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16. ABSTRACT

This report summarizes and evaluates information relevant to a preliminary interim assessment of adverse health effects associated with specific chemicals or compounds. The Office of Emergency and Remedial Response (Superfund) uses these documents in preparing cost-benefit analyses under Executive Order 12991 for decision-making under CERCLA. All estimates of acceptable intakes and carcinogenic potency presented in this document should be considered as preliminary and reflect limited resources allocated to this project. The intent in these assessments is to suggest acceptable exposure levels whenever sufficient data are available. The interim values presented reflect the relative degree of hazard associated with exposure or risk to the chemical(s) addressed. Whenever possible, two categories of values have been estimated for systemic toxicants (toxicants for which cancer is not the endpoint of concern). The first, RfD_S or subchronic reference dose, is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs during a limited time interval. The RfD is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs for a significant portion of the lifespan. For compounds for which there is sufficient evidence of carcinogenicity, q_1^*s have been computed, if appropriate, based on oral and inhalation data if available.

17. KEY WORDS AND DOCUMENT ANALYSIS		
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HEALTH EFFECTS ASSESSMENT FOR STYRENE

ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
OFFICE OF HEALTH AND ENVIRONMENTAL ASSESSMENT
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OH 45268

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PREFACE

This report summarizes and evaluates information relevant to a preliminary interim assessment of adverse health effects associated with styrene. All estimates of acceptable intakes and carcinogenic potency presented in this document should be considered as preliminary and reflect limited resources allocated to this project. Pertinent toxicologic and environmental data were located through on-line literature searches of the TOXLINE, CANCERLINE and the CHEMFATE/DATALOG data bases. The basic literature searched supporting this document is current up to March, 1987. Secondary sources of information have also been relied upon in the preparation of this report and represent large-scale health assessment efforts that entail extensive peer and Agency review. The following Office of Health and Environmental Assessment (OHEA) sources have been extensively utilized:

U.S. EPA. 1984b. Health and Environmental Effects Profile for Styrene. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste, Washington, DC. EPA/600/X-84/325. NTIS PB88-182175.

U.S. EPA. 1985a. Integrated Risk Information System (IRIS). Reference dose (RfD) for oral exposure for styrene. Online. (Verification date 10/09/85.) Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH.

U.S. EPA. 1985b. Reportable Quantity Document for Styrene. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC.

U.S. EPA. 1987. Integrated Risk Information System (IRIS). Carcinogenicity Assessment for Lifetime Exposure to Styrene. Online: Input pending. (Verification date 11/09/87.) Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH.

U.S. EPA. 1988. Drinking Water Criteria Document for Styrene. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Drinking Water, Washington, DC.

The intent in these assessments is to suggest acceptable exposure levels for noncarcinogens and risk cancer potency estimates for carcinogens whenever sufficient data were available. Values were not derived or larger uncertainty factors were employed when the variable data were limited in scope, which tended to generate conservative (i.e., protective) estimates. Nevertheless, the interim values presented reflect the relative degree of hazard or risk associated with exposure to the chemical(s) addressed.

Whenever possible, two categories of values have been estimated for systemic toxicants (toxicants for which cancer is not the endpoint of concern). The first, RfD_S (formerly AIS) or subchronic reference dose, is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs during a limited time interval (i.e., for an interval that does not constitute a significant portion of the lifespan). This type of exposure estimate has not been extensively used, or rigorously defined, as previous risk assessment efforts have been primarily directed towards exposures from toxicants in ambient air or water where lifetime exposure is assumed. Animal data used for RfD_S estimates generally include exposures with durations of 30-90 days. Subchronic human data are rarely available. Reported exposures are usually from chronic occupational exposure situations or from reports of acute accidental exposure. These values are developed for both inhalation (RfD_{SI}) and oral (RfD_{SO}) exposures.

The RfD (formerly AIC) is similar in concept and addresses chronic exposure. It is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs for a significant portion of the lifespan [see U.S. EPA (1980b) for a discussion of this concept]. The RfD is route-specific and estimates acceptable exposure for either oral (RfD_O) or inhalation (RfD_I) exposure with the implicit assumption that exposure by other routes is insignificant.

Composite scores (CSs) for noncarcinogens have also been calculated where data permitted. These values are used for identifying reportable quantities and the methodology for their development is explained in U.S. EPA (1984).

For compounds for which there is sufficient evidence of carcinogenicity, RfD_S and RfD values are not derived. For a discussion of risk assessment methodology for carcinogens refer to U.S. (1980b). Since cancer is a process that is not characterized by a threshold, <u>any</u> exposure contributes an increment of risk. For carcinogens, q_1^*s have been computed, if appropriate, based on oral and inhalation data if available.

ABSTRACT

In order to place the risk assessment evaluation in proper context, refer to the preface of this document. The preface outlines limitations applicable to all documents of this series as well as the appropriate interpretation and use of the quantitative estimates presented.

The available data indicate that styrene is carcinogenic by the oral (NCI, 1979) and inhalation (Jersey et al., 1978) routes in mice and rats, respectively. U.S. EPA (1987, 1988) derived a q_1^* of 3×10^{-2} $(mg/kg/day)^{-1}$ for oral exposure that is adopted as the estimate of oral carcinogenic potency for the purpose of this document. A q_1^* of 2.0×10^{-3} $(mg/kg/day)^{-1}$ corresponding to a unit risk for air of 6×10^{-7} $(\mu g/m^3)^{-1}$ was derived for inhalation exposure to styrene from the inhalation study using rats (U.S. EPA, 1987). The appropriateness of this study for high- to low-dose extrapolation, however, is being evaluated based upon pharmacokinetic considerations. Styrene is placed in U.S. EPA weight-of-evidence Group B2, probable human carcinogen (U.S. EPA, 1987, 1988).

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LIST OF ABBREVIATIONS

AADI	Adjusted acceptable daily intake
CNS	Central nervous system
CS	Composite score
DNA	Deoxyribonucleic acid
HA	Health Advisory
LOAEL	Lowest-observed-adverse effect level
MFO	Mixed function oxidase
MTD	Maximum tolerated dose
NOAEL	No-observed-adverse effect level
ppm	Parts per million
RBC	Red blood cell
RfD	Reference dose
RfD_S	Subchronic reference dose
RNA	Ribonucleic acid
SAP	Serum alkaline phosphatase
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SMR	Standard mortality ratio
SNARL	Suggested no adverse response level
STEL	Short-term exposure limit
TLV	Threshold limit value
TWA	Time-weighted average

1. ENVIRONMENTAL CHEMISTRY AND FATE

Selected physical and chemical properties and environmental fate of styrene are presented in Table 1-1.

Zoeteman et al. (1980) estimated the aquatic half-life to be ~14 hours, based on a river reach study. The volatilization half-life has been estimated to be ~3 hours based on a calculated Henry's Law constant of 5.2×10^{-3} atm/m^3•mol at 25°C. In addition to volatilization, removal by photochemical degradation, biodegeneration and adsorption to sediments (as indicated by monitoring data) may be significant removal processes (U.S. EPA, 1984b). The atmospheric half-life is based on experimentally determined rate constants for the reaction of vapor phase styrene with both ozone and hydroxyl radicals. Considering the reactivity of styrene in air, physical removal processes are not likely to be important (U.S. EPA, 1984b). Biodegradation screening studies in soil indicate biodegradation in soils may occur. Experimental evidence exists, which indicates styrene may persist in certain soils for at least 2 years (U.S. EPA, 1984b). On soil surfaces, volatilization, oxidation, hydrolysis and acid-catalyzed polymerization of stryene are expected to reduce its half-life to a much lower value than its half-life in subsurface soil.

TABLE 1-1

Selected Physical and Chemical Properties
and Environmental Fate for Styrene

Property	Value	Reference
CAS number:	100-42-5	
Chemical class:	Unsaturated substituted benzene	
Molecular weight:	104.16	
Vapor pressure at 20°C	5 mm Hg	U.S. EPA, 1984b
Water solubility at 20°C:	300 mg/ℓ	U.S. EPA, 1984b
Log octanol/water partition coefficient:	2.95	Hansch and Leo, 1985
Bioconcentration factor:	13.5, goldfish (Carassius auratus)	NLM, 1986
Half-lives in		
Air:	1-3 hours	U.S. EPA, 1984b
Water:	3-14 hours, (river) estimated	NLM, 1986; Zoeteman et al., 1980
Soil:	4-22 weeks, (subsurface aquifer) estimated	Wilson et al., 1983

2. ABSORPTION FACTORS IN HUMANS AND EXPERIMENTAL ANIMALS

2.1. ORAL

Data regarding the absorption of styrene from the gastrointestinal tracts of humans could not be located in the available literature. Data from experiments on rats, however, suggest that absorption is rapid and complete. Plotnick and Weigel (1979) administered a single 20 mg/kg gavage dose of ^{14}C-styrene in corn oil to rats and determined that <10% of the dose of radioactivity remained in the gastrointestinal tract at 8 hours posttreatment. At the end of 24 hours, fecal excretion accounted for <2% of the dose and urinary execretion accounted for ~90% of the dose of radioactivity. In a similar study with somewhat larger doses, Sauerhoff et al. (1976) determined fecal excretion at 72 hours to account for 4 and 1.5% of the dose of radioactivity from a 50 and 500 mg/kg dose of ^{14}C-styrene in corn oil. Urinary excretion accounted for 95 and 90%, and expired air contained 1 and 9% of the administered dose of radioactivity at the low and high doses, respectively.

