchodilators may be required to treat bronchospasm (Ellenhorn and Barceloux 1988). Furthermore, cardiac monitoring has been suggested. Supportive treatment may be needed for neurological effects of styrene exposure (Haddad and Winchester 1990).

Styrene is metabolized by the body, and most styrene that is absorbed is excreted in the urine as metabolites of the parent compound. Styrene is cleared rapidly from the human body. Its half-life is several hours in the blood and about 2-4 days in subcutaneous adipose tissue (see Section 2.3). No method is commonly used to enhance the elimination of the absorbed dose of styrene.

In humans, central nervous system depression and upper respiratory tract irritation were reported following acute exposure to higher styrene concentrations (see Section 2.2). Studies in animals indicate that chronic styrene exposure causes liver and kidney effects and may induce cancer. Styrene oxide was found to be the active mutagenic metabolite of styrene in several studies (de Raat 1978; Donner et al. 1979; Norppa et al. 1979, 1980a, 1980b, 1981, 1984, 1988; Pohlova et al. 1985; Vainio et al. 1976). Based on these studies, it can be concluded that styrene is a typical indirect mutagen that needs metabolic activation to be able to bind covalently to macromolecules (e.g., nucleic acids). In one of the possible metabolic pathways, styrene oxide is further metabolized to hydroxyphenylethyl mercapturic acid. The reaction utilizes glutathione (Bond 1989). It has been demonstrated that mutagenic activity of styrene oxide was decreased in the presence of glutathione in *S. typhimurium* TA 100 (Yoshikawa et al. 1980). This experiment, therefore, suggests that glutathione may reduce the mutagenic effects of styrene oxide.

The formation of styrene oxide may also contribute to other effects following styrene exposure. It is well established that glutathione decreases the cytotoxicity of many reactive chemicals by acting as a scavenger of toxic metabolites. It was found that exposure of rodents to high levels of styrene caused depletion of glutathione content in the liver cells of these animals (Das et al. 1978; Vainio et al. 1979). It was suggested that glutathione decreases the hepatotoxicity by preventing styrene oxide reaction with other endogenous macromolecules. Similarly, depletion of glutathione was found in all regions of rat brain following exposure to styrene oxide (Dixit et al. 1982; Trenga et al. 1990). The authors speculated that the depletion of brain glutathione may lead to an increased concentration of free styrene oxide with

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increased binding to cellular nucleophiles. This process would contribute to oxidative injury to neuronal and glial cells and may be a part of styrene-induced neurotoxicity. It should be noted, however, that styrene itself, being a lipophilic compound, may disrupt the nerve membrane function in a manner similar to anesthetic agents.

Although results from in vitro studies in bacteria and in vivo animal studies demonstrate that exogenous glutathione precursors may decrease the effects of styrene toxicity, it is not known whether this treatment would be beneficial in humans. For low level exposure cases it is unlikely that endogenous glutathione levels would be decreased to a significant extent. Therefore, it is unlikely that exogenous glutathione precursors such as N-acetylcysteine may be effective in mitigating the toxic effects of styrene. Exogenous doses of reducing agents may be useful following acute high dose exposure to styrene. In this case, a significant depletion of glutathione may occur as a result of the presence of high levels of styrene oxide. However, there is no clinical data available to date which supports the use of this treatment.

2.9 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of styrene is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of styrene.

