DRAFT

For Review Only

Public Health Goal for STYRENE in Drinking Water

Prepared by

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

February 2010

LIST OF CONTRIBUTORS

PHG PROJECT MANAGEMENT	REPORT PREPARATION	SUPPORT
Project Director	***Authors***	***Administrative Support***
Anna M. Fan, Ph.D.	Jean Rabovsky, Ph.D.	Hermelinda Jimenez
	Amy J. Dunn, M.P.H.	Michael Baes
PHG Program Leader	James F. Collins, Ph.D.	Janet Rennert
Robert A. Howd, Ph.D.	Joseph P. Brown, Ph.D.	
	Thomas A. McDonald, Ph.D.	
	Martha Sandy, Ph.D.	
Comment Coordinator	John Faust, Ph.D.	***Library Support***
Michael Baes	Brian Endlich, Ph.D.	Charleen Kubota, M.L.S.
	Lauren Zeise, Ph.D.	
		Web site Posting
	Primary Reviewers	Laurie Monserrat, M.S.
	Jolanta Bankowska, Ph.D.	
	Michael DiBartolomeis, Ph.D.	
	Final Reviewers	
	Anna M. Fan, Ph.D.	
	George V. Alexeeff, Ph.D.	
	Robert A. Howd, Ph.D.	

PREFACE

Drinking Water Public Health Goals
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.
8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.

9. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Public Health (DPH) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations or technical feasibility, drinking water standards adopted by DPH are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DPH shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By state and federal law, MCLs established by DPH must be at least as stringent as the federal MCL, if one exists.

PHG documents are used to provide technical assistance to DPH, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not intended to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA web site at www.oehha.ca.gov.

TABLE OF CONTENTS

LIST OF CONTRIBUTORS ..... **II**
PREFACE ..... **III**
TABLE OF CONTENTS ..... **V**
PUBLIC HEALTH GOAL FOR STYRENE IN DRINKING WATER **1**
SUMMARY **1**
INTRODUCTION **3**
CHEMICAL PROFILE **4**
Chemical Identity 4
Physical and Chemical Properties 4
Production and Uses 5
ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE **5**
Air 6
Soil 7
Water 7
Food 8
Other Sources 8
METABOLISM AND PHARMACOKINETICS **9**
Summary 9
Absorption 11
Ingestion 11
Inhalation 11
Dermal Absorption 13
Distribution 13
Metabolism 16
Excretion 34
TOXICOLOGY **37**
Genetic Toxicity 37
Cytogenetic Alterations 37
DNA Strand Breaks 50
Gene Mutation 54
DNA Adducts 60

Genetic Toxicity Studies - Summary and Conclusions 66
Dose Response for Genotoxicity 68
Species Comparison 69
Structure-activity 70
Developmental Toxicity 71
Developmental Effects - Human Studies 71
Miscarriages 71
Congenital Malformations 72
Decreased Birth Weight 74
Developmental Effects – Animal Studies 75
Non-neurotoxic Developmental Endpoints in Animals 75
Effects in Offspring prior to Parturition 80
Postnatal Observations 81
Developmental Neurotoxicity 81
Supplementary Information 92
Developmental Toxicity Summary 92
Reproductive Toxicity 93
Male Reproductive Effects - Human Studies 93
Semen Quality and Quantity 93
Paternal Exposure to Styrene (Miscarriages) 95
Male Reproductive Effects – Animal Studies 96
Male Reproductive Effects – Other Relevant Data 98
Female Reproductive Effects - Human Studies 99
Menstrual Function 99
Reproductive Hormone Function in Women 99
Female Reproductive Effects – Animal Studies 100
Endocrine Disruptor Effects 101
Summary 101
Other Toxicity in Animals 102
Acute Toxicity 102
Subchronic Toxicity 103
Respiratory Effects 103
Liver Toxicity 103

Kidney Toxicity 103
Hematologic Effects 104
Ototoxicity 104
Chronic Toxicity 104
Respiratory Effects 104
Liver Toxicity 105
Kidney Toxicity 106
Immunotoxicity 106
Neurotoxicity 107
Acute Neurotoxic Effects 107
Chronic Neurotoxic Effects 107
Carcinogenicity in Animals 108
Administration by the Oral Route 110
Studies using Inhalation Exposure 114
Studies by Injection 125
Discussion of Bioassay Results 126
Summary 132
Other Toxicity in Humans 132
Acute Toxicity 132
Subchronic Toxicity 134
Liver Toxicity in Workers 134
Asthma in Workers 134
Ototoxicity in Workers 134
Immunotoxicity 134
Neurotoxicity 136
Acute Neurotoxic Effects 136
Chronic Neurotoxic Effects 136
Styrene Oxide Neurotoxicity 141
Chronic Toxicity in Humans 142
Respiratory Effects 142
Cardiovascular Toxicity 142
Liver Toxicity 142
Kidney Toxicity 143

Hematologic Effects 143
Carcinogenicity in Humans 143
Styrene Monomer and Polystyrene Manufacturing 144
Fabrication of Glass-Reinforced Plastics 149
Styrene-butadiene Rubber Manufacturing 166
Case-control Studies in a Range of Occupational Settings 183
Ecological study 184
Summary and Conclusions 185
Mechanisms of Carcinogenicity 189
Direct Evidence 189
Structure Activity Considerations 193
Indirect Evidence 193
Summary 193
DOSE-RESPONSE ASSESSMENT 195
Noncarcinogenic Effects 195
Neurotoxicity Dose-Response in Humans 195
Respiratory Dose-Response 196
Hepatotoxicity Dose-Response 197
Carcinogenic Effects 198
Cancer Potency Estimates from Human Data 198
Models for Estimating Cancer Potency from Human Data 199
Population Risk Estimates 202
Summary of Human Cancer Potency Estimates 207
Cancer Potency Estimates from Animal Data 207
Physiologically-based Pharmacokinetic Analysis of Rodent Data 208
Calculation of Cancer Potency 216
Linear Multistage (LMS) Method 216
Benchmark Response Method (BMR) 220
CALCULATION OF PHG 229
Noncarcinogenic Effects 229
Carcinogenic Effects 233
Summary 235

RISK CHARACTERIZATION..... 235
OTHER GUIDANCE VALUES AND REGULATORY STANDARDS 238
REFERENCES..... 240

PUBLIC HEALTH GOAL FOR STYRENE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) proposes a Public Health Goal (PHG) of 0.5 parts per billion (ppb) (0.5 micrograms/liter [µg/L]) for styrene in drinking water, based on its carcinogenicity in rodents. Styrene is a high-production-volume chemical, widely used in such products as styrofoam (polystyrene) and other plastics, and a minor constituent of cigarette smoke and auto emissions. It has significant acute and chronic toxic effects, including both genotoxicity *in vitro* and production of various types of tumors in animal studies. The proposed PHG is based on a chronic inhalation study by Cruzan *et al*. (2001) in mice, in which there were significant dose-related increases in bronchioloalveolar adenomas and combined adenomas and carcinomas.

Styrene is a widely-used organic chemical, produced at a rate of about 10 billion pounds per year in the United States. It is used primarily to make rubber and plastics. The general population may be exposed to styrene by eating food that has been packaged in polystyrene, by breathing contaminated air from industrial sources, auto exhaust, or incineration emissions, by smoking cigarettes, and by ingestion of contaminated drinking water. Occupational exposures are usually orders of magnitude greater than environmental exposures. For example, workers in the reinforced plastics industry (RPI) might be exposed to 2 grams/day of styrene, whereas residents living within one kilometer of a production facility might be exposed to 600 µg/day. The latter value also approximates the styrene exposure of a one pack/day cigarette smoker.

This document provides a comprehensive review of the toxicology and epidemiology literature on styrene and its primary reactive metabolites and degradation products. The potential of styrene to induce cancer and non-cancer effects is described. Dose response relationships are characterized for cancer and for sensitive non-cancer endpoints.

The potential of styrene to cause cancer is evaluated after review of the numerous epidemiological studies, cancer bioassays, and other relevant studies on styrene and its metabolites. The human studies consider three main occupational settings: manufacture of styrene monomer and polystyrene, production of reinforced plastics, and manufacture of styrene-butadiene rubber. The strongest evidence of a cancer risk comes from the reinforced plastics industry (increased leukemias and malignant lymphomas), because of the high exposure levels, a relative lack of confounders, and large cohorts.

With regard to direct, epidemiological evidence, the International Agency for Research on Cancer (IARC) in 2002 found “limited” evidence of carcinogenicity for styrene in humans. In IARC parlance this means a positive association between styrene exposure and cancer is found for which a causal interpretation is considered to be credible, but IARC could not rule out chance, bias, or confounding with reasonable confidence to identify the chemical as a known human carcinogen. The human data reviewed by IARC, along with more recent epidemiology studies, are described here. OEHHA similarly finds suggestive but not definitive direct evidence of cancer in the human

cancer data. The excess malignancies observed most frequently are of the lymphatic and hematopoietic systems.

Indirect evidence of human carcinogenicity comes from animal cancer bioassays and studies of genotoxicity and pharmacokinetics of styrene. Several animal cancer bioassays for styrene indicate its carcinogenic potential for humans. Lung tumors were observed in female and male mice exposed by inhalation for their lifetime and in male mice exposed by gavage. Lung tumors developed in the progeny of mice exposed to styrene *in utero* and then after birth by gavage. Female rats developed mammary gland tumors when exposed by inhalation or drinking water ingestion.

Genotoxic damage has been reported in lymphocytes of styrene-exposed reinforced plastics workers, manifested as chromosomal aberrations, sister chromatid exchange (SCE), single-strand breaks (SSBs)/DNA damage, mutations in the *hprt* gene or glycophorin A locus, and formation of styrene-derived DNA adducts. Since several cancer studies in humans occupationally exposed to styrene have observed an increased risk of lympho-hematopoietic cancers with increased exposure to styrene, these findings of genotoxicity in the lymphocytes of styrene-exposed workers are particularly relevant. Strong correlations among a number of independently measured genotoxicity endpoints and styrene-hemoglobin adducts have been reported among styrene-exposed boat builders. Supporting data for a genotoxic mechanism of styrene action are available from many *in vitro* and *in vivo* test systems with styrene and its metabolites. The weight of evidence strongly suggests that styrene is genotoxic in humans, rodents, and non-mammalian species.

An NTP expert panel (NTP, 2008b) recently recommended that styrene should be listed in the NTP Report on Carcinogens as "reasonably anticipated to be a human carcinogen based on limited evidence of carcinogenicity in humans and sufficient evidence in animals." OEHHA also concludes that there is sufficient evidence that styrene causes cancer in animals. While several epidemiological studies of styrene and cancer in workers exposed in reinforced plastics and other industries have been published, the data do not show proof of carcinogenicity in humans.

The scientific literature includes numerous studies in animals and humans on reproductive and developmental toxicity of styrene, as well as on other toxic effects. The weight of evidence indicates that styrene crosses the placenta and can have adverse and lasting effects on the developing brain. Animal studies show that styrene exposure of pregnant dams increases embryo mortality and fetal resorption, decreases fetal weight, and decreases testicular function in male fetuses. This document also includes a discussion on the mechanism of carcinogenicity and discusses the metabolism and pharmacokinetics of styrene.

For noncarcinogenic effects the range of health-protective concentrations for multiroute exposures was 4 to 200 ppb. The most health protective value of 4 ppb was derived using the benchmark approach for data on bronchiolar effects in male mice (Cruzan *et al*., 2001). The drinking water concentration estimated to be fully protective against non-cancer effects of styrene, for a lifetime of exposure, is therefore proposed to be 4 ppb.

OEHHA concludes that styrene should be considered to be a carcinogen for development of a health-protective level in drinking water. The proposed PHG for styrene is based on

the chronic inhalation exposure study of Cruzan *et al*. (2001) in CD-1 mice. The study was conducted recently, was a lifetime study, and used an adequate number of male and female animals (70/sex/exposure level) and several concentrations of styrene (0, 20, 40, 80, and 160 ppm). The study provided adequate data to derive a dose response cancer potency slope for lung bronchioloalveolar adenomas and carcinomas, and is supported by several additional studies and lines of evidence concerning potential carcinogenic effects of styrene. A health-protective, *de minimis* risk value of 0.5 ppb for multiroute exposure to styrene in drinking water was estimated from this study, which corresponds to a theoretical upper limit cancer risk value of one in one million people exposed for a lifetime.

A Maximum Contaminant Level (MCL) of 0.1 mg/L (100 µg/L, 100 ppb) was established by the California Department of Public Health (DPH) (Title 22 CCR, Division 4, Chapter 15, Article 4, Section 64431) in 1994. California's current detection limit for the purposes of reporting (DLR) for styrene in drinking water is 0.5 µg/L (0.5 ppb), which is identical to the proposed PHG. U.S. EPA set a Maximum Contaminant Level Goal (MCLG) and Maximum Contaminant Level (MCL) of 0.1 mg/L (100 ppb) for styrene in 1991. The federal MCL is based on potential liver, kidney, or circulatory system problems above this level (U.S. EPA, 2002).