Experiments by Withey (1976) suggest that the nature of the vehicle affects the rate of gastrointestinal uptake in rats. When styrene at a dose of 3.147 mg in aqueous solution was given by gavage, blood levels peaked within 10 minutes and declined rapidly. When 32.61 mg was given in vegetable oil, blood levels did not peak until ~100 minutes and the rate of decline was much less rapid, indicating a prolonged absorption phase.

2.2. INHALATION

The respiratory uptake of styrene has been investigated in humans and rats. U.S. EPA (1984b, 1988) summarized the results from several human inhalation studies at concentrations ranging from 50-80 ppm (210-340 mg/m^3). Rapid absorption from the lungs was suggested by Astrand et al.

(1974) who noted that the concentration of styrene in alveolar air plateaued in ~1 minute after exposure began. Experiments by several investigators indicate that the rate of uptake increases with exercise (Ramsey et al., 1980; Wigaeus et al., 1983; Engstrom et al., 1978). However, Engstrom et al. (1978) also reports that physical exercise reduces styrene elimination time considerably. Estimated uptake ranged from 78-125 mg/hour at rest to ~420 mg/hour with strenuous exercise on a stationary bicycle using a mouthpiece and breathing valve.

The pharmacokinetics of inhaled styrene has been investigated in rats at concentrations higher than those used in humans. Anderson et al. (1984) exposed adult male F344 rats to 100, 200, 401 or 799 ppm (426, 852, 1710 or 3400 mg/m^3) for 6 hours, measured arterial concentrations of styrene and estimated rates of uptake. Rates of uptake were 6.13, 12.04, 21.12 and 39.26 mg/kg/hour, respectively. The rate of uptake was somewhat dependent on the rate of metabolism; pretreatment with phenobarbital or previous exposure to styrene induced the metabolism of styrene and increased the rate of uptake. Administration of pyrazole inhibited both metabolism and the rate of uptake.

3. TOXICITY IN HUMANS AND EXPERIMENTAL ANIMALS

3.1. SUBCHRONIC

3.1.1. Oral. Several subchronic oral studies with styrene have been performed using mice, rats and dogs, and these experiments have been reviewed in two recent U.S. EPA (1984b, 1988) analyses. For the purpose of this document, only those studies >90 days in length are discussed. Ponomarkov and Tomatis (1978) administered a single 1350 mg/kg dose of styrene in olive oil by gavage to 29 pregnant 0_{20} mice on day 17 of gestation. Weaned offspring were treated once weekly with 1350 mg/kg (192.86 mg/kg/day). Vehicle-treated controls consisted of nine pregnant females and their offspring, which were treated for life. Untreated controls were also maintained. Treatment with styrene was halted after 16 weeks because of overt toxicity and high mortality in the treated mice compared with controls. At 20 weeks, mortality had reached 50% in treated males and 20% in treated females; survival was ~100% in untreated controls. The liver was the most common site of lesions in dead mice, suggesting that this was an important target organ; multiple centrilobular liver necrosis was common. Common lesions in other organs included hyperplasia of the spleen and severe lung congestion.

In an investigation of the effects on neurotransmitter function in the corpus striatum, Agrawal et al. (1982) administered styrene in peanut oil by gavage to six male 8-week-old albino rats at 0, 200 or 400 mg/kg/day for 90 days. There were no effects on rate of body weight gain or the weight of the corpus striatum. Binding of ^{3}H-spiroperidol to dopamine reception in the corpus striatum was increased, however, at both treatment levels; the authors felt this may indicate increased sensitivity of dopamine receptors because of the destruction of dopamine neurons as a result of exposure to styrene.

To further investigate the effects on the liver, Srivastava et al. (1982) administered styrene in ground nut oil to groups of five young adult male albino rats at 0, 200 or 400 mg/kg, 6 days/week (0, 171.43 or 342.86 mg/kg/day) for 100 days. There were no statistically significant effects on rate of body weight gain or liver weights. Significant alterations occurred in a number of mitochondrial and microsomal drug metabolizing enzyme activities. Activity of benzo[a]pyrene hydroxylase and aminopyrene-N-demethylase increased and glutathione-S-transferase decreased, all in a dose-related manner. Mitochondrial succinic dehydrogenase and ß-glucuronidase activities were decreased at both treatment levels and acid phosphatase activity was decreased at 400 mg/kg (342.86 mg/kg/day). Glucose-6-phosphatase activity was unaffected. Evidence of liver damage, including elevated SGOT and SGPT activities and focal necrosis (incidence not reported), was observed at 400 but not at 200 mg/kg.

Results of an earlier study indicate that female rats may be more resistant than males to the effects of styrene on the liver. Wolf et al. (1956) administered styrene in a vehicle of olive oil and gum arabic to groups of 10 female Wistar derived rats at 66.7, 133, 400 or 667 mg/kg, 5 days/week (47.64, 95.64, 285.71 or 476.43 mg/kg/day) for 6 months. The rats were ~2 months old at the start of treatment. A group of 20 vehicle-treated females was maintained as controls. Growth depression and increased liver and kidney weights were noted at 400 and 667 mg/kg (285.71 and 476.43 mg/kg/day), but there was no histopathological evidence of liver damage and no effects on hematology at either of these levels. No adverse effects of any kind were observed at 66.7 or 133 mg/kg (47.64 or 95.64 mg/kg/day).

A dog study by Quast et al. (1979) clearly defines the NOAEL and LOAEL for oral toxicity of styrene. These data have also been summarized in an

abstract (Quast et al., 1978). Purebred beagle dogs (4/sex/group) were administered styrene in peanut oil to beagle dogs (sex, number and age not specified) at 0, 200, 400 or 600 mg/kg/day for 560 consecutive days. There were no treatment-related effects on body weight, organ weights, urinalysis or clinical chemistries (serum urea nitrogen, SGPT, SGOT and SAP) at any level. Hematological effects included decreased packed RBC volume at 400 and 600 mg/kg/day and a dose-related increase in the presence of Heinz bodies in the RBCs at $\geq$400 mg/kg/day. Histopathological changes, observed only in the liver, included increased iron deposits in the reticuloendothelial cells at $\geq$400 mg/kg/day and increased numbers of acidophilic crystalline intranuclear inclusions in the hepatocytes at 600 mg/kg/day. No effects were noted at 200 mg/kg/day except for one dog that had slightly increased iron deposits in the liver and sporadic low-level occurrence of Heinz bodies in RBCs. The 200 mg/kg/day level was considered a NOAEL and 400 mg/kg/day a LOAEL for liver and hematological effects in dogs in this study.

3.1.2. Inhalation. Several subchronic inhalation studies have been performed with styrene in many laboratory species. These studies have been extensively reviewed by U.S. EPA (1984b, 1988) and are briefly summarized in Table 3-1. Several studies using rats exposed to 300 ppm, 6 hours/day, 5 days/week for 28-119 days or 145.41 mg/kg/day indicated transient biochemical alterations (Vainio et al., 1979; Savolainen and Pfaffli, 1977; Savolainen et al., 1980) or changes in nerve conduction velocity (Seppalainen, 1978) of questionable biological significance. Of greater significance is the observation of histopathological alterations in the liver after exposure for 2 weeks to the above described protocol (Vainio et al., 1979).