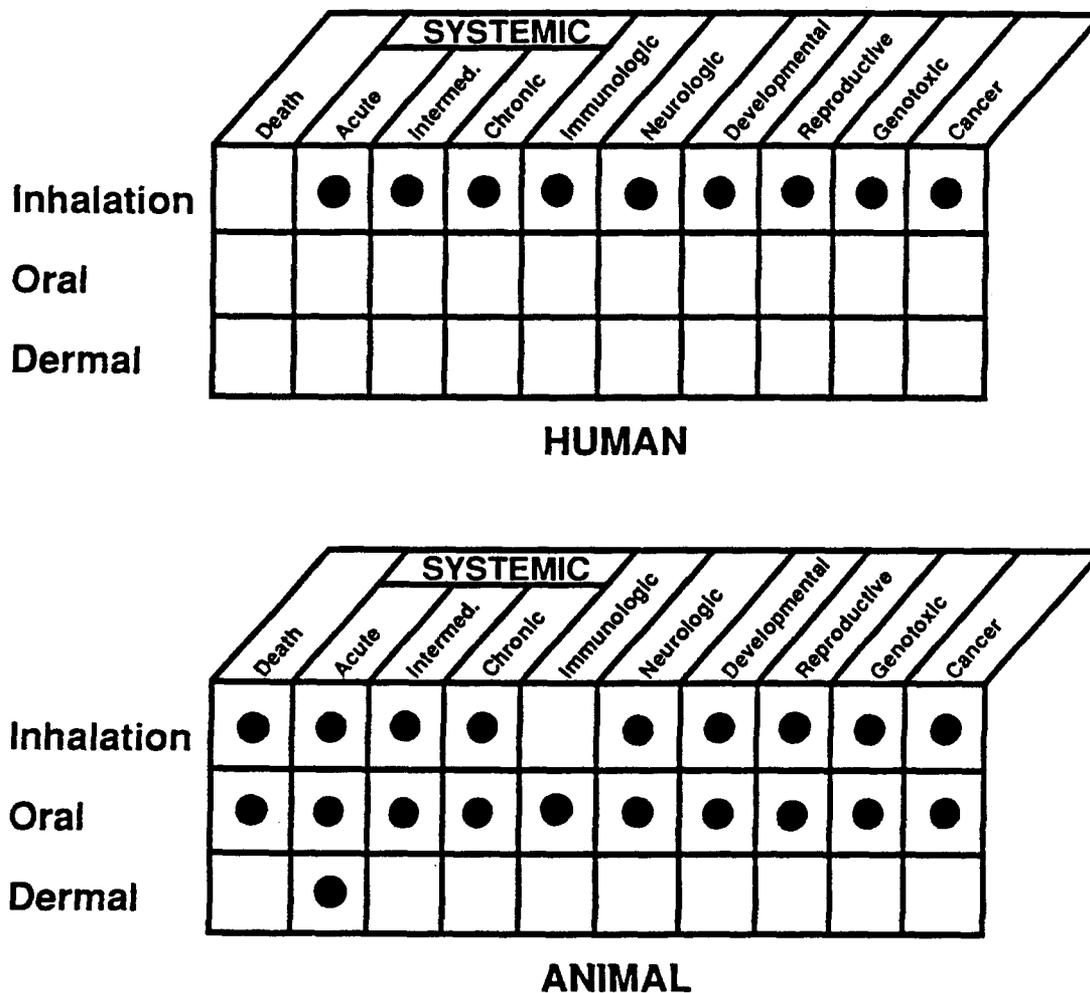
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of Styrene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to styrene are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of styrene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs" information (i.e., data gaps that must necessarily be filled).

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FIGURE 2-4. Existing Information on Health Effects of Styrene



● Existing Studies

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There is information on most categories of human toxicity via the inhalation route from occupational studies. However, data are not available on humans exposed to styrene by the oral or dermal routes. Data from animal studies are more extensive, with studies available for most areas of toxicity resulting from exposure via the oral and inhalation routes; however, there are no data on immunologic effects via inhalation. Little is known about the effects of dermal exposure to styrene in animals.

2.9.2 Data Needs

Acute-Duration Exposure. The possibility for brief human exposure to high concentrations of styrene exists in occupational settings, and might also exist near major spills. Exposure of the general public to episodic high concentrations of styrene at hazardous waste sites, in the home, or in the general environment is unlikely. The respiratory tract and central nervous system are the likely target organ systems for inhaled styrene (Alarie 1973; Carpenter et al. 1944; DeCeaurriz et al. 1983; Kankaanpaa et al. 1980; Murray et al. 1978; Spencer et al. 1942; Stewart et al. 1968). The data are not considered sufficient to establish an inhalation acute-duration MRL. Episodic high-level exposures to styrene from contaminated food or water are unlikely. There are no data on humans orally exposed to styrene, and the animal data are not considered sufficient to derive an oral acute-duration MRL. Thus, additional single-dose oral and inhalation studies are needed to better define toxicity thresholds. However, the potential carcinogenicity of styrene prevents the design of controlled laboratory exposures in humans. Dermal exposure to styrene at significant levels is unlikely except in the case of workplace spills and dermal absorption is probably low based on limited human studies. However, the almost complete lack of dermal toxicity data in animals and humans creates a degree of uncertainty on this issue. Therefore, single dose dermal studies would be useful in determining target organs and thresholds for dermal exposure. In designing these types of studies, precautions should be taken to avoid concomitant inhalation exposure.