INTRODUCTION

The purpose of this document is to summarize the information available on styrene pertinent to development of a Public Health Goal (PHG). This document describes the adverse health effects of styrene in humans and animals, and predicts levels of styrene associated with insignificant risks of ill health. The dose-response data are used to develop a proposed PHG for styrene in drinking water. Previously OEHHA used styrene dose-response data to develop acute and chronic inhalation Reference Exposure Levels (RELs) for styrene (OEHHA, 1999, 2000).

Many styrene studies have been conducted since the U.S. EPA reviewed styrene toxicity in the late 1980s for the oral reference dose (RfD), which led to the current federal MCLG and MCL, and the early 1990s for the inhalation reference concentration (RfC). U.S. EPA has not completed a cancer risk assessment for styrene (U.S. EPA, 2009). The new epidemiology reports, animal studies, and *in vivo* and *in vitro* genotoxicity and mutagenicity data have led OEHHA to conclude that the health-protective level should be decreased by a considerable amount, compared to the existing drinking water standards. Styrene is a mutagenic and genotoxic carcinogen, and a linear, no threshold dose-response curve is judged to be appropriate for its carcinogenic risk assessment.

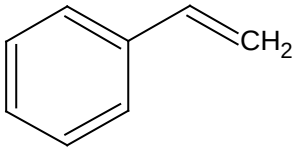
The California Department of Public Health (DPH, formerly Department of Health Services) uses the health-based information provided by OEHHA, along with other criteria such as economic and technical feasibility, to set a California MCL. The California MCL serves as the State's drinking water standard, and must be equal to or lower than the federal MCL. In accordance with the California Safe Drinking Water Act of 1996 (HSC 116365), DPH will reevaluate the State MCL to determine whether it may be feasible to decrease the MCL to bring it closer to the proposed PHG. We invite comments on this document and the proposed PHG for styrene in drinking water.

CHEMICAL PROFILE

Chemical Identity

The chemical formula, structure, synonyms and identification number for styrene are presented in Table 1.

Table 1. Chemical Identity of Styrene

Chemical Name	Styrene
Synonyms	Ethenylbenzene, vinylbenzene, cinnamene
Chemical formula	C_8H_8
CAS Number	100-42-5
Chemical structure	

Physical and Chemical Properties

Important physical and chemical properties of styrene are given in Table 2, based on information given in IARC (1994a) and ATSDR (1992). Styrene is a colorless liquid with a pungent odor, which is slightly soluble in water and very soluble in benzene.

Table 2. Physical and Chemical Properties of Styrene[1]

Property	Value or Information
Molecular weight	104.16 daltons
Color	Colorless to yellowish
Physical state	Liquid
Odor	Sweet, sharp
Odor threshold in water in air	 0.73 mg/L 0.011 mg/L (Amoore and Hautala, 1983) 0.32 ppm (1.36 mg/m^3) (“)
Melting point	-30.6°C
Boiling point	145.2°C
Flash point	31°C
Autoignition temperature	490°C
Solubility in water	 300 mg/L

Property	Value or Information
in organic solvents	Soluble in alcohol, ether, acetone, carbon disulfide
Density	0.906 @ 20°C
Partition coefficients Octanol-water (K_{ow}) Log K_{ow}	 891 2.95
Vapor pressure	6.40 mm Hg @ 25°C
Henry's law constant	2.61×10^{-3} atm-m^3/mol
Conversion factors	1 mg/m^3 = 0.23 ppm, 1 ppm = 4.26 mg/m^3

[1] data from IARC (1994a), ATSDR 1992, and HSDB (2007) except where indicated

Production and Uses

Styrene is one of the world's major organic chemicals. Production of styrene monomer consumes a large part of the world's capacity of benzene, in the form of ethylbenzene (HSDB, 2007). Styrene is used primarily to make rubber and plastics, in the following proportions: polystyrene, 66 percent; acrylonitrile-butadiene-styrene (ABS) resins and styrene-acrylonitrile resins, 11 percent; styrene-butadiene rubber (SBR), 7 percent; unsaturated polyester resins, 5 percent; miscellaneous resins, 5 percent; other uses, 6 percent (HSDB, 2007). U.S. production in 2000 was more than 10 billion pounds, and an additional million pounds were imported into the U.S. (HSDB, 2007).

Products made from styrene include packaging, insulation (electrical and thermal), fiberglass (reinforced plastics), pipes, automotive parts, drinking cups and other food-use containers, and carpet backing (ATSDR, 1992). These products mainly contain polystyrene; however most also contain a residue of unlinked styrene. Styrene monomer may also occur from resin breakdown in these products (ATSDR, 1992). Styrene is present in combustion products such as tobacco smoke and automobile exhaust.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Styrene's production and use in plastic and resin manufacture result in its release to the environment through various waste streams. Monitoring data indicate that the general population may be exposed to styrene via ingestion of food which has been packaged in polystyrene, by ingestion of contaminated finished drinking water, by inhalation of air contaminated by industrial sources, auto exhaust, or incineration emissions, and by inhalation of tobacco smoke (IARC, 1994a). Exposure to styrene may occur during the use of miscellaneous products containing styrene such as floor waxes and polishes, paints, adhesives, putty, metal cleaners, auto body fillers, and varnishes (NIOSH, 1983). Styrene also occurs naturally in the sap of some trees (HSDB, 2007).

Worst-case non-occupational exposure estimates for styrene were calculated by U.S. EPA (1988): 0.5 µg/day from drinking water, 30 µg/day from food, and 65,000 µg/day from air (includes indoor and outdoor sources but not contributions from active cigarette smoking); these estimates were based on the highest levels estimated or monitored and,

therefore, reflect a high potential exposure rather than typical exposure for the general population. To demonstrate the relative significance of different routes of exposure, Fishbein (1992) estimated daily intake of styrene from different sources:

worker in reinforced plastics industry	2 g/day
worker in styrene polymerization	100 mg/day
living within 1 km of a production unit	600 µg/day
breathing heavily polluted urban air	400 µg/day
breathing typical urban air	6 µg/day
breathing indoor air	6 to 1,000 µg/day
drinking polluted water	2 µg/day
cigarette smoke (20 cigarettes per day)	400 to 960 µg/day.

Air

A vapor pressure of 6.40 mm Hg at 25°C indicates that styrene, if released to air, will exist solely as a vapor in the ambient atmosphere. Vapor-phase styrene is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals and ozone; the half-lives for these reactions in air are estimated to be 7 and 16 hours, respectively. Direct photochemical or photolytic reactions for styrene are slow (HSDB, 2007).

In 2000, 1.1 million pounds of styrene were emitted into the air in California by facilities that report under the Air Toxics Hot Spots program (CARB, 2001). Ambient atmospheric styrene levels from the vicinity of six reinforced plastic processors in the U.S. ranged from 0.07 to 690 ppb, while in communities farther away from these processors, styrene was detected at concentrations of 0.4 to 5.6 ppb (HSDB, 2007).

Outdoor ambient air concentrations of styrene have been reported in several studies, with levels generally dropping over time. In studies conducted in the mid-1980s, the concentration of styrene ranged from 0.5 to 3 ppb in Los Angeles, California and was 0.09 ppb in Contra Costa County, California; an earlier study (mid-1960s) found that styrene concentrations ranged from 1 to 15 ppb in four California cities (HSDB, 2007). Statewide, mean ambient air levels in California decreased from 0.27 ppb styrene in 1991 to 0.08 ppb in 1997, with a reported median of 0.05 ppb in 2000 (CARB, 2000).

In indoor air, the mean styrene concentrations are frequently somewhat higher than those in ambient outdoor air (<1 to 6 µg/m^3 (i.e., 0.23 to 1.41 ppb)], due to smoking as well as off gassing from some styrene containing household products (IARC, 1994a). Certain solvent-based adhesives used to finish interiors of office buildings emit styrene (HSDB, 2007). The mean concentration of styrene in the indoor air of homes with smokers and nonsmokers was 2.11 µg/m^3 (range, 0.49 to 7.02 µg/m^3 [0.50 ppb, 0.12 to 1.65 ppb]) and 1.47 µg/m^3 (range, 0.43 to 4.96 µg/m^3 [0.35 ppb, 0.10 to 1.16 ppb]), respectively (HSDB, 2007). The average emission factor of styrene for six commercial brands of cigarettes was 147 µg/cigarette (range, 121 to 191 µg/cigarette) (Hodgson *et al.*, 1996). In new carpets, styrene had an emission rate of 16.6 mg/m^2 in the first 24 hours and 25.9 mg/m^2 in the first 168 hours (HSDB, 2007). Among 178 buildings in the United Kingdom, carpet was determined to be the source of styrene in one case at 0.9 ppm in air. The concentration of styrene released from a tufted textile floor covering with SBR backing

under equilibrium conditions ranged from 5.6 ng/L at 23°C to 14.9 ng/L at 71°C (HSDB, 2007). The emission rate of styrene from dry-process photocopiers ranged from 40 to 220 µg/hr per copier while idle and 300 to 12,000 µg/hr per copier during operation (HSDB, 2007). The emission factor of styrene from urethane cushions ranged from 92 to 94 µg/m^2-hr measured for 6 hours in a 4 L dynamic chamber, 118 to 192 µg/m^2-hour measured for 6 hours in a 52 L chamber, and <1 to 8 µg/m^2-hour measured for 96 hours in a 52 L chamber (HSDB, 2007).

Soil

If released to soil, styrene is expected to have low mobility based upon an estimated octanol-soil partition coefficient (K_{oc}) of 960. Volatilization from moist soil surfaces is expected to be an important fate process. For example, in 1.5 cm deep samples of a loamy soil, 26 percent of 2 mg/kg styrene added volatilized in 31 days. Based upon its vapor pressure, styrene may volatilize from dry soil surfaces. Biodegradation by aerobic microorganisms may lead to extensive or complete destruction of styrene in soil. It was found that 97 and 87 percent of ^{14}C- styrene added to soil at 2.0 g/kg was converted to ^{14}C-CO_2 in 16 weeks in a landfill soil and sandy loam soil, respectively (HSDB, 2007).

Water

In the National Drinking Water Contaminant Occurrence Database (NCOD), a repository of drinking water quality data, the concentration of styrene in Public Water System (PWS) drinking water derived from surface water averaged 18.4 µg/L (range, 0.044 to 660 µg/L) for 36 PWSs with detectable styrene (out of 1,490 PWSs analyzed) (U.S. EPA, 2001). The concentration of styrene in drinking water derived from ground water under the influence of surface water was 0.2 µg/L for one of 96 PWSs with analyses, and drinking water derived from ground water averaged 1.5 µg/L (range, 0.03 to 10 µg/L) for 90 of 9,023 PWSs with analyses (U.S. EPA, 2001). In the mid-1980s (HSDB, 2007), styrene was not detected in 945 finished water supplies throughout the U.S. which use ground water sources. In the NCOD, the concentration of styrene in “other” surface water averaged 0.33 µg/L (range, 0.2 to 0.6 µg/L) for 5 of 503 stations with analyses (U.S. EPA, 2001).

If released into water, styrene is expected to adsorb to suspended solids and sediment based upon the Koc (HSDB, 2007). In lake water, 10 to 20 percent mineralization was observed in three weeks with samples containing 2.5 to 1,000 µg/L styrene. Degradation of styrene is rapid in sewage under aerobic conditions (HSDB, 2007).

Volatilization from water surfaces is expected to be rapid. Under laboratory conditions, 50 percent of 2 to 10 mg styrene/liter (depth not specified) was lost by volatilization in 1 to 3 hours in lake-water samples and in six to seven hours in distilled water (HSDB, 2007). A bioconcentration factor of 13.5 for goldfish suggests low bioconcentration in aquatic organisms. Styrene is not expected to undergo hydrolysis in the environment due to the lack of hydrolysable functional groups (HSDB, 2007).

Food

Styrene migrates into food from both rigid and expanded polystyrene foam containers (WHO, 1983). Styrene levels ranged from 3.9 to 240 ppb (average, 20.6 ppb) in 234 table-ready food items from the U.S. Food and Drug Administration's (FDA) Total Diet Study, with the highest concentration in fruit yogurt (Heikes *et al.*, 1995). This study also reported the styrene concentration in sandwich cookies (216 ppb), margarine (9.28 ppb), butter (22.4 ppb), and cake doughnuts (23.0 ppb). The level of volatile organic residues appears to vary directly with the fat content of the food (Heikes *et al.*, 1995). Earlier studies had found styrene in yogurt packaged in polystyrene containers at concentrations of 2.5 to 34.6 ppb, with styrene content in yogurt increasing with time (IARC, 1979). In scrambled eggs made from eggs stored in polystyrene containers, styrene monomer increased from 1 ppb in farm fresh eggs to 14 ppb in farm fresh eggs stored for two weeks in polystyrene containers; scrambled eggs made from "supermarket eggs" packaged in polystyrene containers had 103 ppb styrene (Matiella and Hsieh, 1991). Styrene was found at 17.2 ppb in homogenized milk after 19 days storage in polystyrene packaging (IARC, 1979). The concentration of styrene in commercial fermented soybean curds was reported as 34 ppb (HSDB, 2007).