TABLE 3-1

Subchronic Inhalation Studies of Styrene

Species/Strain	Sex/Number	Age or Weight	Concentration (mg/kg/day)	Exposures	Duration (days)	Effects	Reference
Rats/Wistar	M/40	adult	145.41	6 hours/day 5 days/week	77	Lung and liver glutathione levels depressed initially. Mixed function oxidase system enhanced in liver and kidney after 2 weeks. Other changes in hepatic and renal enzyme activities. Histological liver alterations after >2 weeks.	Vainio et al., 1979
Rats/NR	NR/20, 15 control	young adult	254.47*	6 hours/day 5 days/week	77	Motor conduction velocity of tail nerve significantly higher ($p<0.05$) after 6 weeks, but not after 8 or 11 weeks.	Seppalainen, 1978
Rats/Wistar	M/40	adult	145.41	6 hours/day 5 days/week	77	Transient increase in serum creatine kinase activity and transient decrease in serum cholesterase. After >9 weeks, significant changes in enzyme activity in the brain, decreased protein content and increased RNA content in the brain.	Savolainen and Pfaffli, 1977
Rats/Wistar	M/26	adult	145.41	6 hours/day 5 days/week	28-119	No effect on behavior. Glial acid proteinase decreased significantly only at 4 weeks.	Savolainen et al., 1980
Rats/Wistar	M&F/50, 28	NR	840.15, 1242.53	7-8 hours/day 5 days/week	148,214	Eye and nose irritation at 1300, weight gain depression at 2000 ppm.	Spencer et al., 1942; Wolf et al., 1956
Guinea pigs/ heterogeneous	M&F/12-94	NR	313.96, 627.91, 966.02	7-8 hours/day 5 days/week	148-214	No effect at 650 ppm. 10% mortality after a few exposures to 1300 ppm from acute lung irritation. Slow weight gain in survivors. No apparent effect on survival at 2000 ppm, but weight gain depressed.	Spencer et al., 1942; Wolf et al., 1956
Rabbits/ heterogeneous	M&F/12, 2	NR	694.01, 1067.71	7-8 hours/day 5 days/week	360, 148	No adverse effects reported.	Spencer et al., 1942; Wolf et al., 1956
Monkeys/rhesus	M&F/2, 2	NR	890.07	7-8 hours/day 5 days/week	$\leq$360	No adverse effects reported.	Spencer et al., 1942; Wolf et al., 1956

*Assumed young adult body weight of 200 g.

NR = Not reported

In earlier studies, rats, guinea pigs, rabbits and monkeys were exposed to 650, 1300 or 2000 ppm (2770, 5540 or 8520 mg/m^3), 7-8 hours/day, 5 days/week for 148-360 days (or 313.96, 627.91 or 966.02 mg/kg/day) (Spencer et al., 1942; Wolf et al., 1956). Rabbits and monkeys appeared to be least sensitive to styrene vapor as no adverse effects on weight gain, survival, gross or histological appearance of selected major organs and tissue or hematological parameters were observed. Rats experienced eye and nose irritation at ≥1300 ppm (840.15 mg/kg/day) and weight gain depression at 2000 ppm (1292.53 mg/kg/day). Histopathological examinations were not performed on rats. No effects on body weight gain, organ weights or gross appearance at necropsy were noted in guinea pigs exposed to 650 ppm (313.96 mg/kg/day). At 1300 ppm (627.91 mg/kg/day), mortality from acute lung irritation occurred in ~10% of the guinea pigs, and survivors gained weight slowly. Survival was unaffected in guinea pigs at 2000 ppm (966.02 mg/kg/day), but depressed rate of body weight gain was noted.

3.2. CHRONIC

3.2.1. Oral. Chronic oral experiments with styrene include a long-term study using rats by Ponomarkov and Tomatis (1978), the NCI (1979) bioassay on rats and mice and a 2-year drinking water study using rats (Belilies et al., 1985) (Table 3-2). In the Ponomarkov and Tomatis (1978) study, a single 1350 mg/kg dose was administered to 21 pregnant BD IV rats on gestation day 17, and 144 male offspring were treated from weaning up to 120 weeks with once weekly doses of 500 mg/kg (71.43 mg/kg/day). Body weight was unaffected by treatment. Several rats died at 50-60 weeks and exhibited small necrotic foci in the liver and moderate congestion of the lungs and kidneys. The liver lesions were not observed in rats that died at ≥80 weeks. Other common observations were lesions in the forestomach and hyperplasia of the epithelium of the renal pelvis.

TABLE 3-2

Chronic Studies of Styrene

Species/ Strain	No./Sex	Age/Weight	Concentration (mg/kg/day)	Exposure	Duration (days)	Effects	Reference
Rat/BD IV	21/F	17th day of gestation	1350 mg/kg	single gavage	1		Ponomarkov and Tomatis, 1978
	144/M	weanling to 840 days	71.4	once weekly gavage	840	Small necrotic foci in the liver and moderate congestion of the lungs and kidneys at 350-420 days. Liver lesions were not observed after 560 days. Common observations include lesions in the forestomach and hyperplasia of the renal pelvis epithelium.	
Rat/F344	50/M 50/F	0.35 kg (assumed)	35.7	gavage 5 days/week	385	Depression of mean body weights in males.	NCI, 1979
			714.3		546	Depression of mean body weights in males.	
			1428.6		546	Depression of mean body weights in males. High mortality of both sexes before 161 days.	
Mice/B6C3F1	50/M 50/F	0.03 kg (assumed)	107.1	gavage 5 days/week	175 +91-day observation period	Slight depression in mean body weights in females.	NCI, 1979
			214.3			Slight depression in mean body weights in females. Decreased survival in both sexes.	
Rat/Charles River CD BS(SD)BR	50/M 70/F	35 days	15.7	inhalation	730	Decreased water consumption. No changes in body weight, food consumption, clinical signs, survival, ophthalmic examination, hemograms, organ weights, or gross or histopathological appearance of many organs or tissues.	Bellies et al., 1985

TABLE 3-2 (cont.)

Species/ Strain	No./Sex	Age/Weight	Concentration (mg/kg/day)	Exposure	Duration (days)	Effects	Reference
Rat/Sprague-Dawley	96/M 96/F	M/0.565 kg F/0.384 kg	M/180.2 F/265.1 M/300.3 F/441.8 initially, M/360.3 F/530.1 first 2 months	Inhalation	M/549 F/621	Survival time reduced in high-dose and control males probably due to an outbreak of murine pneumonia. Initial mean body weight depression in treated males (up to 263 days) and in high-dose females (up to 506 days). Sporadic depression in absolute liver and kidney weights in treated males but increased absolute and relative liver weights in females. No effect seen on hematology, urinalysis or clinical chemistries. Minor lesions in the lungs were observed in treated females.	Jersey et al., 1978

NCI (1979) treated groups of 50 male and 50 female F344 rats by gavage at 1000 or 2000 mg/kg, 5 days/week (714.29 or 1428.57 mg/kg/day) for 78 weeks followed by a 27-week observation period. Controls consisted of 20 rats/sex. High mortality in high-dose rats of both sexes early in the course of treatment led to the establishment of another treatment group at 23 weeks that received 50 mg/kg (35.71 mg/kg/day assuming a similar treatment regimen) for 103 weeks followed by a 1-week observation period. Another group of concurrent controls was started at this time. A dose-related depression in mean body weights was apparent in all treated groups of males. An early and marked increase in mortality was observed in high-dose rats of both sexes. Hepatic necrosis was noted in several high-dose rats of both sexes and was considered to be related to the high mortality observed in this group.

NCI (1979) also treated groups of 50 male and 50 female B6C3F1 mice with 150 or 300 mg/kg, 5 days/week (107.14 or 214.29 mg/kg/day) for 28 weeks followed by a 13-week observation period. Concurrent vehicle-treated controls consisted of 20 mice/sex. A dose-related but very slight depression in mean body weights was observed in female but not male mice. Survival was decreased in high-dose mice of both sexes, and the Tarone test indicated a dose-related trend in decreased survival in males but not in females. Histopathological examination revealed no increase in nonneoplastic lesions in treated mice compared with controls.

In a combination chronic toxicity reproduction study (Belilies et al., 1985), groups of 50 male and 70 female 35-day-old Charles River CDBS(SD)BR rats were provided drinking water containing styrene at 125 or 250 ppm nominal concentrations for 2 years. Analysis of styrene in drinking water indicated that the average concentrations were 112 and 221 ppm (15.68 and 30.94 mg/kg/day). Controls consisted of 76 males and 106 females.

Treatment had no effect on body weight, food consumption, clinical signs, survival, ophthalmic examination (conducted at weeks 51 and 104), hemograms, organ weights, or gross or histopathological appearance of many organs and tissues. The latter three parameters were evaluated for 10 rats/sex/group at a 52-week interim sacrifice, at time of natural death or moribund sacrifice, or at termination. A dose-related decrease in water consumption was noted in both sexes in both treated groups.