Intermediate-Duration Exposure. Intermediate-duration exposure studies in animals and humans confirm that the upper respiratory tract and central nervous system are the target organ systems for inhaled styrene (Kulig 1988; Ohashi et al. 1986; Pryor et al. 1987; Rosengren and Haglid 1989; Spencer et al. 1942). However, additional studies are needed, as the data are not considered sufficient to derive an intermediate-duration inhalation MRL. Oral exposure studies of intermediate-duration are limited. Animal studies indicate that renal and neurological end points need further evaluation (Johnston et al. 1983; Vainio et al. 1979; Viau et al. 1987). The data are considered sufficient to derive an oral intermediate-duration RfD of 0.2 mg/kg/day based on liver enzyme changes in rats (Srivastava et al. 1982). However, additional data would be valuable since the critical study does not define a NOAEL. Basic information on the adverse effects of intermediateduration dermal exposure to styrene in animals is also needed due to the sparsity of available data.

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Chronic-Duration Exposure and Cancer. Chronic studies are available that investigated the adverse health effects of styrene on workers in the plastics industry (Harkonen et al. 1978, 1984; Hotz et al. 1980; Lemasters et al. 1989; Lorimer et al. 1978; Mutti et al. 1984a,b; Thiess and Friedheim 1978). Although the lung and liver are both affected by chronic exposure, neurological effects such as decreased short term memory or impaired visuomotor performance seem to be the most sensitive indicators of toxicity (Harkonen et al. 1984; Mutti et al. 1984a). Based on a LOAEL of 25 ppm identified by Mutti et al., a chronic inhalation MRL of 0.06 ppm has been derived. Further research to define the dose-response curve more fully and to identify a chronic inhalation NOAEL for neurological effects would be valuable and would help reduce uncertainty in the MRL. Data on chronic oral exposure to styrene is only available through animal studies (Beliles et al. 1985; Conti et al. 1988; NC1 1979b; Quast et al. 1979). In these studies, the most sensitive indicator of toxicity appears to be Heinz body formation in red blood cells (Quast et al. 1979), and the EPA has calculated a chronic oral RFD based on this study (IRIS 1991). However, as discussed above (Section 2.4), there is some doubt regarding the chronic oral NOAEL, and whether hematological effects are really more sensitive than neurological effects. Moreover, decreased survival has been noted in rats at exposure levels only slightly higher than the no-effect level for hematological effects (Conti et al. 1988). Therefore, no chronic oral MRL has been derived. Further studies on the effects of oral exposure, with special emphasis on neurological or neurobehavioral effects, would be valuable. Although chronic dermal exposure by the general public is not likely, there may be some potential for dermal contact with soil at hazardous waste sites. Therefore, data on longterm effects of dermal contact with styrene would be useful.

Taken together, the animal and human data indicate that styrene may possibly be a weak human carcinogen. Although data from epidemiological studies are limited due to concurrent chemical exposures and small cohorts, the data are suggestive of some carcinogenic potential in humans (Coggon et al. 1987; Hodgeson and Jones 1985; Matanoski and Schwartz 1987; Meinhardt et al. 1982; Nicholson et al. 1978; Okun et al. 1985; Ott et al. 1980; Wong 1990). Studies in rats and mice have indicated that styrene may be a weak animal carcinogen via the oral and inhalation routes. Clarification of the data is needed in several areas. Interpretation of existing animal bioassays is complicated by the marginal statistical significance of elevated tumor incidences and by the lack of adequate dose response data. Almost all of the available epidemiological studies involve concurrent exposures to other chemicals. Additional studies that account for these issues would be valuable. Finally, the role of the metabolism of styrene to styrene oxide in humans and animals needs to be clarified. This might best be accomplished by studies of industrially exposed (worker) populations.