Styrene has been detected as a natural constituent of foods and beverages, the highest measured levels occurring in cinnamon; enzymatic degradation of cinnamic acid derivatives was proposed as the possible source of styrene (IARC, 1994a).

Styrene was detected, but not quantified, in eight of eight human breast milk samples selected from a total of 42 samples collected from U.S. women in four cities (Pellizzari *et al.*, 1982). The samples selected were those with the greatest number of peaks or those containing intense, unique (not observed in other samples) peaks.

Other Sources

Occupational exposure to styrene may occur through inhalation and dermal contact at workplaces where styrene is produced or used (HSDB, 2007). NIOSH (NOES Survey 1981 to 1983) estimated that 333,212 workers (86,902 of these are female) are potentially exposed to styrene in the U.S. (NIOSH, 1983). Occupational exposure occurs in polystyrene manufacture at levels generally less than 21 mg/m^3 (5 ppm), though occasional values of 210 mg/m^3 (50 ppm) or more have been reported (WHO, 1983). In reinforced plastics applications, concentrations of styrene found during the production of reinforced plastics were generally much higher than those found in the polystyrene production plants. Peak concentrations during earlier periods were as high as 6,300 mg/m^3 (1,500 ppm) (WHO, 1983), although current levels are generally much lower. Average styrene exposures in reinforced plastics/composites plants can range from 40 to 100 ppm, with individual TWA and short-term exposures as high as 150 to 300 ppm and 1,000 to 1,500 ppm, respectively (HSDB, 2007). Other workplaces with exposure include non-production departments of pulp, paper, and paper product mills, where occupational exposure to styrene was 9.9 ppm for maintenance, building construction, and cleaning workers (HSDB, 2007).

METABOLISM AND PHARMACOKINETICS

Summary

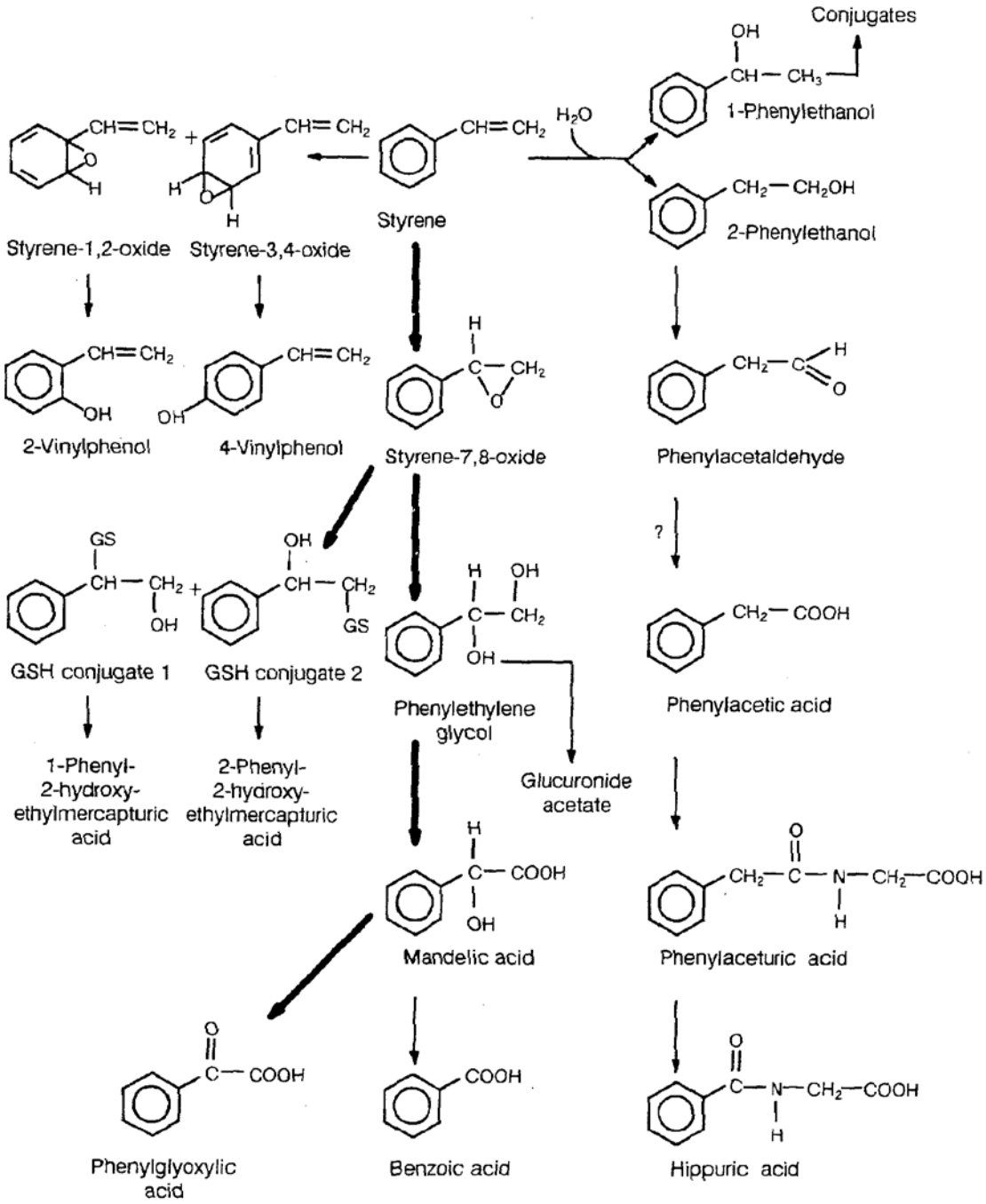
Styrene is substantially and efficiently absorbed by inhalation and ingestion but only minimally absorbed by skin after dermal contact. Cytochrome P_{450}-dependent enzymes in humans and rodents (mice and rats) catalyze the biotransformation of styrene to the active metabolite styrene-7,8-oxide and, to a lesser extent, styrene-3,4-oxide, as per the general scheme shown in Figure 1. The principal P450 in human liver is CYP2E1 (Wenker *et al*., 2001a), but other P450s are involved in both humans and animals (Nakajima *et al*., 1994a; Carlson, 2003). Non-cytochrome P_{450}-dependent heme proteins, such as myeloperoxidase and prostaglandin synthase, also metabolize styrene. Saturation of styrene metabolism in humans occurs with prolonged exposure to styrene in air in the approximate range of 100 to 200 ppm (430 to 870 mg/m^3).

The liver appears to be quantitatively the most important organ for the cytochrome P_{450}-dependent metabolism, but other organs and tissues, such as the lungs and lymphocytes, can convert styrene to styrene-7,8-oxide. Kinetic studies on isolated liver microsomal fractions reveal more than one form of styrene-metabolizing cytochrome P_{450}-dependent enzymes, and the analysis of the formation of styrene-7,8-oxide production provides some evidence for interspecies differences. Furthermore, polymorphisms in cytochrome P_{450}-dependent enzymes might result in subpopulations that are more sensitive to styrene toxicity (Rueff *et al*., 2009).

Styrene-7,8-oxide is detected in the blood of humans exposed under controlled laboratory conditions or during work activities. In humans and rodents, styrene-7,8-oxide has been detected as adducts of hemoglobin or DNA. Styrene-7,8-oxide undergoes detoxification reactions to non-reactive metabolites and other products depending on the balance of the activation and detoxification pathways. In humans, the major pathway is epoxide hydrase-dependent hydrolysis to styrene glycol with subsequent metabolism to the urinary metabolites mandelic acid (MA) and phenylglyoxylic acid (PGA). In rodents, styrene-7,8-oxide metabolism may occur through hydrolysis or conjugation to reduced glutathione via glutathione-S-transferase; the final products are mercapturic acids. Urinary MA and PGA, alone or in combination, are biomarkers of styrene exposure and are measured primarily for exposure assessment purposes, particularly in the workplace.

While urinary excretion is an effective clearance pathway, fat is an effective repository for absorbed styrene (and its metabolites) and can provide a source of internal styrene exposure after cessation of external exposure due to the high lipophilicity of styrene (K_{ow} = 891). Humans exposed under controlled conditions to styrene vapor retain styrene in fat tissue with individual differences in the rate of release. Reported half-lives of one to four days suggest that continuous environmental exposures to styrene might result in an accumulation of the chemical in body tissue and removal from the source of styrene exposure for short lengths of time may not be sufficient to clear styrene and its metabolites from the body.

Figure 1. Metabolic Pathways of Styrene[1]



[1]Figure from IARC, 1994a

Absorption

Ingestion

Styrene can be absorbed through the gastrointestinal (GI) tract, which is important for food and drinking water pathways. However, quantitative GI absorption studies were not identified during a search of the published scientific literature. Csanady *et al*. (1994) assumed 100 percent bioavailability of orally administered styrene in rodents.

Inhalation

Uptake and absorption studies carried out in humans and rodents demonstrate that airborne styrene is absorbed by the lung and across the alveoli into the blood. The data also suggest that some styrene is retained in the respiratory tract. Pulmonary uptake depends on the styrene concentrations in air and follows first-order kinetics.

The majority of studies available in the published scientific literature on human exposure are for the inhalation pathway. Data from several human exposure studies are presented in Table 3. The pulmonary uptake of styrene among humans exposed to styrene in the air ranges from about 61 to 71 percent. Considering these data together, 65 percent absorption of styrene from inhalation exposure in humans is a reasonable estimate for use in risk assessment.

Table 3. Fractional Uptake of Styrene by Humans Exposed by Inhalation[1]

Subjects	Percent Styrene Absorbed	Styrene Concentration / Duration of Exposure	Reference
No information provided	61	22 ppm/8 hrs	Bardodej and Bardodejova, 1970
Men (n = 14) at rest	62	50 to 150 ppm/30 min	Astrand *et al.* 1974
Men (n = 7) at rest or light exercise	63	50 ppm/30 min	Engstrom *et al.*, 1978
Men (n = 8), light exercise	68	69 ppm/2 hrs	Wigaeus *et al.*, 1983
No information provided	71	5, 9, 23, 47 ppm/4 hrs (x 2)	Wieczorek and Piotrowski, 1985
Men (n = 5) Women (n = 1)	64	48 to 52 ppm/2 hrs	Norstrom *et al.*, 1992
Men (n = 4), light exercise	66	50 ppm/2 hrs	Johanson *et al.*, 2000
Men (n = 20), light exercise	62	24 or 85 ppm/1 hr	Wenker *et al.*, 2001a

[1] Presented as percent of inspired styrene.

Among human volunteers exposed by inhalation for two hours to 69 ppm styrene, the *in vivo* blood/alveolar air ratio was 62, whereas the blood/inspiratory air ratio was 7.5 (Wigaeus *et al.*, 1983). Systemic absorption of inhaled styrene has been measured by the presence of styrene in arterial blood. Wigaeus *et al.* (1983) reported arterial blood levels of 21 μmol/L, or 2.2 ppm, in men exposed to 69 ppm styrene under light exercise.

Astrand *et al.* (1974) measured the arterial blood levels of styrene in humans exposed at air levels of 50 and 150 ppm under conditions of rest, light (50 watts), or intermediate (100 W) exercise for 30 minutes. At each activity level, the arterial blood concentration of styrene increased with increasing styrene air levels and breathing rate, ranging from 0.5 ppm (50 ppm concentration, at rest) to a maximum of 12 ppm with intermediate exercise and exposure to 150 ppm styrene. In men exposed to 50 ppm, an alveolar ventilation rate of 8 L/min was measured at rest and 23 L/min when performing light exercise (50 Watts). An 8 percent increase in alveolar styrene level and a 4-fold increase in arterial styrene blood concentration were observed in men performing light exercise while exposed to 50 ppm styrene, as compared with men exposed to 50 ppm styrene while at rest. Similar effects of increased activity and increased ventilation rate on alveolar and arterial blood styrene levels were observed at exposures up to 150 ppm styrene. The greater increase in arterial blood styrene levels relative to the alveolar styrene levels indicates that styrene is rapidly removed from the alveoli into the circulating blood, as would be predicted for a highly soluble chemical such as styrene.

Styrene uptake by inhalation appears to exhibit linear kinetics at air levels up to about 85 ppm styrene. Among workers exposed to air styrene levels from ten to 80 ppm, there was a linear relationship between styrene-7,8-oxide in venous blood and air styrene levels or venous blood styrene levels (Korn *et al.*, 1994). The increase was 3-fold when measurements were taken under light exercise. Human volunteers exposed to 24 or 85 ppm styrene for one hour under light exercise exhibited the same increases in total uptake, area under the curve, and cumulative urinary metabolite excretion as the increase in styrene air levels (Wenker *et al.*, 2001b).