3.2.2. Inhalation. Jersey et al. (1978) exposed Sprague-Dawley rats (96 of each sex) to styrene at 0, 600 or 1000 ppm (265.1 and 441.8 mg/kg/day for female rats and 180.2 and 300.3 mg/kg/day for male rats), 6 hours/day, 5 days/week for up to 18.3 months (549 days) for males and 20.7 months (621 days) for females. The high-dose rats were initially exposed to 1200 ppm (530.1 and 360.3 mg/kg/day for females and males, respectively), but the concentration was reduced to 1000 ppm at 2 months because of narcosis in the males. Interim sacrifices were performed at 6 months (5/sex/group) and at 12 months (6/sex/group); survivors were sacrificed at 24 months. Survival in control and high-dose males was markedly reduced, compared with the low dose, which was due primarily to an outbreak of murine pneumonia. A depression in mean body weights was noted in both groups of treated males during the first 263 days and in high-dose females for the first 506 days of treatment. Sporadic depressions in absolute liver and kidney weights were noted in treated males, but increased absolute and relative liver weights were observed in treated females. Neither sex had effects on hematology, urinalysis or clinical chemistries. No treatment-related histopathological changes were noted in males; minor lesions in the lungs occurred in both treated groups of females. Neoplastic changes in this experiment are discussed in Section 4.2.2.

3.3. TERATOGENICITY AND OTHER REPRODUCTIVE EFFECTS

3.3.1. Oral. Murray et al. (1976, 1978a) investigated the developmental toxicity of styrene administered by gavage to groups of 29-39 mated Sprague-Dawley rats at 0, 180 or 300 mg/kg/day on days 6-15 of gestation. Maternal toxicity was manifested as decreased body weight gain in both treated groups. There were no effects on mortality, pregnancy, implantation/dam, live or resorbed fetuses/litter or fetal body weights or crown rump lengths. In addition, examination of fetuses indicated no increase in the incidence of gross external, skeletal or soft tissue malformations.

As a part of the drinking water toxicity study described in Section 3.2.1., Beliles et al. (1985) performed a 3-generation reproduction study. The F_0 generation consisted of 10 male and 20 female rats exposed to 0, 125 or 250 ppm (17.5 or 35.0 mg/kg/day) that were mated after ~90 days treatment. Subsequent generations were obtained by mating rats at ~110 days of age. Only one litter/generation was produced. The F_0 parents were returned to the chronic study after weaning the F_1 offspring. Although statistically significant differences were observed sporadically in various reproductive parameters, no dose-related trends were evident and the investigators concluded that there were no treatment-associated effects on fertility. In addition, there were no effects on relative organ weights, histopathology or cytogenetics in any of the generations of offspring.

3.3.2. Inhalation. Tenuous data weakly associate exposure of pregnant women to styrene with an increased incidence of spontaneous abortion. Hemminki et al. (1980) analyzed the frequency of spontaneous abortion among ~9000 female chemical workers in Finland from 1973 to 1976. In the investigators analysis, the number of spontaneous abortions was related to the number of pregnancies (births + induced abortions + spontaneous abortions;

this is referred to as the rate of spontaneous abortion). The ratio of spontaneous abortions in each branch of the chemical industry refers to the number of spontaneous abortions related to the number of births. The rate of spontaneous abortions was 8.54% ($p<0.01$) in the Union of Chemical Workers with a total of 52 spontaneous abortions, and 15.0% ($p<0.01$) in the subgroup labeled "styrene production and use" with a total of 6 spontaneous abortions.

The control populations labeled "all women in Finland" had 15,482 spontaneous abortions or a 5.52% rate of spontaneous abortion. The ratio of spontaneous abortions in the Union of Chemical Workers and in the styrene industry were similarly elevated, 15.57% ($p<0.001$) and 31.59% ($p<0.001$), respectively, in relation to 7.98% in the control population.

In a smaller scale study, Harkonen and Holmberg (1982) analyzed the obstetrical histories of 67 plastics lamination workers exposed to styrene, and 67 age-matched textile and food processing workers. There were no significant differences in the number of pregnant women or in the incidence of spontaneous abortions between the groups.

Holmberg (1977) interviewed 43 Finnish mothers of children born with CNS defects and determined that two had been exposed regularly during pregnancy to styrene and a number of other chemicals in the reinforced plastics industry. The defects in the two chemical-exposed infants were anencephaly and hydrocephaly. Based on the Finnish fertility rate, the investigator estimated that ~12 births should have occurred among reinforced plastics working women during the study period and that, based on the national reported rate for anencephaly and hydrocephaly in the population as a whole, a 300-fold increase in the incidence of these defects had occurred. However, a very small sample size was evaluated.

Several developmental toxicity studies have been performed using laboratory animals exposed by inhalation (Table 3-3). A teratogenic response was not observed in rats (Murray et al., 1978a,b; Ragule, 1974), mice and hamsters (Kankaanpaa et al., 1980) or rabbits (Murray et al., 1978a,b). Fetotoxicity (increased dead and resorbed fetuses) was observed in hamsters at 1000 ppm (4260 mg/m^3) (988.96 mg/kg/day assuming a 7 day/week exposure regimen) 6 hours/day (Kankaanpaa et al., 1980) and in rabbits (marginal increase in the incidence of unossified fifth sternebrae) at 600 ppm (2556 mg/m^3) (392.38 mg/kg/day, assuming a 7 day/week exposure regimen) 7 hours/day (Murray et al., 1978a,b). Ragule (1974) reported resorptions in rats at 0.35 ppm (1.49 mg/m^3) (0.16 mg/kg/day) 4 hours/day throughout gestation, but this Russian study was not reported in sufficient detail to permit adequate review. Of the studies reviewed in Table 3-3, only Murray et al. (1978a,b) evaluated maternal toxicity. There was no evidence of maternal toxicity in rabbits at 600 ppm (392.38 mg/kg/day), but at 300 and 600 ppm (237.0 and 475.0 mg/kg/day) rats showed decreased food consumption and reduced body weight gain during days 6-9 of gestation. Exposures in both species were for 7 hours/day on days 6-15 of gestation.

3.4. TOXICANT INTERACTIONS

Styrene is rapidly metabolized and eliminated from the body. Toxicant interaction studies have focused on the effect of exposure to other chemicals or dietary modifications on the metabolism of styrene. Ikeda et al. (1972) and Ikeda and Hirayama (1978) noted that the simultaneous administration of toluene or trichloroethylene with styrene (both chemicals given by intraperitoneal injection or by inhalation) resulted in reduced excretion of urinary metabolites of styrene, compared with excretion following styrene administration alone. When given by the intraperitoneal route, the inhibitory effect of toluene was reduced by the coadministration of phenobarbital.

TABLE 3-3

Developmental Toxicity of Styrene by Inhalation to Laboratory Animals

Species/Strain	No. Dams at Start	Concentration or Dose (mg/kg/day)	Exposure Duration	Maternotoxic, Fetotoxic and Teratogenic Effects	Reference
Rats/Sprague-Dawley	29-30/group	0, 237.0, 475.0	7 hours/day, days 6-15 of gestation	Reduced maternal body weight gain and decreased food consumption at both exposures. Average fetal crown-rump length decreased significantly only at 300 ppm. The incidence of skeletal variations was significantly higher in the 300 ppm group only, but the incidences were consistent with the range of incidences in historical controls. No significant differences in the incidences of malformations.	Murray et al., 1978a,b
Rats/NR	NR	0.158, 0.543 and 5.43	4 hours/day, throughout gestation	Resorption reported at all exposure levels. No significant increase in the number of malformations.	Ragule, 1974
Mice/BMR/T6T6	NR	0, 346.14	6 hours/day, days 6-16 of gestation	Embryotoxicity: increased ($p<0.1$) incidences of dead or resorbed fetuses. The number of live fetuses/litter was not significantly altered. No teratogenic effects were observed. A higher percentage of fetuses with minor skeletal abnormalities.	Kankaanpaa et al., 1980
Hamsters/Chinese	NR	0, 296.69, 494.48, 741.72, 988.96	6 hours/day days 6-18 of gestation	A significantly ($p<0.001$) greater number of dead or resorbed fetuses at 1000 ppm. The number of live fetuses/litter at 1000 ppm (2.4) was not significantly different from the control (5.3). No malformations were reported in any of the hamsters.	Kankaanpaa et al., 1980
Rabbits/New Zealand	20/group	0, 196.19, 392.38	7 hours/day, days 6-15 of gestation	No significant effects were observed on maternal toxicity. No significant effects on the mean incidence of implantation/litter, live fetuses/litter or resorptions/litter, or mean fetal body weight or crown-rump length. Gross external and cleft palate examinations of all fetuses and gross visceral examinations of 1/3 of the fetuses/litter revealed no malformed fetuses in any groups. There was a significant ($p=0.049$) increase in the incidence of unossified fifth sternebrae among litters of the 600 ppm group, but within the range of historical controls.	Murray et al., 1978a,b

NR = Not reported

In an in vitro system, Sato et al. (1980, 1981) observed that microsomes from rats exposed continuously to low levels of ethanol in the drinking water for 3 weeks before sacrifice metabolized styrene more rapidly than did microsomes from control (no ethanol) rats. Removal of ethanol for as little as 24 hours before sacrifice resulted in a loss of the effect on microsomal enzyme induction. When single graded doses of ethanol were given by gavage 18 hours before sacrifice, maximum enzyme induction occurred at 4 g/kg and less enzyme induction was noted at 5 g/kg. The enzyme inducing effects of ethanol appear to be dose-dependent until a point of diminishing returns is reached. Incubation of control microsomes with added ethanol resulted in depressed styrene metabolism, suggesting that the accelerated metabolism observed with microsomes from ethanol-treated rats was due to enzyme induction rather than to the presence of alcohol in the incubation system.