Genotoxicity. On-going studies by Perera (Columbia University) and Rappaport (University of California) will address links between styrene exposure and cytogenic response. The results of genotoxicity tests for styrene both *in vivo* and *in vitro* are frequently conflicting, and the genotoxic potential of styrene is not clear (Andersson et al. 1980; Beliles

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et al. 1985; Hogstedt et al. 1979; Meretoja et al. 1977, 1978; Watanabe et al. 1981). The reasons for the mixed or conflicting genotoxicity results may be differences in the metabolism or detoxification of styrene in the various test systems employed. The role of the metabolite styrene oxide in genotoxicity assays on styrene should be fully evaluated, preferably in mammalian in vivo systems. Toxicokinetic studies evaluating the presence, level, and activity of styrene oxide in humans will influence the interpretation of genotoxicity studies on styrene and their relevance to public health.

Reproductive Toxicity. Additional studies are needed to determine the potential effects of styrene exposure on spontaneous abortion rates in female workers in the styrene industry. The exposures in existing studies were not quantified and therefore, interpretation of results is difficult (Harkonen and Holmberg 1982; Hemminki et al. 1980; Lindbohm et al. 1985). A single three-generation study showed no styrene-related reproductive effects; however, one reproductive study in animals indicated altered testicular function (Beliles et al. 1985; Salomaa et al. 1985). Additional reproductive data on occupationally-exposed males would be useful in evaluating the existing animal data that indicates altered testicular function.

Developmental Toxicity. Data on the developmental effects of inhalation exposure to styrene are available in humans and animals. Developmental effects were not generally observed in animal studies, but some fetal- and embryo-toxicity was observed (Kankaanpaa et al. 1980). This information, in combination with observations of reduced birth weight in the offspring of female workers in the styrene industry (Lemasters et al. 1989), indicates that additional repeated dose animal studies and epidemiological studies would be useful. Little information is available on the potential developmental effects of oral exposure to styrene. The single negative study in rats (Murray et al. 1978) should be supplemented by confirmatory studies in other species.

Immunotoxicity. Immunotoxicity data in humans are limited to one study in which styrene exposure had no effect on serum alpha, beta and gamma globulin concentrations in workers (Chmielewski et al. 1977). In another study, it was found that styrene epoxide was more sensitizing to humans than styrene itself (Sjoberg et al. 1984). Limited data in animals indicate that oral exposure to styrene has some immunotoxic potential (Sinitskij 1969). It would be useful to investigate the potential for styrene-induced immunotoxicity in future studies.

Neurotoxicity. Central nervous system depressant effects in humans from inhalation exposure to styrene are well known (Carpenter et al. 1944; Harkonen et al. 1978; Mutti et al. 1984a,b; Stewart et al. 1968). Although the threshold for neurologic effects is not well defined, the studies of Harkonen et al. (1979) and Mutti et al. (1984a) provide sufficient dose-response data to permit derivation of a chronic inhalation MRL. Since this is based on a LOAEL, further studies which define the chronic NOAEL, as well as acute- and intermediate-duration NOAELs, would be valuable especially at levels of styrene causing problems with coordination and psychological function. These

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and other neurological effects may play a role in the rate of workplace accidents and the level of performance. Additional studies in mammalian animal models are needed to determine if styrene causes chronic damage to the central and/or peripheral nervous systems and to determine the associated mechanism of toxicity. Also, information is needed to determine if neurotoxicity can result from exposure to styrene via the oral route.

Epidemiological and Human Dosimetry Studies. Since acute effects such as upper respiratory tract irritation and eye irritation have been frequently noted in occupational health studies and in laboratory experiments, additional epidemiological studies of workers are needed to determine the chronic respiratory effects of styrene. Since liver function evaluation of workers exposed to styrene has resulted in equivocal results (Harkonen et al. 1984; Hotz et al. 1980; Lorimer et al. 1978; Thiess and Friedheim 1978), additional studies are necessary and should include complete profiles of serum hepatic enzymes of exposed workers. Further epidemiological information is needed to determine if exposure to styrene causes reproductive effects, developmental effects, or cancer.

Biomarkers of Exposure and Effect. Available studies indicate that there are good quantitative relationships between styrene metabolites (MA and PGA) in the urine and styrene exposure levels in humans (Harkonen et al. 1978; Mutti et al. 1984a). Efforts to establish styrene oxide as a biomarker would also be valuable, since this metabolite may underlie many of styrene's toxic effects. However, methods which focus on these metabolites are mainly useful for determining exposure within 1 day of exposure. Efforts to identify biomarkers of prior exposures would also be valuable.