Pulmonary uptake and systemic absorption of styrene following inhalation have been studied in rats and mice. Ghantous *et al.* (1990) reported the presence of styrene metabolites in the nasal mucosa and olfactory bulb of mice exposed to approximately 104 to 208 μg vaporized styrene over a ten-minute interval via inhalation. Among male Sprague-Dawley rats and male CD1 mice exposed to 50 ppm styrene for 45 minutes, uptake efficiencies of 4 to 17 percent by the nasal-laryngeal region were reported by Morris (2000). The uptake efficiencies decreased as the air styrene concentration increased from 5 to 200 ppm styrene, and as the flow rate through the nasal-laryngeal region was increased from 50 to 150 percent of the minute volume. In animals treated with metapyrone, an inhibitor of cytochrome P_{450}-dependent metabolism, nasal-laryngeal uptake efficiencies were reduced at low styrene concentrations which effectively eliminated the styrene concentration-dependence of the uptake efficiencies. The authors suggested that at low styrene levels a portion of styrene retention by the nasal-laryngeal area is due to metabolism and formation of a styrene metabolite.

Boogaard *et al.* (2000a) reported pulmonary uptake of 113 μmol/kg-hour for male Sprague-Dawley rats and 186 μmol/kg-hour for male CD1 mice exposed in nose-only

chambers for six hours to 160 ppm styrene. The arterial blood concentration of styrene equivalents (styrene and metabolites) in rats exposed for eight hours was 49 nmol/g tissue at 45 ppm styrene in air, and 375 nmol/g at 240 ppm (Carlsson, 1981). Cruzan *et al.* (1998; 2001) measured the pulmonary absorption of styrene by rats and mice as the blood levels of styrene and styrene-7,8-oxide. In rats, absorption was measured after a 95-week inhalation exposure to 50 to 1,000 ppm styrene. The relationship between blood styrene and air styrene was supralinear, whereas the relationship for styrene-7,8-oxide was linear. Among mice exposed for 74 weeks to air styrene levels of 20 to 160 ppm, the concentration dependencies of blood styrene and blood styrene-7,8-oxide were supralinear. Under the conditions of the experiment, saturation of styrene metabolism occurred in rats and mice, and saturation of metabolism of styrene-7,8-oxide occurred in rats but not in mice.

Dermal Absorption

Styrene liquid and vapor are modestly absorbed through intact skin. Berode *et al.* (1985) exposed the hands of 13 men to undiluted styrene liquid for 15 to 30 minutes and then monitored the urinary excretion of styrene metabolites. The authors calculated that the amount dermally absorbed was about 4 percent of what would be absorbed from inhalation of 50 ppm styrene for eight hours. Using the results of Riihimaki and Pfaffli (1978) and Wieczorek (1985) on dermal uptake of styrene vapors by humans exposed to 322 to 763 ppm for two to 3.5 hours, as reported by McDougal *et al.* (1990), and assuming an adult human inhalation rate of 19 m^3/day, a dermal absorption fraction for styrene vapor of 0.021 can be estimated. Limasset *et al.* (1999) reported two styrene workers, exposed for two hours to 42 to 59 ppm air styrene and wearing only respiratory protective wear, excreted a similar level of the styrene metabolite mandelic acid as did two workers who wore total body protective wear. The authors concluded that dermal uptake was negligible compared to pulmonary uptake.

In experiments comparing styrene uptake in rats with closely clipped fur exposed for four hours to 3,000 ppm styrene vapor or fresh air, dermal uptake was estimated as 9 percent of the total absorbed through both the inhalation and dermal routes (McDougal *et al.*, 1990). Dermal absorption of neat liquid styrene in rats was approximately 15 percent following 24 hrs of exposure (Morgan *et al.* (1991).

Distribution

Styrene uptake into blood and fatty tissues in humans and rodents has been reported in terms of the styrene partition coefficients (Table 4). Human data are generally taken from blood levels following inhalation exposure, whereas tissue levels are reported for experimental animal studies. Fat serves as a repository for styrene in both humans and animals.

Table 4. Measured Styrene Partition Coefficients

Compartments	Species			References
	Rat	Mouse	Human	
Tissue/Air				
Lung/Air	70	70		Schwegler and Filser, 1989
Blood/Air	40			Andersen *et al.*, 1984; Gargas *et al.*, 1989
"	110	100	48	Schwegler and Filser, 1989; Csanady and Filser, 1994
"			62	Wigaeus *et al.*, 1983[a]
"			7.5	Wigaeus *et al.*, 1983[b]
Tissue/Blood				
Fat/Blood	86			Andersen *et al.*, 1984; Gargas *et al.*, 1989
"	*41*[c]	*44*	94	Schwegler and Filser, 1989; Csanady and Filser, 1994
"			3.1	Wigaeus *et al.*, 1983
Liver/Blood	3.5			Andersen *et al.*, 1984; Gargas *et al.*, 1989
"	*1.2*	*1.2*	2.7	Schwegler and Filser, 1989; Csanady and Filser, 1994
Muscle/Blood	*1.2*			Andersen *et al.*, 1984; Gargas *et al.*, 1989
"	*0.85*	*1.3*	2.0	Schwegler and Filser, 1989; Csanady and Filser, 1994

[a] Alveolar air, following 30 min exposure.
[b] Inspiratory air, following 30 min exposure.
[c] Numbers in *italics* were calculated from the authors' reported partition coefficients for blood/air and tissue/air.

Wigaeus *et al.* (1983) calculated a 13 percent uptake of styrene into fat during a 30 minute interval by eight men exposed to 69 ppm styrene vapor. The release of styrene from the adipose tissue of four of the men was incomplete by 21 hours after styrene exposure ended. For two men, the levels at 21 hours were 71 and 80 percent of the levels measured at 30 minutes. For the other two men, the levels at 21 hours were greater than those at cessation of exposure and decreased to 80 and 67 percent of the 30-minute levels at seven days. The authors estimated a styrene half-life in adipose tissue of two to four days and observed that the person with the largest amount of body fat appeared to clear the styrene at the slowest rate. Wigaeus *et al.* (1984) also measured venous blood levels of non-conjugated styrene-7,8-oxide and styrene glycol among four men exposed for two hours to 69 ppm air styrene and reported 0.05 μmol/L styrene-7,8-oxide and 2.1 μmol/L styrene glycol between five and 30 minutes exposure. Pierce *et al.* (1996) reported an *in vitro* fat/styrene vapor partition coefficient for humans (n = 7) of 3,184 ± 84 (SEM).

Distribution studies in laboratory animals were primarily conducted for inhalation exposures to radiolabeled styrene. Gargas *et al.* (1989) reported tissue/air styrene partition coefficients in isolated rat tissues as 3,476 for fat, 139 for liver, and 47 for muscle. By way of comparison, the liquid/air partition coefficient for saline was 1.4 and that for olive oil was 3,548. Withey and Collins (1979) measured styrene concentrations in perirenal fat of male Wistar rats after a five hour inhalation exposure to 53; 510; 1,100; 1,500; or 2,100 ppm styrene and found styrene levels of 9; 236; 416; 1,252; or 2,436 μg/g, respectively. Bergman (1983) exposed male NMRI mice to [^{14}C-ring]-styrene by inhalation for ten minutes and measured disposition of the label over a 48-hour period. The radioactive tracer was diluted with an unreported amount of unlabeled styrene, so the total administered styrene dose is unknown. The administered dose of radioactive styrene was reported to be 6.9 mg. Twenty-four hours following the ten-minute exposure, whole-body autoradiograms showed radiolabel in body fat. Radiolabel retained in kidney, liver, and bronchi provides evidence of firmly bound styrene metabolites in these organs.

Styrene distribution was measured in male Wistar rats exposed by inhalation to 50 to 2,000 ppm for five hours (Withey and Collins, 1979). The rate of styrene uptake into blood increased as a function of air styrene levels between one and five hours and was supralinear during the initial hour of exposure. The tissue/blood ratios showed that the distribution pattern among the organs changed with the air styrene levels. At low styrene levels (54 ppm), the tissue/blood styrene ratio was 2 for kidney and less than 1 for heart, lungs, liver, spleen, and brain. The ratio in kidney decreased to about 1 with increasing air styrene levels. In lung, the tissue/blood ratios were not statistically different from 1 between 470 and 2,240 ppm in air. In liver, a statistically significant increase of 1.5 to 2.2-fold in the tissue/blood styrene ratio was measured after exposure to 2,250 ppm styrene, compared to 54 ppm exposure.

7-[^{14}C]-Styrene was administered to F344 rats, B6C3F$_1$ mice, and CD1 mice via nose-only inhalation for six hours, and the distribution of radioactive label 48 hours post-exposure was reported for lung, liver, muscle, and abdominal fat (Sumner *et al.*, 1997). Among the animal strains tested, the rank order, based on body weight, of the concentration of radiolabel was B6C3F$_1$ mice > CD1 mice > F344 rats for lung tissue; no strain differences were observed for the other three tissue types.

Boogaard *et al.* (2000a) measured tissue levels of [^{14}C]-styrene in rodents following a six hour inhalation exposure to 160 ppm [^{14}C (ring-labeled)]-styrene and found only a negligible amount remaining 42 hours after exposure. Styrene was retained in the bronchi of rats and mice. Autoradiographs taken 42 to 45 hours after exposure showed radioactivity in lung tissue. The authors concluded that radioactivity was mainly located in discrete regions of the lungs and presumably in the bronchi. However, criteria for distinguishing the bronchi from other regions within the conducting airways or alveolar region (e.g., autoradiographs of sectioned lung slices) were not presented.

The systemic uptake of styrene from the lung has also been evaluated by measuring the levels of the urinary metabolites mandelic acid (MA) and phenylglyoxylic acid (PGA). The combined excretion of MA and PGA has been reported to be from 54 to 86 percent of the parent compound (Guillemin and Bauer, 1979; Wenker *et al.*, 2001b). Based on pulmonary uptake, MA was reported to represent from 14 to 57 percent (Astrand *et al.*,

1974; Guillemin and Bauer, 1979 Wigaeus *et al.*, 1983; Johanson *et al.*, 2000) of the total urinary metabolites.

Savolainen and Vainio (1977) administered 577 μmol [^{14}C]-styrene (position of label not specified but assumed to be on the aliphatic side-chain) by intraperitoneal (i.p.) injection to male Sprague-Dawley rats, and reported that the distribution of radiolabel at three hours post-injection was: kidney (445 nmol/kg) ≈ brain ≈ duodenum > liver > spinal cord > lungs > blood (23 nmol/mL). At six hours post-injection, the level of radiolabel increased in blood by 13 percent ($p<0.0005$) and the levels in duodenum and brain decreased by 20 and 29 percent, respectively ($p<0.005$ and $p<0.0025$). At 24 hours post-exposure, 10 to 25 percent of the radiolabel remained in these tissues.

The distribution of unmetabolized styrene and the metabolite styrene-7,8-oxide was studied in NMRI mice administered styrene via a single i.p. injection of 1 to 5 mmol 7-[^{14}C]-styrene/kg(bw) (Lof *et al.*, 1984). Within one hour following injection, the highest styrene concentrations occurred in the pancreas, while intermediate levels occurred in liver and kidney. The lowest levels occurred in lung, brain, and blood. At two hours post-injection, the highest styrene concentrations were found in subcutaneous adipose tissue. At two hours post-injection, a supralinear increase in tissue levels of styrene with increasing administered dose was observed for each of the above tissues except pancreas. Styrene-7,8-oxide was detected in each of the above tissues; the highest concentration occurred in subcutaneous fat. The rank order of tissue/blood ratios of styrene-7,8-oxide, taken 30 minutes after i.p. injection of 2.7 mmol/kg styrene and measured by two different analytical methods, was: subcutaneous fat (ratio by method 1 = 14; ratio by method 2 = 19) >liver (7; 8) > lung (3; 4) > kidney (2; 2) > blood (1;1). A linear increase in tissue levels of styrene-7,8-oxide was observed with increasing administered dose for all tissues examined two hours post-injection.

Metabolism

Styrene-7,8-oxide is the primary and most widely studied bioactive metabolite of styrene (see Figure 1). It is further metabolized to less reactive forms for elimination or possibly to other reactive species such as arene oxide, styrene-3,4-oxide, and 4-vinylphenol. The enzyme-catalyzed reactions, which result in the formation of styrene-7,8-oxide, involve metabolism of the vinyl side-chain of styrene. Evidence for metabolism of the aromatic ring has also been described.

For the purposes of discussion, styrene metabolism is divided into three major phases in this document: 1) bioactivation of styrene to the epoxide metabolite, styrene-7,8-oxide, 2) detoxification of styrene-7,8-oxide to styrene glycol or to styrene-7,8-oxide-reduced glutathione conjugates, and 3) formation of the urinary styrene metabolites MA and PGA.