Nakajima et al. (1982) investigated the effects of dietary changes on the ability of rat microsomes to metabolize styrene. Decreased food intake and decreased dietary sucrose content increased microsomal metabolism of styrene. Similar results were obtained with a high protein, high fat diet free of carbohydrates.

4. CARCINOGENICITY

4.1. HUMAN DATA

4.1.1. Oral. Pertinent data regarding the carcinogenicity of styrene to humans by the oral route could not be located in the available literature.

4.1.2. Inhalation. A number of epidemiological investigations of workers exposed to styrene have been performed to determine the association of occupational exposure with cancer (Lemen and Young, 1976; Block, 1976; Hodgson and Jones, 1985; McMichael et al., 1976; Meinhardt et al., 1978, 1982; Ott et al., 1980; Nicholson et al., 1978; Frentzel-Beyme et al., 1978; Hardell et al., 1981). These studies generally are limited by small cohort size, poorly quantitated duration and intensity of exposure, exposure to multiple chemicals and the presence of the healthy worker effect. These studies have been reviewed in detail in several recent U.S. EPA (1984b, 1988) analyses, and it is beyond the scope of this document to repeat that effort here. Only the conclusions from these analyses are presented.

The National Institute for Occupational Safety and Health (NIOSH, 1976) held a briefing on April 30, 1976 to review the hazards of styrene-butadiene production. Five cases of leukemia in a B.F. Goodrich styrene-butadiene rubber plant were described by Lemen and Young (1976). The leukemias were of diverse histologic types and there was no apparent association with a specific job classification. Three cases of leukemia in workers in a U.S. chemical synthetic rubber plant were also described (Lemen and Young, 1976).

Block (1976) described a study of six chemical plants in the Kentucky area including one synthetic rubber plant. Of the 72 death certificates obtained for workers employed between 1950 and 1975, leukemia was indicated as the cause of death in two cases and Hodgkin's disease was the cause in another two cases. An additional leukemia death was reported for a worker

in 1976 (after the study cutoff date). There were three deaths from leukemia reported in the other five chemical plants with no styrene or butadiene exposure. IARC (1979) reviewed these reports and concluded the data were inadequate to indicate an association between an increased risk of leukemia and styrene or butadiene exposure; however, further study was justified.

Hodgson and Jones (1985) conducted a mortality study of 622 men who worked for at least 1 year in the production, polymerization and processing of styrene at a chemical site in the United Kingdom during the period 1945-1974. This included 131 men who were potentially exposed to styrene among other chemicals in laboratories and 491 individuals who would have had mixed chemical exposures but had specific potential exposure to styrene in the production of styrene monomer, the polyerization of styrene, or the manufacture of finished products. A significant elevation of lymphoma deaths in the exposed group was reported. However, the number of lymphoma deaths was small and the workers were exposed to other chemicals in addition to styrene.

McMichael et al. (1976) conducted a retrospective cohort study of 6678 male workers in a tire manufacturing plant. Workers were placed in one of six categories based on job classification; only 2-3% were exposed to styrene-butadiene for >2 years. Risk ratios for deaths from lymphatic and hematopoietic cancer, lymphatic leukemia and stomach cancers were calculated to be 6.2, 3.9 and 2.2, respectively. Although the risk ratio for lymphatic and hematopoietic cancer was statistically significant, it was based on only four deaths, the biological significance of which is unclear.

The most extensive investigation of styrene in the workplace was by Meinhardt et al. (1978) concerning two styrene-butadiene rubber factories

(plant A and plant B). Mean styrene concentration in plant A was 0.94 ppm (4.0 mg/m^3) (0.272 mg/kg/day assuming 8-hour workdays, 5 days/week, 70 kg body weight and 20 mg/m^3 breathing rate) and in plant B was 1.99 ppm (0.577 mg/kg/day assuming 8-hour workdays, 5 days/week, 70 kg body weight and 20 m^3/day breathing rate); butadiene was also present at substantially greater concentrations. These concentrations probably do not reflect past exposure because changes in the manufacturing processes were expected to have reduced concentrations. In plant A, 9 deaths from neoplasms of the lymphatic and hematopoietic tissues in 252 total deaths resulted in an SMR of 155. Reevaluation using a subgroup of white males exposed before changes were made in the manufacturing process, resulting in reduced exposure, resulted in an SMR for 212 for overall lymphatic and hematopoietic neoplasms and an SMR of 278 for leukemia and aleukemia. In plant B, an SMR of 78 was calculated for neoplasia of lymphatic and hematopoietic tissue, which was attributed to the healthy worker effect. The investigators noted that the SMRs calculated for plant A were not statistically significant and that, because of the high background incidence of leukemia in the general population, the observed incidence would have to be ~4 times larger than the expected incidence to be statistically significant using the consecutive two-sided test ordinarily used by NIOSH. Applying the one-sided test, the mortality from leukemia and aleukemia for plant A was marginally significant ($p \leq 0.05$) and the authors concluded that the findings "suggested" an association between styrene-butadiene exposure and mortality from lymphatic and hematopoietic cancers.

Ott et al. (1980) reported the results of a retrospective cohort mortality study of Dow Chemical Co. plants involved in the manufacture of styrene products. Statistically significant increases in incidences of leukemia (6

observed, 1.6 expected) and lymphatic leukemia (4 observed, 0.5 expected) were noted. The largest SMR was determined for workers in one category exposed to styrene, polystyrene dust, ethylbenzene, oligomers of styrene, inorganic colorants and various solvents.

Nicholson et al. (1978) studied a cohort of 560 male workers exposed for at least 5 years in a styrene-polystyrene factory. For 116 workers, exposure concentration was <1 ppm (<4 mg/m^3) (<0.29 mg/kg/day, making usual assumptions); for the rest of the workers, exposures were generally 5-20 ppm (20-85 mg/m^3) (1.45-5.79 mg/kg/day, making usual assumptions) with some wide variations. Unspecified levels of benzene and ethylbenzene were also present. For all causes of death, including cancer, observed deaths were fewer than expected, which was attributed to the healthy worker effect. There appeared to be no differences between high and low exposure groups. Although there were no specific increases in cause-specific morlality, the incidence of leukemia (5/104 deaths) indicated to the investigators a need for further study.

The healthy worker effect was also evident in data from a styrene-polystyrene factory in Germany. In this study of 1960 workers divided into those employed before and after plant modernization reduced exposures, fewer total deaths occurred than were expected in the exposure groups (Frentzel-Beyme et al., 1978). There were also fewer cancer-related deaths than expected, although the incidences of some rare tumors were increased sporadically in single age groups. These increases were not associated with extent or duration of exposure and were considered to be artifacts.

Hardell et al. (1981) conducted a matched case-control study of males aged 25-85 years with malignant lymphomas who were admitted to the Department of Oncology in Unea in a 4-year period. Exposure histories were

obtained by questionnaire. An elevated relative risk (4.6; 95% confidence limits 1.9-11.4) was noted for a category including exposure to styrene, benzene, trichloroethylene and perchloroethylene.

The U.S. EPA (1984b, 1988) noted the limitations of these epidemiology studies and declined to draw conclusions regarding the carcinogenicity of styrene to humans.

4.2. BIOASSAYS

4.2.1. Oral. Oral cancer bioassays with styrene include the NCI (1979) study using rats and mice, a 1-year drinking water study using rats (Belilles et al., 1985) and a long-term study in which the offspring of two strains of mice and one strain of rats from treated dams were treated once weekly for life (Ponomarkov and Tomatis, 1978). In the NCI (1979) experiment, (see Section 3.2.1.), rats were given styrene at 500, 1000 or 2000 mg/kg, 5 days/week for 78-103 weeks (357.14, 714.29 or 1428.57 mg/kg/day). Early mortality in high-dose rats resulted in inadequate numbers in these groups at risk for late developing tumors. Adequate numbers of rats survived in the low and middle groups, however. No tumor incidence was significantly elevated in any of these groups compared with controls, nor did time to tumor appear to be shortened for any tumor type.