There are currently no biomarkers specific for the effects of styrene that are not also typical of other central nervous system depressants. Further research is needed to evaluate potential biomarkers of effect in the areas of chromosome aberrations, psychomotor decrement, hepatic glutathione depletion, and adipose tissue retention of styrene. These potential biomarkers should be evaluated in terms of long-term or chronic exposure periods, and their specificity for exposure to styrene.

Absorption, Distribution, Metabolism, and Excretion. Styrene oxide (styrene epoxide) has been identified as an intermediate metabolite of styrene (Drummond et al. 1989; Engstrom et al. 1976; Korn et al. 1984, 1987; Leibman 1975; Lof et al. 1983; Withey and Collins 1979; Young et al. 1979). However, styrene oxide has only been found in minute amounts in human studies (Lof et al. 1986a). The presence of styrene oxide, a mutagen and carcinogen, may account for some conflicting results and/or interspecies variation in mutagenicity tests and cancer bioassays. The role, if any, of styrene oxide in the overall toxicity of styrene needs to be evaluated by additional metabolism studies to confirm its presence, level, and duration in human tissues. The toxicokinetics of styrene exposure via inhalation are reasonably well defined. However, oral and dermal exposure data are needed to better characterize absorption rates and the elimination ratios of the metabolites (MA and PGA).

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Comparative Toxicokinetics. Interspecies variations in styrene metabolism have been established by noting, for example, different ratios of MA and PGA in different species (Ramsey et al. 1980; Ramsey and Young 1978; Young et al. 1979). Mendrala et al. (1991) reported species differences in the *in vitro* metabolism of styrene and styrene oxide which indicated that mice and rats had a higher capacity to produce styrene oxide from than humans. Efforts should continue to identify which animal model best approximates human metabolism of styrene. Although urinary metabolites of styrene in man are known, the occurrence and significance of styrene oxide needs to be evaluated.

Mitigation of Effects. Recommended methods for the mitigation of acute effects of styrene intoxication include mechanical ventilatory support, administration of oxygen, and drug therapy for bronchospasm, if exposure is by inhalation (Bronstein and Currance 1988; Ellenhorn and Barceloux 1988). Thorough washing or flushing with water is recommended for dermal/ocular exposure. There is disagreement concerning the use of emetics to prevent absorption of styrene following ingestion due to potential of aspiration into the lung (Bronstein and Currance 1988; Ellenhorn and Barceloux 1988; Haddad and Winchester 1990). Supportive treatment is indicated for neurological effects of styrene exposure (Haddad and Winchester 1990). No information was located concerning mitigation of effects of lower-level or longer-term exposure to styrene. Further information on techniques to mitigate such effects would be useful in determining the safety and effectiveness of possible methods for treating styrene-exposed populations in the vicinity of hazardous waste sites. This includes further studies on the mechanism(s) of styrene toxicity, so that methods may be developed to interfere with or block styrene's toxic actions in the body.

2.9.3 On-going Studies

A number of research projects are in progress investigating styrene. These projects are summarized in Table 2-6.

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TABLE 2-6. On-going Studies on the Health Effects of Styrene

Investigator	Affiliation	Research description	Sponsoring agency
S. M. Rappaport	University of California	Occupational health study	NIOSH
W. J. Nicholson	Mt. Sinai School of Medicine	Epidemiological study	NIEHS
F. P. Perera	Columbia University	Biological markers	NCI
A. M. Jeffrey	Columbia University	Biologically effective doses in humans and mice	NCI
J. Roycroft	NTP	Two-year inhalation bioassays in rats and mice	NCI
M. Kogevinas	IARC	Epidemiological study on 20,000 styrene workers	IARC
R. Nolan	Dow	Metabolism/Kinetics	SIRC
J. Mattson	Dow	Ototoxicity studies	SIRC
J. Filser	GSF Toxicology Institute, Munich	Metabolism/Kinetics	ECETOC
W. Lutz	Institute of Toxicology, Zurich	DNA-binding	ECETOC

DNA = Deoxyribonucleic acid; ECETOC = European Chemical Industry Ecology and Toxicology Center; IARC = International Agency for Research on Cancer; NCI = National Cancer Institute; NIEHS = National Institute of Environmental Health Sciences; NIOSH = National Institute for Occupational Health and Safety; NTP = National Toxicology Program; SIRC = Styrene Information and Research Center