Bioactivation - Styrene-7,8-oxide Levels in Humans and Animals

Styrene-7,8-oxide has been detected in the blood of humans exposed to styrene under controlled conditions or in workplace settings. Six men exposed for two hours to 69 ppm styrene in air with light exercise [defined as 50 watts of work or a heart rate of 93 bpm (Engstrom *et al.*, 1978)] exhibited a venous blood styrene-7,8-oxide level of 6 μg/L (Wigaeus *et al.*, 1984). Four men exposed for two hours to 50 ppm styrene in air with

light exercise exhibited an average of 0.8 μg/L styrene-7,8-oxide in the venous blood (Johanson *et al.*, 2000).

Eight men previously exposed in the workplace to a mean level of 10 ppm styrene were exposed under controlled conditions to 69 ppm styrene in air for two hours with light exercise, and exhibited venous blood styrene-7,8-oxide levels of 2 (limit of detection) to 4 μg/L (Lof *et al.*, 1986). Seven workers exposed to styrene (air concentration not specified) during the manufacture of unsaturated polyester containers exhibited an average of 0.09 μg/L styrene-7,8-oxide in the blood compared to an average of <0.02 μg/L among four office workers (Christakopoulos *et al.*, 1993). Among 13 male workers exposed to air styrene levels from 10 to 73 ppm in a reinforced polyester resin factory, venous blood styrene-7,8-oxide levels of 0.9 (limit of detection) to 4 μg/L were found (Korn *et al.*, 1994). Styrene-7,8-oxide was not detected in the blood of control subjects.

Tornero-Velez *et al.* (2001) reported blood styrene-7,8-oxide levels from about 0.06 to 0.14 μg/L among 35 styrene workers in the reinforced plastics industry. The authors commented that comparisons among field studies are difficult due to: 1) differences in analytical procedures, 2) the reactivity of styrene-7,8-oxide towards tissue macromolecules, and 3) the possible contamination of styrene-related styrene-7,8-oxide with airborne styrene-7,8-oxide formed by chemical oxidation of styrene.

Styrene-7,8-oxide has been detected in styrene-exposed laboratory animals. Male $B6C3F_1$ mice exposed by inhalation to 250 ppm styrene exhibited an eight hour cumulative styrene-7,8-oxide blood level of 3.1 μM-hour (Leavens *et al.*, 1996). Female and male CD-1 mice, exposed to 0, 20, 40, 80, or 160 ppm styrene by inhalation six hours/day for 74 weeks, exhibited blood styrene-7,8-oxide levels from 0.01 to 0.29 μM (1 to 30 μg/L) at the three highest doses. The dose-response suggested saturation of styrene-7,8-oxide metabolism (Cruzan *et al.*, 2001). NMRI mice received 521 mg styrene/kg (bw) by i.p. injection and exhibited a blood styrene-7,8-oxide level of 0.6 μg/gm wet weight tissue (Lof *et al*., 1984). Fischer 344 rats (n = 10), exposed by inhalation to 1,000 ppm styrene six hours/day for four days, exhibited a ten-hour cumulative styrene-7,8-oxide blood level of 3.3 μg styrene-7,8-oxide/gm blood-hour (Mendrala *et al.*, 1993). Sprague-Dawley rats, exposed by inhalation to 0, 50, 200, 500, or 1,000 ppm styrene for six hours/day for 95 weeks, exhibited blood styrene levels from 0.29 to 30 μg/mL in females and 0.43 to 33 μg/mL in males. Styrene-7,8-oxide blood level at an air styrene level of 50 ppm was below the level of quantitation, while the levels at 200, 500, and 1,000 ppm were 0.028, 0.092, and 0.15 μg/mL in females and 0.066, 0.12, and 0.18 μg/mL in males, respectively (Cruzan *et al.*, 1998).

As a marker of exposure, styrene-7,8-oxide has been measured in humans and animals in the form of either hemoglobin (Hb) adducts or DNA adducts. Johanson *et al.* (2000) reported a styrene-7,8-oxide-Hb level of 0.3 pmol/g globin among four men exposed to 50 ppm air styrene under light exercise. No adducts were found in pre-exposure samples. Styrene-7,8-oxide-Hb adducts were also detected in 11 styrene exposed workers in a glass fiber factory, whereas none were detected in workers from a piano manufacturing plant (Liu *et al.*, 2001). The air styrene levels ranged from 4.6 to 69 ppm and the major Hb adduct (2-phenylethylcysteine) was present at 1.6 to 2.8 mmol/g globin. Adducts in which the styrene-7,8-oxide was adducted to Hb carboxyl groups or the thiol of a

cysteine group were present at lower levels. The results showed a dose-response relationship between the air level of styrene and the 2-phenylethylcysteine residue, but not with the 1-phenylethyl cysteine or albumin adducts. Christakopoulos *et al.* (1993) monitored workers exposed to styrene (air levels not specified, but inferred from urinary metabolites) during the manufacture of unsaturated polyester containers. The authors reported an average styrene-7,8-oxide-Hb adduct level of 28 pmol/g among seven styrene-exposed workers and ≤ 13 pmol/g among three unexposed office workers..

Severi *et al.* (1994) were unable to detect styrene-7,8-oxide-Hb adducts in the blood of 52 styrene workers exposed to an average of 7.3 ppm styrene, although the authors noted that the urinary metabolite levels were lower in their study than those measured by Christakopoulos *et al.* (1993).

Rappaport *et al.* (1996) concluded from their analysis of styrene-7,8-oxide adducts of Hb and albumin in styrene workers that the major contributor to the adducts was chemically produced airborne styrene-7,8-oxide rather than styrene-7,8-oxide endogenously formed from inhaled styrene. Airborne styrene-7,8-oxide may be produced chemically from styrene in the presence of workplace oxidizing agents. In 12 fiberglass-reinforced plastics factories, Tornero-Velez *et al.* (2000) reported a ratio of air styrene to air styrene-7,8-oxide of about 1,000:1. The air styrene-7,8-oxide hypothesis was considered in a physiologically-based pharmacokinetic (PBPK) model (Tornero-Velez and Rappaport, 2001). Among the assumptions of the model is the restriction of styrene metabolism to the liver. However, there is evidence for the metabolism of styrene in human lung and blood (Nakajima *et al.*, 1994a; Belvedere and Tursi, 1981) as well as evidence for variability in styrene metabolism in the human population (e.g., Kim *et al.*, 1997; Wenker *et al.*, 2000). Rappaport *et al.* (1996) also commented that styrene was the major contributor to the internal styrene-7,8-oxide dose in 5 of 20 subjects. As previously discussed, humans exposed to styrene under controlled conditions, including those that prevented the formation of exogenous styrene-7,8-oxide, exhibited measurable levels of blood styrene-7,8-oxide (Wigaeus *et al.*, 1984; Johanson *et al.*, 2000) and styrene-7,8-oxide-Hb adducts (Johanson *et al.*, 2000). These data support the endogenous formation of styrene-7,8-oxide following inhalation exposure to styrene in the workplace.

Male NMRI mice were exposed by i.p. injection to doses from 1 to 5 mmol styrene/kg. Three to 60 nmol styrene-7,8-oxide/g Hb were observed two hours after treatment (Nordqvist *et al.*, 1985). The authors injected a different group of mice with 0.04 to 1 mmol styrene-7,8-oxide/kg and two hours later observed Hb adducts at levels that ranged from 0.1 to 13 nmol/g Hb. Nordqvist *et al.* (1985) reported the binding of styrene-7,8-oxide to plasma proteins in the samples used for Hb adduct analyses. The authors noted a supralinear dose-response relationship for Hb binding and suggested that the results may be due to: 1) saturation of styrene-7,8-oxide metabolism; 2) the increased importance of metabolic activation of styrene by erythrocytes; or 3) the decreased rate of styrene-7,8-oxide elimination. Styrene-7,8-oxide-Hb adducts, at levels of about 0.05 to 0.65 nmol/g Hb, were observed in the blood of female and male mice injected i.p. with about 0.4 to 2.5 mmol styrene/kg (Osterman-Golkar *et al.*, 1995). Male Sprague-Dawley rats also exhibited increasing levels of the Hb adducts after the same styrene exposure. The authors reported that the dose-response curve in mice suggested that the further metabolism of styrene-7,8-oxide was saturated at about 1.0 nmol styrene/kg. A similar

dose-response curve was observed when the mice were injected with styrene-7,8-oxide (at lower doses compared to styrene). Deviation from linearity was observed to a lesser extent in rats.

Bioactivation - Cytochrome P_{450}-Dependent Enzymatic Metabolism

Cytochrome P_{450}-dependent monooxygenases in the liver, lung, and blood are the primary enzymes involved in converting styrene to styrene-7,8-oxide. Formation of styrene-7,8-oxide in tissue can be assayed by: 1) direct analysis following extraction and chromatography (Carlson, 1997), 2) chemical conversion to the stable styrene glycol (Belvedere and Tursi, 1981; Mendrala *et al.*, 1993), or 3) the enzymatic conversion of styrene-7,8-oxide to styrene glycol (Nakajima *et al*., 1994a; Wenker *et al*., 2001a).

Quantitatively, liver is the most important organ for cytochrome P_{450}-dependent metabolism of styrene to styrene-7,8-oxide in humans and laboratory animals, particularly after ingestion exposure (Elovaara *et al.*, 1991; Nakajima *et al.*, 1994a,b; Kim *et al.*, 1997; Carlson, 1997). Nakajima *et al.* (1994a) reported cytochrome P_{450}-dependent styrene-7,8-oxide formation in isolated microsomal fractions from human liver (0.7 and 1.9 nmol/min-mg protein at 0.085 mM and 1.85 mM styrene, respectively). Carlson *et al.* (2000) reported the formation of styrene-7,8-oxide by the microsomal fraction from 5 of 6 human livers to be 0.91 nmol/mg protein-minute in the presence of 2 mM styrene. Mendrala *et al.* (1993) reported one kinetic form of cytochrome P_{450}-dependent styrene-7,8-oxide formation in human liver microsomes over a range of 0.02 to 2.5 mM styrene. Wenker *et al.* (2001a) reported two kinetic cytochrome P_{450}-dependent styrene metabolizing forms in the microsomal fraction of human liver (at 0.016 and 1.1 mM styrene).

Cytochrome P_{450}-dependent metabolic activity in lung tissue and human erythrocytes and lymphocytes is also significant for the biotransformation of styrene following inhalation exposure to styrene (Nakajima *et al.*, 1994a; Carlson, 1998; Belvedere and Tursi, 1981).

The metabolic capacity of lung, although less than that of liver, is important for defense against inhaled and blood derived toxicants (Minchin and Boyd, 1983). In lung, cytochrome P_{450}-dependent activities range from 0.006 to 0.012 nmole/min-mg protein) (Nakajima *et al.*, 1994a). Green *et al.* (2001) demonstrated *in vitro* styrene metabolism in the nasal tissue of rats and mice with a relatively higher level in the microsomal fraction of the nasal olfactory epithelium compared to the nasal respiratory epithelium. Nasal metabolism of styrene in male CD1 mice exposed to 160 ppm for six hours has also been suggested by toxicological data (Foster, 1999).

Among the 40 lung cell types, bronchiolar non-ciliated Clara cells and alveolar epithelial Type II cells are associated with cytochrome P_{450}-dependent monooxygenase activity (Devereux *et al.*, 1979; Rabovsky *et al.*, 1989). Clara cells in the bronchioles possess metabolic, secretory, and stem cell functions (Hook *et al.*, 1990). Type II cells, located on the alveolar epithelial surface, function in xenobiotic metabolism, surfactant synthesis and secretion, water transport, and repair of injured alveolar epithelium (Castranova *et al.*, 1988). Although data on isolated Type II cells in the absence of Clara cells were not found, Hynes *et al.* (1999) reported a higher level of styrene-7,8-oxide formation from styrene in fractions enriched with Clara cells compared to fractions enriched with Type II cells from mouse or rat. These authors also reported a lower rate of styrene metabolism

in rat Clara cells, compared to mouse. In a study on the histochemical properties of mouse lung tumors induced by styrene, Brown (1999) reported evidence for Type II cells in the tumors. These results suggest an inconsistency between the mouse lung tumors that are associated with alveolar Type II cells (Brown, 1999) and the higher level of styrene metabolic activation associated with Clara cells (Hynes *et al.*, 1999).