Mice were treated at 150 or 300 mg/kg, 5 days/week for 78 weeks (107.14 or 214.29 mg/kg/day) followed by a 13-week observation period (NCI, 1979). Although survival was reduced in high-dose mice of both sexes, adequate numbers survived at risk for late developing tumors. No tumor type was significantly increased in treated female mice compared with controls. In male mice, a dose-related increase in combined alveolar/bronchiolar carcinomas and adenomas was noted (0/20 concurrent controls, 6/44 low group, 9/43 high group), which was significant for trend by the Cochran-Armitage

test (p=0.023) and significant at the high group by the Fisher Exact test (p=0.024). NCI (1979) noted an incidence of combined adenomas and carcinomas of the lung in historical untreated controls of 32/271 (12%) and concluded that the results of this experiment "suggested that the administration of styrene may have been associated with the increased combined incidence of alveolar/bronchiolar adenomas or.... carcinomas in male mice..."

As part of a chronic toxicity reproduction study, Belles et al. (1985) treated groups of 76 male and 106 female rats with 125 or 250 ppm (17.5 or 35.0 mg/kg/day) styrene in the drinking water for 2 years (see Section 3.2.1.). Survival was unaffected and sufficient for observation of late-developing tumors. Comprehensive gross and histopathological examination performed at interim sacrifice (10/sex/group at 52 weeks), maternal death or moribund sacrifice or at termination yielded no evidence of carcinogenicity. It did not appear that the MTD had been reached in this study, since there were no effects on mortality, body weight or clinical signs.

Ponomarkov and Tomatis (1978) administered single doses of styrene (99% pure) in olive oil to 29 female O_{20} mice at 1350 mg/kg, to 15 females C57B1 mice at 300 mg/kg and to 21 female BD IV rats at 1350 mg/kg on day 17 of gestation. Following weaning, the offspring of the C57B1 mice were treated once weekly with 300 mg/kg (42.86 mg/kg/day) and the offspring of the rats were treated once weekly with 1350 mg/kg (192.86 mg/kg/day) for life. Offspring of the O_{20} mice were treated once weekly with 1350 mg/kg (192.86 mg/kg/day), but treatment was terminated at 16 weeks because of overt toxicity and early mortality. Vehicle-treated progeny controls consisted of 42 O_{20} and 25 C57B1 mice and 75 BD IV rats; untreated controls were also maintained.

Early deaths occurred among the 45 male and 39 female styrene-treated offspring of the O_{20} mice (average age of death was 32 weeks for males and 49 weeks for females compared with 88 and 85 weeks for males and females, respectively, in the oil-treated controls). Nevertheless, a significant increase in the incidence of lung tumors classified as adenomas and carcinomas was observed for both sexes ($p<0.02$ for males and $p<0.001$ for females, calculated from study results using Fisher's exact test) when compared with the olive oil-treated controls. Characteristically, O_{20} mice have a high spontaneous rate of lung adenomas and carcinomas; in this study, the concurrent control incidence was 42%-67% (male and female) with an age at tumor onset ranging from 53-57 weeks. The lung tumor rates (based on the survivors at the time the first tumor was observed) were 20/23 and 32/32 in treated males and females, respectively, compared with 8/19 and 14/21 in male and female vehicle-treated controls, and 34/53 and 25/47 in male and female untreated controls, respectively. Lung tumors were observed in mice dying at an earlier age in the treated group as compared with the controls. The female treated mice had a much higher ratio of carcinomas to adenomas than the vehicle controls (1.3 treated compared with 0.4 in the vehicle controls) but a similar ratio to the untreated controls, perhaps suggesting vehicle related inhibition of carcinogenesis. The high spontaneous background rate of lung tumors in O_{20} mice raises a question as to how to interpret this increase in tumor incidence. The observed significant increase in tumor incidence cannot be dismissed, therefore, and is thought to be of a highly suggestive nature given the high statistical significance of the response and the reduced latency period for tumor induction in terms of indicating a tumorigenic potential.

In C57B1 mice a slight, but insignificant, increase in liver carcinomas was observed in treated animals (3/24). Although this increase is not statistically significant when compared with male vehicle controls (0/12), it is statistically significant (p=0.022 calculated from study results using Fisher's exact test) when compared with untreated controls (0/47) and vehicle controls combined. Pooling both control groups is acceptable because there is no difference between their tumor responses. No significant tumor-related effects were observed in BDIV rats.

4.2.2. Inhalation. Jersey et al. (1978) exposed groups of 85 male and 85 female Sprague-Dawley rats to atmospheres containing styrene at 0, 600 or 1200 ppm (0, 2560 or 5110 mg/m^3), 6 hours/day, 5 days/week (0, 290.82 or 581.64 mg/kg/day) for ~2 years. After 2 months, the concentration in the high group was reduced to 1000 ppm (4260 mg/m^3, 484.7 mg/kg/day) because of narcosis in the males. Exposures of each sex were terminated when mortality reached 50% for that sex in either test group (18.3 and 20.7 months for males and females, respectively). Survivors were sacrificed at 24 months. High mortality from murine pneumonia in control and 1000 ppm (484.7 mg/kg/day) males precluded reliable interpretation of tumor incidence data in males. In females, statistically significant increases in tumor incidences included grossly observed ovarian tumors at 1000 ppm (484.7 mg/kg/day) and mammary adenocarcinomas at 600 ppm (290.82 mg/kg/day). The incidence of grossly visible ovarian tumors was 0/85 in controls and 5/85 at 1000 ppm (484.7 mg/kg/day), but microscopic examination resulted in a reduction in incidence to 3/85 in the high group. The incidence of mammary adenocarcinomas was 1/85 in controls and 7/85 at 600 ppm (290.82 mg/kg/day), but the investigators noted the incidence in concurrent controls was unusually low compared with historic controls. A more biologically significant

observation was the combined incidence of leukemia and lymphosarcomas: 1/85 (1.18%) in controls, 6/85 (7.06%) at 600 ppm and 6/85 (7.06%) at 1000 ppm (484.7 mg/kg/day). Although the incidence in either treated group was not significantly elevated above concurrent controls, marginal significance (p=0.04, Fisher Exact test) was obtained when data from the treated groups were combined. The investigators presented the incidence data for leukemia-lymphosarcoma in historic controls. A total of 11 cases occurred in 808 (1.36%) female Sprague-Dawley rats that had been part of 10 other experiments. The incidence in controls in these experiments ranged from 0-2.64%. When the incidence in either treated group (6/85) is compared with that of historic controls, the results are statistically significant by the Fisher Exact test (p=0.0033, analysis at SRC).

4.3. OTHER RELEVANT DATA

Maltoni et al. (1982) investigated the ability of styrene to induce brain tumors in rats exposed by gavage or inhalation for 52 weeks. In these experiments, groups of 40 male and 40 female Sprague-Dawley rats were treated with styrene in olive oil at 0, 50 or 250 mg/kg, 4-5 days/week (0, 35.71 or 178.57 mg/kg/day assuming 5 days/week), or other similar groups were exposed to 0, 25, 50, 100, 200 or 300 ppm (0, 107, 213, 426, 852 or 1278 mg/m^3), 4 hours/day, 5 days/week (0, 8.08, 16.16, 32.31, 64.63 or 96.94 mg/kg/day). The rats were examined at the time of spontaneous death. In neither study did the incidence of grossly or histologically identified brain tumors in treated rats significantly exceed the incidence in controls.

Styrene oxide, a metabolite of styrene, has been tested for carcinogenicity by gavage in rats and mice (NTP, 1986). The results of this bioassay have not yet been published, but an unpublished report from the contracting laboratory (Lijinsky, n.d.) indicates that styrene oxide was

associated with stomach tumors in both rats and mice. In an earlier gavage study with styrene oxide, a dose-related and highly significant increase in the incidence of carcinomas of the forestomach was observed in rats treated with 50 or 250 mg/kg, 4-5 days/week (35.71 or 178.57 mg/kg/day, assuming 5 days/week) for 52 weeks and observed up to week 156 (Maltoni et al., 1979).

Styrene oxide was negative for skin tumors (Weil et al., 1963; Van Duuren et al., 1963) and for skin and lung tumors (Kotin and Falk, 1963) in mouse skin painting studies. In the last study, however, malignant lymphoma occurred in 3/20 (16%) of C3H mice painted with a total of 20 μm styrene oxide. Further information was not available.