Cruzan *et al*. (2009) investigated the activity of the CYP2F isoforms in mouse, rat, and human lungs, relative to metabolism of styrene. They conclude that rat CYP2F4 appears to be equally active as the mouse CYP2F2 isoform in metabolizing styrene and related chemicals. However, the amount of the enzyme is less in the rat Clara cells, and the resulting levels of metabolites are lower, which they consider insufficient to cause cytotoxicity in the rat lung. They found that microsomes prepared from human lungs produced little or no styrene metabolites, and that human lungs contain fewer Clara cells than mice or rats. Cruzan *et al.* also provide the opinion that humans should be much less sensitive than mice to toxicity due to the metabolites, because of differences in the morphology of their Clara cells. They conclude that it is unlikely that chemicals that cause mouse lung tumors via CYP2F2 metabolism will cause lung tumors in humans via the corresponding CYP2F1 isozyme. However, styrene is associated with many toxicity endpoints, including pneumotoxicity, and information on the apportionment of tissue-specific metabolism to each toxicity endpoint in the three species is not available.

The metabolic characteristics of cytochrome P_{450}-mediated styrene metabolism in one tissue cannot be directly extrapolated to other tissues, because cytochrome P_{450}-dependent monooxygenases represent a large family of distinct isozymes which show a lack of concordance among tissues. For example, cytochrome P_{450}-dependent isozymes identified in human whole lung tissue (e.g., CYP1A and -2F1) were not present in human lymphocytes. CYP1A1, first identified in human liver, was only observed in the lungs of current smokers (Hukkanen *et al.*, 1997). Krovat *et al*. (2000) reported that the inducible expression of two cytochrome P_{450}-dependent isozymes observed in a liver cell line was minimal in blood cell lines.

Nakajima *et al.* (1994a) measured human styrene metabolism associated with individual human cytochrome P_{450}-dependent isozymes by infecting HepG2 cells with Vaccinia virus-expressed human cytochrome P_{450} forms. The rank order of styrene metabolizing activity (and activity relative to CYP2B6) was CYP2B6 (100) > -2F1 (87) > -1A2 (53) ≈ -2E1 (53) > -2C8 (40) > -3A3/4 (20) > -4B1 (11) > -3A5 (10). Because many P450 isozymes can activate styrene, it is likely that nearly all adult humans can form styrene-7,8-oxide. Some of these isozymes have also been detected in children and neonates. CYP3A4 (the major cytochrome P450 isozyme in adult liver) and CYP1A2 activities have been detected in neonates (< 6 months old); their activity is lower than in adults (Dorne *et al*., 2005). CYP3A4 and CYP1A2 are present in children and their activity is about 20-25 percent greater than in adults. There are no reports on the ability of CYP3A7, the major fetal cytochrome P450, to activate styrene. In rats, Nakajima *et al.* (1994b) report data that are consistent with roles for CYP2E1 and CYP2C in liver styrene metabolism and for CYP2B1/2 in lung metabolism. In mouse liver, CYP2E1, -2B and -2F1 but not CYP1A, appear to play a role in the conversion of styrene to styrene-7,8-oxide (Carlson, 1997; Carlson *et al.*, 1998).

Cytochrome P_{450}-mediated epoxidation of styrene might occur in the lung following inhalation exposure. The cytochrome P_{450}-dependent isozymes CYP2E1 and CYP3A were identified in human lung using immunochemical techniques (Wheeler *et al.*, 1992). Willey *et al.* (1996) reported the presence of CYP1A1, -1B1, -2E1, and -4B1 in human primary bronchiolar epithelial cells of non-smokers. In a mouse lung microsomal fraction, CYP2E1 and -2F, and perhaps to a minor extent CYP1A and/or -2B, may be important for bioconversion of styrene to styrene-7,8-oxide (Carlson, 1997; Carlson *et al.*, 1998).

Data on the enantiomeric forms (R, S) of styrene-7,8-oxide suggest that mouse and rat lung Clara cells and Type II cells contribute to cytochrome P_{450}-dependent styrene-7,8-oxide formation and that differences between them can be detected in the enriched cell preparation (Hynes *et al.*, 1999). In mouse lung the R/S ratio for styrene-7,8-oxide formation is about four for both Clara cell- or Type II cell-enriched preparations. In rat lung, the R/S ratio is one for Clara cell-enriched preparations and 0.5 in Type II cell-enriched preparations. Relevance of the enantiomer selectivity of cytochrome P_{450}-dependent styrene-7,8-oxide formation by the two cell types is unclear. For styrene-induced cytogenetic alterations in CD-1 mice, the R/S ratio was 0.6 to 0.7 (Sinsheimer *et al.*, 1993). For mutagenicity against *Salmonella typhimurium* (TA100) the ratio ranged from 1.3 to > 2 (Pagano *et al.*, 1982; Seiler, 1990; Sinsheimer *et al.*, 1993). *In vitro* inhibitor experiments with isolated Clara cells suggested a role for CYP2F in the activation of styrene to styrene-7,8-oxide (Hynes *et al.*, 1999). The apparent lack of involvement of CYP2E in Clara cell styrene bioactivation may reflect an intrinsic property of Clara cell CYP2E or it may be due to a lack of uptake of the CYP2E inhibitor diethyldithiocarbamate by the Clara cell preparation. The authors suggested that styrene metabolism in lung tissue occurs in the bronchiolar epithelial Clara cell and not the alveolar Type II cell.

Hukkanen *et al.* (1997) reported CYP2E1 in human lung, bronchiolar-alveolar macrophages, and blood lymphocytes, and –2B6/7 and –2F1 in human lung and bronchiolar-alveolar macrophages. Hodges *et al.* (2000) reported CYP2E1 in human bone marrow and bone marrow derived cells. Bernauer *et al.* (2000) identified CYP2E1 immunochemically in human bone marrow stem cells. CYP1B1, -2B6, and -2E1, and ethanol inducible CYP3A3/4 were identified in human blood monocytes and macrophage subsets (Baron *et al.*, 1998). Other authors detected the messenger RNA (mRNA) expression of potential styrene-metabolizing enzymes, which suggests that these tissues could be sources of human *in-situ* cytochrome P_{450}-mediated epoxidation of styrene.

Cytochrome P_{450}-dependent metabolism can be characterized by the kinetic properties of the isozymes. Commonly used K_m and V_{max} values for human and rodent cytochrome P_{450}-dependent enzymes from liver that catalyze the transformation of styrene to styrene-7,8-oxide are provided in Table 5. Studies on the kinetic properties of cytochrome P_{450}-dependent styrene-7,8-oxide formation in human lung or blood were not found in the published scientific literature.

Table 5. K_m and V_{max} Values of Enzymes Involved in Styrene Metabolism in Isolated Liver Microsomal Fractions[1]

Species	Activity					
	Cytochrome P_{450}		Epoxide Hydrase		Glutathione Transferase	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
Mouse	0.05[a] 0.03(S)[a]	13[a] 8.5(S)[a]	0.74[a]	0.15[a]	0.26(SO)[a]	47(SO)[a]
Rat	0.06(SD)[a] 0.03(SD,S)[a] 0.09(F)[a] 0.07(F,S)[a]	9.3(SD)[a] 7.9(SD, S)[a] 11(F)[a] 14(F, S)[a]	0.029 (R, SO, W)[d] 0.16 (S, SO, W)[d] 0.13(SD)[a] 0.23(F)[a]	12 (R, SO)(W)[d] 44 (S, SO)(W)[d] 14[a] 15[a]	0.17(SO)[a]	39(SO)[a]
Human	0.09[a] 0.07[b] 0.12[b] 0.01[c]	2.1[a] 1.2[c] 3.0[c] 0.96[c]	0.01[a] 0.02(R, SO)[e] 0.14(S, SO)[e]	15[a]	4.7(SO)[f] 0.04[f] 0.9[f]	34(R, SO)[e] 168(S, SO)[e] 22(SO)[f] 9[f] 23[f]

Abbreviations: SD = Sprague-Dawley rat; F = Fischer 344 rat; W = Wistar rat; S = animals pre-exposed to styrene; R and SO refer to the individual enantiomeric forms of styrene-7,8-oxide.

[1] K_m in mM and V_{max} in nmol/min-mg protein.

[a] Mendrala *et al.*, 1993.

[b] Nakajima *et al.*, 1994a. The K_m values for Vaccinia virus-expressed CYP2B6 and CYP2E1 catalyzed styrene metabolism were 0.18 and 0.1 mM, respectively.

[c] Wenker *et al.*, 2001a. The K_m and V_{max} are associated with a high affinity form.

[d] Watabe *et al.*, 1981.

[e] Wenker *et al.*, 2000.

[f] Pacifici *et al.*, 1987.

Nakajima *et al.* (1994a) identified a high and low affinity form (0.07 and 0.12 mM, respectively) of cytochrome P_{450}-dependent enzymes for styrene in liver. When CYP2B6 and CYP2E1 mediated reactions were analyzed separately, the observed K_m values of 0.18 mM and 0.1 mM, respectively, appeared to reflect the high K_m form present in human liver. Wenker *et al.* (2001a) also detected a high affinity liver form with a K_m of 0.011 mM and a low affinity form. The low affinity form was not characterized by its K_m but rather by an intrinsic clearance (V_{max}/K_m, first-order rate constant, Siegel, 1975), which was 0.007 times that of the high affinity form.

The *in vitro* cytochrome P_{450}-dependent enzyme V_{max} data suggest that humans metabolize styrene to styrene-7,8-oxide at a slower rate than rats or mice (by about 16 and 27 percent, respectively). When *in vitro* liver microsomal V_{max} values are re-calculated based on total animal or unit body weight, the V_{max} for human liver cytochrome P_{450}-dependent styrene metabolism is about 5 to 8 percent of the V_{max} for mice or rats (Mendrala *et al.*, 1993). These differences between humans and rodents are

minimized when V_{max} values are calculated based on body weight raised to an exponent of two-thirds ($BW^{2/3}$) (Anderson *et al.*, 1983) or three-fourths ($BW^{3/4}$) (U.S. EPA, 1992).

Enantioselectivity of Styrene Metabolism

Styrene metabolism is enantioselective due to the presence of chirality in metabolites such as styrene-7,8-oxide, styrene glycol, MA, and the mercapturic acids. Enantioselectivity may impact styrene tumorigenicity and genotoxicity if such activities are also enantioselective. An important aspect of styrene metabolism is that the enantioselectivity will depend on the properties of the enzymes that catalyze the formation and on the further metabolism of the chiral metabolites.

In humans, the enantioselectivity of styrene metabolism has been studied in workers and in isolated tissue fractions. Korn *et al.* (1984) observed a levorotatory/dextrarotatory (L/D) ratio of urinary MA from styrene-exposed workers of 1.6 in men (n = 11) and 1.4 in women (n = 6). Hallier *et al.* (1995) reported the excretion of MA from 20 male styrene-exposed workers with an R/S ratio of 1 ± 0.3 (range: 0.7 to 2.2). Among three workers exposed to 99 ppm styrene, MA stereoisomers were excreted with an R/S ratio of 1.2, 1.3, and 1.1 (Drummond *et al.*, 1989). The R/S ratio of styrene-7,8-oxide formed from 2 mM styrene by isolated human liver microsomal fractions from six donors was reported as 0.7 ± 0.4, where the individual R/S ratios were 1.1, 1.0, 0.9, 0.5, 0.1, and 0 (i.e., no R-styrene-7,8-oxide formed) (Carlson *et al.*, 2000). In the one (out of six) lung sample that exhibited styrene metabolic activity, the R/S ratio was 1.2 (Carlson *et al.*, 2000). The *in vitro* enantioselectivity of cytochrome P_{450}-dependent styrene-7,8-oxide formation in human liver (n = 20) was studied by Wenker *et al.* (2001a) at two styrene levels, 0.016 and 1.1 mM. The authors reported a 15 percent selectivity for the S-form at low substrate concentration and a seven percent selectivity for the R-form at high styrene concentration. They further commented that the data were not normally distributed. The results suggest that the enantioselectivity of styrene metabolism in humans is not absolute and may depend on exposure levels.

In an *in vitro* rat reconstituted styrene metabolizing liver cytochrome P_{450}-dependent system, the R/S ratio of styrene-7,8-oxide varied from 0.6 to 1.3 depending on pretreatment with non-styrene inducers (Foureman *et al.*, 1989). In an *in vitro* rat liver microsomal study, the cytochrome P_{450}-dependent formation of styrene-7,8-oxide from styrene proceeded with an R/S ratio of 0.8 (Watabe *et al.*, 1981). In an isolated rat liver microsomal fraction, styrene-7,8-oxide formation from styrene proceeded with an R/S ratio of 0.6 (Hynes *et al.*, 1999). The R/S ratio of styrene-7,8-oxide formed from styrene in isolated rat lung microsomal fraction was 0.5 and that formed in a lung cell preparation enriched for alveolar Type II cells was 0.5 (Hynes *et al.*, 1999).