Styrene and some of its metabolites have been tested for mutagenicity in several prokaryotic, eukaryotic and mammalian systems. The most comprehensive review and analysis of these studies is U.S. EPA (1988), from which the following generalizations are made. Styrene has been uniformly negative in in several strains of Salmonella typhimurium without metabolic activation, but both positive and negative results were obtained with metabolic activation (Busk, 1979; Milvy and Garro, 1976; De Flora, 1981; Stoltz and Withey, 1977; Loprieno et al., 1978; De Meester et al., 1977, 1981; Vainio et al., 1976; Poncelet et al., 1980; Simmon et al., 1977). Styrene oxide, on the other hand, yielded consistently positive results in S. typhimurium strains TA1535 and TA100 with or without metabolic activation (De Meester et al., 1977, 1981; Busk, 1979; De Flora, 1981; Glatt et al., 1975; Loprieno et al., 1978; Milvy and Garro, 1976; Vainio et al., 1976; Drinkwater et al., 1978). Negative results were obtained with styrene in several forward mutations and a gene conversion test in yeast (Loprieno et al., 1976; Bauer et al., 1980). Results in forward mutation tests in V79 human lymphocytes were positive only in the presence of metabolic activation (Loprieno et al., 1976; Beije

and Jenssen, 1982). Positive results were obtained for recessive lethal mutations in Drosophila melanogaster (Donner et al., 1979). In this test, metabolic induction with phenobarbital increased the frequency of mutation. Styrene oxide was positive in many mutation tests in eukaryotes (Loprieno et al., 1976; Sugiura et al., 1979; Beije and Jenssen, 1982; Amacher and Turner, 1982; Donner et al., 1979).

Mixed positive and negative results were reported for clastogenic effects of styrene in several in vitro and in vivo tests in mammalian systems (Matsuoka et al., 1979; Ishidate and Yoshikawa, 1980; Linnainmaa et al., 1978a,b; de Raat, 1978; Norppa et al., 1980a,b, 1981; Meretoja et al., 1978a; Conner et al., 1979, 1980, 1982). Generally, metabolic activation appeared to be required in the in vitro systems. Styrene oxide yielded positive and dose-related clastogenic results in in vitro systems (de Raat, 1978; Norppa et al., 1980a; Linnainmaa et al., 1978a,b) and metabolic activation actually decreased the intensity of the effect (de Raat, 1978). Styrene oxide did not produce clastogenic effects in in vivo systems (Fabry et al., 1978; McGregor, 1981; Norppa et al., 1979).

U.S. EPA (1988) also summarized several studies in which peripheral lymphocytes from workers exposed to styrene were examined for chromosomal damage (Meretoja et al., 1977, 1978b; Fleig and Thiess, 1978; Andersson et al., 1980; Camurri et al., 1983; Hogstedt et al., 1979; Watanabe et al., 1981; Thiess et al., 1980). Several investigators reported positive effects, particularly for concentrations in the workplace $\geq$50 ppm (213 mg/m^3) ($\geq$14.49 mg/kg/day assuming 8-hour workdays, 5 days/week). WHO (1983) evaluated these studies and concluded that the biological significance of these clastogenic effects is unknown.

4.4. WEIGHT OF EVIDENCE

Human epidemiological data are inadequate to either confirm or refute the carcinogenic activity of styrene; however, the results of three chronic animal bioassays (Jersey et al., 1978; NCI, 1979; Ponomarkov and Tomatis, 1978) collectively provide sufficient animal evidence. Strong supporting evidence is provided by metabolic and genotoxicity studies, some of which have been published recently. When the animal bioassay data are considered collectively along with the metabolism/genotoxicity data, there is a reasonable basis for classifying styrene as having a "sufficient" level of evidence and therefore as a Group B2 chemical using EPA's Guidelines for Carcinogen Risk Assessment. The guidelines provide several avenues for reaching a sufficient level of animal evidence. In this analysis, the bioassay data alone were considered to be strong enough for at least a "marginal" call of sufficient animal evidence.

The classification of marginally-sufficient animal evidence comes from animal bioassays showing statistically significant increased tumor incidences in the B6C3l male mouse (alveolar/bronchiolar adenomas and carcinomas by multiple dose gavage) (NCI, 1979), in male and female O_{20} mice (lung adenomas and carcinomas by dose gavage), in male C57Bl mice (liver carcinomas by gavage) (Ponomarkov and Tomatis, 1978), and in female Sprague-Dawley rats (leukemia/ lymphosarcoma, by inhalation) Jersey et al., 1978).

The guidelines encourage the use of additional considerations to support or limit the strength of the bioassay evidence. In the case of styrene, the evidence for genotoxicity in short-term animal test systems along with recent data showing tha styrene and its epoxide metabolite form DNA adducts and the epoxide has been detected in humans exposed to styrene is very

supportive of a carcinogenic potential. Equally important is the fact that the epoxide metabolite has been tested in rodents and found to be clearly carcinogenic in this biassay. These additional considerations together with the bioassay data give a collective weight of evidence in the sufficient category, Group B2.

5. REGULATORY STANDARDS AND CRITERIA

ACGIH (1986a,b) recommends a TWA-TLV for styrene monomer of 50 ppm (~215 mg/m^3) and an STEL of 100 ppm (~425 mg/m^3) based primarily on the association of styrene with lymphoid or hematopoietic tumors at 600 and 1000 ppm (290.82 and 484.7 mg/kg/day) in rats in the Jersey et al. (1978) 2-year inhalation experiment. OSHA recommended an 8-hour TWA of 100 ppm, an acceptable ceiling concentration of 200 ppm (850 mg/m^3) and an acceptable maximum peak of 600 ppm (2560 mg/m^3) for $\leq$5 minutes in any 3-hour period (OSHA, 1985).

NAS (1977) derived an ADI of 0.133 mg/kg/day based on the NOAEL of 133 mg/kg/day in the rat gavage study by Wolf et al. (1956). An uncertainty factor of 1000 was applied and a SNARL of 0.9 mg/ℓ was estimated. U.S. EPA (1988) derived 1-day HAs for ingestion of styrene in drinking water of 22.5 mg/ℓ for a 10 kg child based on a NOAEL of 22.5 mg/kg/day from a human inhalation study (Stewart et al., 1968). No data were identified in the available literature that would be suitable for derivation of a 10-day HA. It was therefore recommended that the longer-term HA of 20 mg/ℓ for a child be adopted as the 10-day HA. A lifetime DWEL of 7 mg/ℓ was based on the NOAEL of 200 mg/kg/day for liver effects in dogs treated by gavage for 560 consecutive days (Quast et al., 1979). An uncertainty factor of 1000 was applied. The same data and uncertainty factor were used by the U.S. EPA (1984b) to derive an RfD of 0.2 mg/kg/day or 14 mg/day for a 70 kg human. This RfD is available on IRIS (U.S. EPA, 1985a).

U.S. EPA (1988) also derived a q_1^* of 3×10^{-2} $(mg/kg/day)^{-1}$ for oral exposure to styrene based on the gavage study in mice by NCI (1979).

The derivation of this potency factor is described more completely in Section 6.3. This assessment is available on IRIS (U.S. EPA, 1987). In addition, an inhalation unit risk of $6 \times 10^{-7} (\mu g/m^3)^{-1}$ based upon the Jersey et al. (1978) rat inhalation study is also described.

6. RISK ASSESSMENT

6.1. SUBCHRONIC REFERENCE DOSE (RfD_S)

Styrene has been shown to be carcinogenic to laboratory animals by both oral and inhalation exposure. RfD_S values, therefore, are not derived.

6.2. REFERENCE DOSE (RfD)

As noted in Chapter 5, recent agency analyses (U.S. EPA, 1984b, 1985a) have derived an RfD for oral exposure to styrene of 0.2 mg/kg/day or 14 mg/day for a 70 kg human from a NOAEL in a 560-day gavage study using dogs (Quast et al., 1979). Because styrene has been identified as a carcinogen, the RfD value previously derived by U.S. EPA is not adopted as the RfD for the purposes of this document. Instead, oral and inhalation cancer potencies are presented in Section 6.3.

6.3. CARCINOGENIC POTENCY (q_1^*)

6.3.1. Oral. Ponomarkov and Tomatis (1978) administered styrene by olive oil gavage to female O_{20} mice, C56Bl mice and BDIV rats once on the 17th day of gestation and then weekly throughout their offspring's lifetimes. A statistically significant increased incidence and earlier onset of lung tumors were observed in the O_{20} offspring, but the high background tumor rate (up to 67%) in this strain makes it unsuitable for a potency estimate. A few rare tumors were observed in the BDIV offspring, but the data are too sketchy for a reliable potency estimate. In the C57Bl mice, a slight but insignificant increase in liver carcinomas was observed in treated animals (3/24). Although this increase is not statistically significant when compared with male vehicle controls (0/12), it is statistically significant ($p=0.022$ calculated from study results using Fisher's Exact Test) when compared with male untreated controls (0/47) and vehicle controls combined.

Pooling both control groups is acceptable because there is no difference between their tumor responses. Details of this study are summarized in Table 6-1.

NCI (1979) administered styrene at levels of 150 or 300 mg/kg (107.14 or 214.29 mg/kg/day) by corn oil gavage to B6C3F1 mice. Exposure was terminated after 78 weeks, and the study was terminated after 91 weeks. Statistically significant increased incidences of lung alveolar/bronchiolar adenomas or carcinomas were observed in both exposed groups with a statistically significant dose-response trend. This study is summarized in Table 6-2. The human slope estimate (q_1*) from this study is 3×10^{-2} (mg/kg/day). This slope estimate was chosen in both U.S. EPA (1987, 1988) documents to best characterize the oral carcinogenic potency of styrene. This selection was based upon the following considerations: that more than one exposure level was evaluated and that a dose-related trend in tumor incidence was observed. In addition, the other oral study (Ponomarkov and Tomatis, 1978) utilized a dosing regimen (one weekly dose) which is less appropriate to the prediction of effects of chronic daily exposure. The inhalation slope estimates support the estimate based upon NCI (1979). In this instance it is considered more appropriate to utilize the somewhat stronger study by the route of interest rather than attempting to derive a slope estimate based upon route extrapolation. In conclusion, the q_1* (slope) estimate of 3×10^{-2} $(mg/kg/day)^{-1}$ is proposed as currently the best estimate for the carcinogenic effects of oral exposure to styrene.

6.3.2. **Inhalation.** Jersey et al. (1978) observed a small increase in the incidence of leukemia and lymphosarcoma in female rats exposed for 20.1 months to styrene at 600 and 1000 ppm (2560 and 4260 mg/m^3) (265.1 and 441.8 mg/kg/day) styrene. Incidences were 1/85 (1.18%), 6/85 (7.06%) and 6/85 (7.06%) in concurrent control, low and high groups, respectively.

TABLE 6-1

Data Used for the Derivation of q_1^*

Compound: styrene

Reference: Ponomarkov and Tomatis, 1978

Species/strain/sex: mice/C57Bl/male

Route/vehicle: gavage/olive oil

Length of exposure (le) = 840 days

Length of experiment (Le) = 840 days

Lifespan of animal (L) = 840 days

Body weight = 0.03 kg (assumed)

Tumor site and type: liver hepatocellular carcinoma

Experimental Doses or Exposure (mg/kg)	Transformed Dose[a] (mg/kg/day)	Human Equivalent Dose[b] (mg/kg/day)	Incidence No. Responding/No. Tested
0	0	0	0/59
300	42.9	3.2	3/24

Human $q_1^* = 9 \times 10^{-2}$ $(mg/kg/day)^{-1}$

[a]TWA dose estimated by dividing single weekly dose by 7

[b]Estimated using a surface area approximation $(W_A/W_H)^{1/3}$

TABLE 6-2

Cancer Data Sheet for Derivation of q_1^*

Compound: styrene

Reference: NCI, 1979

Species/strain/sex: mouse/BCC3F1/male

Route, vehicle: gavage/corn oil

Length of exposure (le) = 91 weeks

Length of experiment (Le) = 78 weeks

Lifespan of animal (L) = 24 months

Body weight = 0.03 kg (assumed)

Tumor site and type: lung alveolar/bronchiolar adenoma/carcinoma

Dose (mg/kg)	Transformed Animal Dose[a] (mg/kg/day)	Human Equivalent Dose[b] (mg/kg/day)	Incidence No. Responding/No. Tested
0	0	0	0/20
150	107.1	8.1	6/44
300	214.3	16.2	9/43

Human $q_1^* = 3 \times 10^{-3}$ $(mg/kg/day)^{-1}$

[a]TWA dose, nominal dose was multipled by 5 days/7 days and 78 weeks/92 weeks

[b]Based on surface area approximation animal dose x $(W_A/W_H)^{1/3}$

The incidence in either treated group is not statistically significant when compared with concurrent controls. When compared with historic controls (incidence 11/808, 1.3%), however, the incidences in the treated groups are statistically significant by the Fisher Exact test (p=0.0033). This significance reflects the greater statistical power of the larger number of historic than concurrent controls, rather than a lower incidence in historic controls. Because the incidence in treated rats compared with historic controls is significant, it is appropriate to compute a q_1^* from data in the Jersey et al. (1978) study. The data used in computation are presented in Tables 6-3 and 6-4. Using the multistage model developed by Howe and Crump (1982), a q_1^* of 2×10^{-3} $(mg/kg/day)^{-1}$ is calculated. This corresponds to a unit risk for air of 6×10^{-7} $(\mu g/m^3)^{-1}$ by assuming a human ventilatory volume of 20 m^3/day, a body weight of 70 kg and complete absorption. This study (Jersey et al., 1978) may not be appropriate for low dose extrapolation because of pharmacokinetic constraints (U.S. EPA, 1987). This issue is being evaluated. In the interim, the slope estimate of 2×10^{-3} $(mg/kg/day)^{-1}$ and the unit risk for air of 6×10^{-7} $(\mu g/m^3)^{-1}$ appear on IRIS (U.S. EPA, 1987).

TABLE 6-3

Cancer Data Sheet for Derivation of q_1^*

Compound: styrene

Reference: Jersey et al., 1978

Species/strain/sex: rat/Sprague-Dawley/female

Route, vehicle: inhalation

Length of exposure (1e) = 20.7 months

Length of experiment (Le) = 24 months

Lifespan of animal (L) = 24 months

Body weight = 0.384 kg (control, at end of exposure period)

Tumor site and type: leukemia and lymphosarcoma

Experimental Doses or Exposure[a]	Transformed Animal Dose[b] (mg/kg/day)	Human Equivalent Dose[e] (mg/kg/day)	Incidence No. Responding/ No. Tested
0	0	0	1/85
600 ppm[c]	265.1	46.75	6/85
1000 ppm[d]	441.8	77.92	6/85

Human $q_1^* = 2 \times 10^{-3}$ $(mg/kg/day)^{-1}$

[a]Exposures were for 6 hours/day, 5 days/week over a 621-day period: 437/621 days.

[b]Transformed doses calculated by expanding to continous exposure, estimating a breathing rate for 0.384 kg rats from the expression [0.105 (body weight/0.113)$^{2/3}$] and expanding exposure to the full experimental period (30 hours/week/168 hours/week; 20.7 months/24 months).

[c]Mean measured concentration = 592 ppm (2522 mg/m^3)

[d]Mean TWA concentration 1007 ppm (4290 mg/m^3) based on 38 days at 1197 ppm and 399 days at 989 ppm.

[e]Transformed using a surface area adjustment $(W_A/W_H)^{1/3}$

TABLE 6-4

Cancer Data Sheet for Derivation of q_1^*

Compound: styrene

Reference: Jersey et al., 1978

Species/strain/sex: rat/Sprague-Dawley/male

Route, vehicle: inhalation

Length of exposure (le) = 24 months

Length of experiment (Le) = 18.3 months

Lifespan of animal (L) = 24 months

Body weight = 0.565 kg (control, at end of exposure period)

Tumor site and type: leukemia and lymphosarcoma

Experimental Doses or Exposure[a] (ppm)	Transformed Animal Dose[b] (mg/kg/day)	Human Equivalent Dose[c] (mg/kg/day)	Incidence No. Responding/ No. Tested
0	0	0	1/62
600	180.2	36.14	5/78
1000	300.3	60.23	1/78

Human $q_1^* = 1 \times 10^{-3}$ $(mg/kg/day)^{-1}$

[a]Exposures were for 6 hours/day, 5 days/week over 549 days out of a 24-month expermental duration.

[b]Breathing rate estimated as $[0.105\ (0.565\ kg/0.113)^{2/3}]$ and expanding for continuous exposure by multiplying by 30 hours/week/168 hours/week; 18.3 months/24 months.

[c]Transformed using a surface area adjustment $(W_A/W_H)^{1/3}$

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APPENDIX

Summary Table for Styrene

Route	Species/Strain/Sex	Experimental Exposure/Dose	Effect	q_1^* $(mg/kg/day)^{-1}$	Reference
Oral	mouse/B6C3F1/male	0, 150, 300 mg/kg 5 days/week for 78 weeks	lung alveolar/ bronchiolar adenomas and carcinomas	$3x10^{-2}$	NCI, 1979
Inhalation	rat/Sprague-Dawley/female	0, 600 or 1000 ppm 6 hours/day, 5 days/week over 20.7 months. Study was terminated at 24 months (0, 235 or 399 mg/kg/day)	leukemia and lymphosarcoma	$2.0x10^{-3}$†	Jersey et al., 1978

†$6x10^{-7}$ $(\mu g/m^3)^{-1}$