The *in vivo* enantioselectivity of styrene metabolism in mice was studied by analyzing urinary metabolites (Linhart *et al.*, 2000). A slight excess of S-styrene-7,8-oxide was produced, compared to R-styrene-7,8-oxide. In mouse liver microsomal fraction, the R/S ratio of styrene-7,8-oxide formed through cytochrome P_{450}-dependent styrene metabolism is one, whereas the ratio produced in mouse lung microsomal fraction is two (Carlson, 1998; Hynes *et al.*, 1999). In isolated mouse lung cells enriched for either bronchiolar Clara cells or alveolar Type II cells, the R/S ratio of styrene-7,8-oxide is about 4 (Hynes *et al.*, 1999). Thus, the relative amounts of R- and S-isomers may be tissue specific.

Polymorphisms of Styrene Metabolism

One or more polymorphisms for styrene metabolism exist in the human population. Kim *et al.* (1997) reported inter-individual variability in cytochrome P_{450}-dependent styrene metabolism in human liver. The authors studied styrene metabolism in isolated liver microsomal fractions of Northern European (Finnish) and Asian (Chinese) subjects. The rate among the Finnish was less than that among the Chinese (0.42 vs. 0.72 nmol/min-mg protein, respectively, $p<0.05$). However, this difference was observed only at a low substrate concentration (0.085 mM) and not at a higher substrate concentration (1.85 mM styrene). The authors also reported differences in some cytochrome P_{450}-dependent isozymes between the two groups. CYP1A2 and -2B6 occurred at lower levels in isolated liver microsomal fractions from the Chinese subjects compared to the Finnish subjects, whereas the reverse was true for CYP2E1. The level of CYP2C8 was similar between the two groups. One explanation for the effect of substrate concentration on the detection of differences in metabolism may be the presence of two kinetic forms of cytochrome P_{450}-dependent styrene-7,8-oxide formation with K_m of 0.08 and 1.2 mM (Nakajima *et al.*, 1994a).

Vodicka *et al.* (2001b) reported associations between two CYP2E1 polymorphisms and genotoxicity endpoints in the lymphocytes of styrene workers. Among the total population (exposed plus controls), DNA single strand breaks were associated with polymorphisms in the 5'-flanking region and in intron 6, whereas the same correlation was not observed when the populations were considered separately. Among the styrene exposed population, the mutation frequency at the *hprt* locus was associated with a polymorphism in the 5'-flanking region of CYP2E1. Within the total population an association was detected for CYP2E1 intron 6. The authors suggested that different outcomes, observed with and without control data, may reflect the effect of non-styrene exposures on some genotoxic endpoints. Cytochrome P_{450}-dependent enzyme gene products may also possess more than one function in terms of substrate specificity.

Jang *et al.* (1997) noted a significant ($p<0.05$) difference in the end-of-exposure urinary PGA between Asian men (73 mg/g creatinine, n = 6) and Caucasian men (117 mg/g creatinine, n = 6) exposed to 50 ppm air styrene for six hours. The authors also noted an increased consumption of ethanol among the Asians compared to the Caucasians, but this difference was not significant. Styrene levels in the breath of individuals on the following day also differed between the Asians and Caucasians (0.07 and 0.04 ppm, respectively, $p<0.05$).

The *in vivo* uptake and distribution of the drug chlorzoxazone was studied in 20 Japanese men and 20 U.S. Caucasian men (Kim *et al.*, 1996). Chlorzoxazone is considered a model substrate for CYP2E1 (Omiecinski *et al.*, 1999), one of the enzymes considered important in the human liver metabolism of styrene (Nakajima *et al.*, 1994a). Following oral administration, Kim *et al.* (1996) observed decreases in Japanese compared to Caucasian subjects for oral clearance, fraction clearance of the drug metabolite, and the plasma concentration-time curve drug metabolite/parent drug ratio. Such a difference in P_{450}-2E1 could affect styrene metabolism and disposition. However, Kim *et al.* (1997) reported that styrene metabolism in the liver microsomal fraction of Chinese Asians was greater than that of U.S. Caucasians at low styrene concentration. Morel *et al.* (1999) also point out that substrate specificity for CYP2E1 is not absolute. Hence, the use of

model substrates may lead to inaccurate conclusions about the detailed characteristics of human styrene metabolism.

Non-cytochrome P_{450}-mediated Bioactivation

An important characteristic of cytochrome P_{450}-dependent enzymes is the presence of the iron-containing porphyrin moiety. Other enzymes that possess a porphyrin moiety are also capable of catalyzing the reaction. Non-cytochrome P_{450}-dependent enzymes that catalyze epoxidation of styrene include prostaglandin synthase (Guengerich, 1990), myeloperoxidase (Tuynman *et al.*, 2000), cytochrome c peroxidase (Miller *et al.*, 1992), and chloroperoxidase (Geigert *et al.*, 1986). Ferrodoxin, a non-heme iron enzyme, also catalyzes epoxidation of styrene (Belvedere *et al.*, 1982).

While the rates of these activities may be less than the cytochrome P_{450}-mediated reactions, under certain exposure conditions where styrene enters the systemic circulation prior to entering the liver, non-cytochrome P_{450}-dependent bioactivation reactions in lung may have implications for styrene-related carcinogenicity. For example, prostaglandin synthase, a lung enzyme located primarily in the alveolar region (Minchin and Boyd, 1983), catalyzes the conversion of styrene to styrene-7,8-oxide (Guengerich, 1990). The epoxidation of benzo(a)pyrene-7,8-diol by myeloperoxidase has been reported in lung cells from mice that lacked the receptor for cytochrome P_{450}-dependent enzyme induction (Petruska *et al.*, 1992). A polymorphism that may reduce the risk of lung cancer has been reported for the human myeloperoxidase gene (London *et al.*, 1997; Cascorbi *et al.*, 2000). These results provide an alternate mode of action for the *in situ* metabolic formation of the carcinogenic agent.

Myeloperoxidase can also catalyze the *in vitro* epoxidation of styrene in bone marrow and lung (Ross, 1996; Tuynman *et al.*, 2000). Belvedere and Tursi (1981) and Belvedere *et al.* (1983) reported the bioactivation of styrene in human blood. Although the rate of styrene-7,8-oxide formation is greater in lymphocytes than in erythrocytes, the requirement for reducing equivalents needed for cytochrome P_{450}-dependent monooxygenation is less stringent in erythrocytes (Belvedere and Tursi, 1981). Ortiz de Montellano and Catalano (1985) observed that hemoglobin-dependent epoxidation of styrene exhibited characteristics different from the cytochrome P_{450}-dependent reaction (e.g., partial scrambling of the reaction stereospecificity). Norppa and Tursi (1984) described enhancement of sister chromatid exchanges (SCEs) in human lymphocyte cultures in the presence of erythrocytes, under conditions where such enhancement was not observed for the known SCE inducer cyclophosphamide.

Co-exposures to Other Chemicals

For humans exposed to styrene in the workplace there are at least three potential chemical co-exposures (butadiene, ethanol, and acetone) that might influence the rate of styrene bioactivation from cytochrome P_{450}-dependent reactions. Co-exposure to a fourth industrial chemical, ethylbenzene, has been associated with increased urinary MA and PGA in humans (Bardodej and Bardodejova, 1970). In addition, pre-exposure to styrene and cigarette smoke induces styrene bioactivation in laboratory animals and humans.

Butadiene is a quantitatively important co-exposure in the butadiene-styrene rubber industry. Measurements of blood styrene and styrene-7,8-oxide in male B6C3F1 mice,

exposed by inhalation to styrene (0, 50, 100, or 250 ppm) and/or 1,3-butadiene (0, 100, or 1,000 ppm), revealed metabolic interactions (Leavens *et al.*, 1996). When styrene uptake was measured as the difference between the levels of air styrene in the chamber, there was no effect of any level of butadiene on styrene uptake into the lung. However, the eight-hour level of styrene in the blood of the mice was increased in the presence of 1,000 ppm butadiene compared to the blood styrene level in the mice exposed only to styrene. The enhancement occurred at all styrene exposure levels. Among the mice exposed to 250 ppm styrene, a 1,000 ppm butadiene co-exposure increased the blood levels of styrene-7,8-oxide compared to levels in mice exposed only to styrene. The styrene-butadiene co-exposure appeared to involve metabolic interactions either in the activation of one or more cytochrome P_{450}-dependent isozymes that act on styrene or from inhibition of enzymes that act on styrene-7,8-oxide.

Ethanol is a known inducer of cytochrome P_{450}-dependent enzyme activity, in particular CYP2E1 (Morel *et al.*, 1999; Omiecinski *et al.*, 1999). Nakajima *et al* (1994b) showed that the livers of Wistar rats which received ethanol metabolized styrene to styrene-7,8-oxide at a greater rate than control rats. CYP2E1 was induced in liver but not in lung. The ethanol-induced activity was dependent on styrene concentration. Studies with perfused livers from male Wistar rats showed that induction by ethanol of styrene metabolism occurred in the fasted state, whereas in the fed state styrene metabolism was suppressed (Sripaung *et al.*, 1995). The changes may have been related to changes in pyridine nucleotide levels, which affect cytochrome P_{450}-dependent metabolism.

The effect of ethanol on cytochrome P_{450}-mediated metabolism of styrene in humans is not known. Girre *et al.* (1994) studied metabolism of the CYP2E1 model substrate chlorzoxazone in alcoholic patients with liver disease and found little difference in the total excretion of the chlorzoxazone metabolite due to ethanol intake compared to non-alcoholic patients. However, two hours after ingestion of the drug, the alcoholic patients exhibited a significant increase in ratio of the plasma metabolite/parent compound; the ratio was reduced following a seven day abstinence. The relationship between the results with the model drug and the transformation of styrene to styrene-7,8-oxide is unknown.

Ethanol may affect other enzymes in the styrene metabolic pathway. Reversible inhibition of styrene glycol dehydrogenase by ethanol resulted in a decrease in urinary MA at the expense of styrene glycol. This effect of ethanol has implications for biomonitoring of MA. The excretion of urinary MA is delayed when ethanol is also ingested. An accurate level of urinary MA can be obtained by carrying out one measurement of MA many hours after the ingestion of ethanol or carrying out MA measurements from start of work-shift until retiring at night (Wilson *et al.*, 1983; Berode *et al.*, 1986). These factors were considered in the establishment of workplace monitoring protocols for styrene (ACGIH, 1997).

Acetone, frequently found in the reinforced plastics industry workplace, is an inducer of cytochrome P_{450}-mediated metabolism of xenobiotics (Morel *et al.*, 1999; Omiecinski *et al.*, 1999). Wigaeus *et al.* (1984) found that, among six males exposed for two hours to 69 ppm styrene vapors with or without a concomitant exposure to 522 ppm acetone, there was no difference in arterial blood styrene levels (about 19 μmol/L) or in arterial blood styrene glycol (about 3.1 μmol/L) levels. Among 44 fiberglass workers who were exposed to about 48 ppm styrene and about 118 ppm acetone, the presence of acetone

appeared not to interfere with the excretion of MA or PGA (De Rosa *et al.*, 1996). The data suggest that simultaneous exposure to acetone does not alter styrene uptake or metabolism among humans. Among rats, an increased rate of styrene metabolism was observed in isolated microsomes from acetone exposed animals compared to sham controls. However, the rate of styrene metabolism in rats exposed to acetone plus styrene was similar to that in rats exposed only to styrene (Elovaara *et al.*, 1991).

Styrene metabolism has been studied in laboratory animals following pre-exposure to styrene. Elovaara *et al.* (1991) observed an increased rate of styrene metabolism (compared to sham controls) in isolated liver microsomal fraction in Wistar rats exposed by inhalation to 493 ppm styrene for 24 hours. The increased rate was consistent with increased liver CYP2E1. At lower styrene vapor levels, no increase in the activity associated with liver CYP2E1 was observed (Truchon *et al.*, 1990). Mendrala *et al.* (1993) observed no effect on liver or lung microsomal metabolism of styrene in Sprague-Dawley or Fischer 344 rats exposed by inhalation to 1,000 ppm styrene for six hours/day for four days. Exposure of Wistar rats by gavage to 0, 5, or 10 mmol styrene/kg-day for four days resulted in increased styrene-7,8-oxide production by liver microsomes, but no enhancement of the reaction in lung microsomes (Nakajima *et al.*, 1994b). Rat liver CYP1A was unaffected by *in vivo* styrene exposure through inhalation (Elovaara *et al.*, 1991) or gavage (Nakajima *et al.*, 1994b).

An increased styrene metabolism in lung in smokers may be due to the presence of styrene in cigarette smoke (Wallace *et al.*, 1996).

Other Epoxide Formation

Ring hydroxylation of styrene to form the arene oxide styrene-3,4-oxide has been considered to play a minimal to non-existing role in mammalian styrene metabolism (Leibman, 1975). However, the presence of styrene-3,4-oxide in exposed humans and experimental animals has been inferred from the measurement of the stable metabolite, vinyl phenol, at a fraction of a percent of the level of urinary MA (Bakke and Scheline, 1970; Pantarotto *et al.*, 1978; Pfaffli *et al.*, 1981). In workers exposed to 130 ppm styrene, Pfaffli *et al.* (1981) reported urinary 4-vinylphenol at about 0.04 nmol/g creatinine, which was estimated to be 0.3 percent of the level of urinary MA. Among 174 styrene-exposed workers in three European cohorts and 26 volunteers exposed to 50 mg/m^3 (11.8 ppm) styrene for 8 h, the glucuronide and sulfate conjugates of 4-vinylphenol represented approximately 0.5-1 percent of the total excretion of styrene metabolites (Manini *et al*., 2003).

In mice and rats, 4-vinylphenol (a.k.a. 4-hydroxystyrene) is short-lived and is metabolized under conditions consistent with a role for cytochrome P_{450}-dependent enzymes (Watabe *et al.*, 1984; Carlson *et al.*, 2001). The 48-hour level of 4-vinylphenol in the Pantarotto *et al.* (1978) rat study was estimated as 0.1 percent of the oral styrene dose. The role of CYP2E1 and -2F2 enzymes in 4-vinylphenol metabolism was suggested by the results of Carlson *et al.* (2001). Watabe *et al.* (1984) concluded from their *in vitro* rat liver microsomal data that one product of 4-hydroxystyrene metabolism may be a reactive 4-hydroxystyrene-7,8-epoxide. While these data suggest that arene oxide formation represents a minor pathway in the metabolism of styrene by humans and

rodents, the *in vitro* mutagenicity of styrene-3,4-oxide (Watabe *et al.*, 1978), suggests that the arene oxide pathway could have implications for styrene carcinogenicity.

Detoxification – Two Pathways

The detoxification of styrene-7,8-oxide depends on individual genetics, environmental factors, and co-exposures to other chemicals. Two major pathways are hydrolysis to styrene glycol and conjugation to reduced glutathione (GSH). In humans and rodents, hydrolysis of styrene-7,8-oxide is catalyzed by epoxide hydrase. Conjugation to reduced GSH is catalyzed by the family of glutathione-S-transferases (GST). The reduced GSH pathway is considered important for the detoxification of styrene-7,8-oxide in rodents. The pathway exists in human tissue; however, its role in the detoxification of styrene-7,8-oxide in humans appears to be less important than in rodent tissue.

Detoxification by Epoxide Hydrase

Epoxide hydrase (or hydrolase) activity towards styrene-7,8-oxide is ubiquitous in animal tissues (Oesch, 1972; Oesch *et al.*, 1977). Among humans and rodents, the rank order of microsomal liver epoxide hydrase activity has been reported as human > rat > mouse (Oesch, 1972; Mendrala *et al.*, 1993). Epoxide hydrase activity towards other substrates is also widespread. Species differences, based on substrate specificity and the presence of more than one form within any tissue of one species, have been reported (Guengerich *et al.*, 1979a,b). In rat, microsomal epoxide hydrase activity towards styrene-7,8-oxide is greatest in liver, intermediate in kidney, low in intestine and lung, and not detectable in brain, heart, spleen, or muscle (Oesch, 1972). Green *et al.* (2001) reported styrene-7,8-oxide hydrase activity in the microsomal fraction of nasal tissue from mice and rats; greater activity occurred in the nasal olfactory epithelium compared to the nasal respiratory epithelium. The authors also reported styrene-7,8-oxide hydrase activity in human nasal tissue (n = 6); however, insufficient tissue was available to separate the nasal olfactory from the nasal respiratory tissue.

Unlike the activity in human lymphocytes, the epoxide hydrase activity in human erythrocytes does not act on styrene-7,8-oxide (McGee and Fitzpatrick, 1985). The lack is relevant to the observed role of red blood cells in styrene induced SCEs in human lymphocytes (Norppa and Tursi, 1984) and in the bioactivation of styrene to styrene-7,8-oxide in blood (Belvedere and Tursi, 1981).

The hydrolysis of styrene-7,8-oxide to styrene glycol is mediated by epoxide hydrase in cytosolic and microsomal fractions (Omiecinski *et al.*, 1993). Within the microsomal fraction, epoxide hydrase exists in a tightly bound membrane complex with a cytochrome P_{450}-dependent enzyme(s) or in a “free” form (Oesch, 1972). Although the presence of the microsomal bound epoxide hydrase in close proximity to cytochrome P_{450}-dependent enzymes suggests a role for this epoxide hydrase in detoxification of styrene-7,8-oxide, the relationship between the variously located forms and the biological effects of styrene have not been elucidated. In one study, the rate of styrene-7,8-oxide hydration in adult human liver microsomes was greater than in the cytosolic fractions (Pacifici *et al.*, 1983).

Kinetic constants (K_m and V_{max}) associated with epoxide hydrase-mediated styrene-7,8-oxide hydrolysis in rodent and human liver have been reported (Table 5). Mendrala *et al.* (1993) reanalyzed the V_{max} data and found that on a body weight basis the rank order was

mouse > rat > human (Table 6). This rank order changes to human > rat > mouse when V_{max} values are calculated in terms of $BW^{3/4}$ (or $BW^{2/3}$). Mendrala *et al.* (1993) also analyzed the kinetic data for epoxide hydrase activity in terms of the half-life for styrene-7,8-oxide. Based on the assumption that the hydrolysis of styrene-7,8-oxide is a first-order process, and using K_m and V_{max} values based on body weights, the authors calculated styrene-7,8-oxide half-lives as 17 minutes for F344 rats, 9 minutes for Sprague Dawley rats, 38 minutes for B6C3F1 mice, and 1.8 minutes for humans. One important aspect of the calculations of Mendrala *et al.* (1993) is the use of kinetic constants associated with styrene-7,8-oxide hydrolysis in liver. Similar calculations that use kinetic constants associated with styrene-7,8-oxide hydrolysis in lung or blood were not reported. In humans, epoxide hydrase activity associated with erythrocytes does not appear to use styrene-7,8-oxide as a substrate (McGee and Fitzpatrick, 1985).

Table 6. *In Vivo* V_{max} Values Estimated from *In Vitro* V_{max} Values from Liver Microsomal Fractions[1]

Enzyme Activity	Basis	Vmax (mg/hour)			
		F344	SD	$B6C3F_1$	Human
Cytochrome P_{450}	Animal	9.2	8.2	1.2	226
	BW	46	41	60	3.2
	$BW^{3/4}$	31	27	23	9.3
Epoxide Hydrase	Animal	13	15	2	1,886
	BW	65	75	100	27
	$BW^{3/4}$	43	50	38	78
Glutathione-S-Transferase	Animal	256	498	45	11,774[a]
	BW	1,280	2490	2,250	168
	$BW^{3/4}$	856	1,665	846	487

F344 = Fischer 344 rat; SD = Sprague-Dawley rat; B6C3F1 mice; BW = body weight.
[1] V_{max} values are expressed as mg/hour per animal or per body weight raised to 1 or 3/4 (U.S. EPA, 1992) power. V_{max} values based on whole animals and body weight are taken from Mendrala *et al.* (1993).
[a] V_{max} value for human glutathione-S-transferase is based on the average of three of five liver samples demonstrating sufficient activity (3 to 40 nmol/min-mg protein) (Mendrala *et al.*, 1993).

Little information is available on the inter-individual variability of epoxide hydrase activity towards styrene-7,8-oxide. Pacifici *et al.* (1983) reported that the specific activity of adult human cytosolic liver epoxide hydrase towards styrene-7,8-oxide was greater than that of fetal cytosolic liver epoxide hydrase. Comparative data on microsomal epoxide hydrase activities were not presented. Viaene *et al.* (2001) reported a normal distribution (5 fold) of blood mononuclear cell microsomal epoxide hydrase activity towards styrene-7,8-oxide among former and currently exposed workers (n = 56). Wenker *et al.* (2000) analyzed epoxide hydrase genotypes (*EPHX*) among the livers of non-styrene-exposed humans and reported that 5of 20 livers were homozygous for the mutated allele of exon 3 (associated with slow hydrolysis rate). A similar distribution (21

percent) of the homozygous mutated allele of exon 3 in the peripheral blood of styrene-exposed workers was reported by de Palma *et al.* (2001). De Palma *et al.* (2001) also determined that there was no association between the haplotypes at the *EPHX* locus and the level of the urinary metabolites MA plus PGA. (The assignment of slow and fast mutated alleles to exons 3 and 4 of epoxide hydrase is based on results of Hassett *et al.* (1994) in which enzyme activity was measured against benzo(a)pyrene-4,5-epoxide.)

Wenker *et al.* (2000) studied distribution of the kinetic parameters K_m and V_{max} for the hydrolysis of the R- and S-enantiomers of styrene-7,8-oxide in microsomal fractions from human liver (n = 20). *In vitro*, the (S)-enantiomer had an approximately six times higher K_m and five times higher V_{max} than the (R)-enantiomer. *In vivo*, when racemic (R+S) SO was used as a substrate, the (R)-enantiomer acted as an inhibitor of the hydrolysis of the (S)-enantiomer. No effect of alcohol or cigarette smoking was observed. However, V_{max} for hydrolysis of the S-enantiomer of styrene-7,8-oxide decreased as the age of the individual increased.

The results of Omiecinski *et al.* (1993) and Hassett *et al.* (1997) suggested a bimodal distribution of epoxide hydrase activity toward benzo(a)pyrene-4,5-epoxide in human lymphocytes (40-fold range in activity) and an 8-fold range of activity in human liver, but no data on epoxide hydrase activity towards styrene-7,8-oxide were presented. Using the same non-styrene substrate, the results were consistent with altered human epoxide hydrase activity that occurs as a result of altered allelic combinations that represent amino acid substitutions (Hassett *et al.*, 1994).

The ratios of epoxide hydrase/cytochrome P_{450}-dependent enzyme activities towards styrene-7,8-oxide and styrene, respectively, were reported in liver, heart, lungs, spleen, and kidneys for each sex in rat, mouse, guinea pig, and rabbit (Cantoni *et al.*, 1978). The ratios were <1 for male and female mouse lung, female rat spleen, male rabbit heart, and male and female rabbit lung, and >1 in the remaining cases. On the basis of epoxide hydrase and cytochrome P_{450}-dependent enzyme activities, species, tissue, and sex differences could lead to different expressions of styrene activation or styrene-7,8-oxide deactivation.

Experimental animal data suggest a minimal effect of prior styrene exposure on epoxide hydrase-mediated styrene-7,8-oxide hydrolysis to styrene glycol. Exposure to airborne styrene had no effect on the rate of styrene glycol formation in microsomes derived from liver or lung of Sprague-Dawley rats or $B6C3F_1$ mice or in the lung microsomes from Fischer 344 rats (Mendrala *et al.*, 1993). Such exposure resulted in a 1.6-fold increase in epoxide hydrase activity in the liver microsomal fraction of Fischer 344 rats. The lack of a relationship between exposure to airborne styrene and microsomal epoxide hydrase (mEH) activity towards styrene-7,8-oxide suggests that prior exposure to styrene among humans does not induce mEH activity (Viaene *et al.*, 2001). A similar observation was reported for a non-styrene-7,8-oxide substrate, benzo(a)pyrene-4,5-epoxide (Omiecinski *et al.*, 1993).

With regard to enantioselectivity, the epoxide hydrase-dependent hydrolysis of styrene-7,8-oxide proceeded with a selection for the R-styrene-7,8-oxide in both tissues (Carlson, 1998). Watabe *et al.* (1981) reported that epoxide hydrase-dependent R-styrene-7,8-oxide hydrolysis was less than that of the S-styrene-7,8-oxide in a rat liver microsomal

fraction. Also, at one time point, as the level of microsomal protein increased (and the proportion of hydrolysis product increased), the R/S ratio of styrene-7,8-oxide hydrolysis products from racemic styrene-7,8-oxide increased. That is, at only 25 percent completion, the R/S ratio was >1, and it decreased to about one by 100 percent completion. Watabe *et al.* (1981) concluded that the high R/S ratio of the hydrolysis product was due to differential inhibitory properties of the enantiomeric styrene-7,8-oxides. Hence the R/S ratios of styrene-7,8-oxide and its metabolites in animal tissues are probably not constant and depend on many factors.

Data on epoxide hydrase activity as a function of age are limited. Activity in fetal mice was detected indirectly by use of a specific inhibitor of the enzyme (Barcellona *et al*., 1987). Kapitulnik *et al*. (1977) detected hydration of styrene-7,8-oxide in the liver microsomes from nine people autopsied including one full-term stillborn, a neonate who died at two days of age, and a two-year old. The epoxide hydrase activity in all three was similar to the levels in the six adults (ages 56-98 years). Pacifici and Rane (1982) detected epoxide hydrolase activity toward [7-^{3}H]styrene 7,8-oxide in the microsomal fraction of human fetal liver, lungs, kidneys, adrenal glands, gut, and placenta. Activity in fetal livers (n=20) was about 40 percent of that found in adults (Pacifici and Rane, 1983). At least one epoxide hydrase isozyme (EPHX1) is reported to be present from the first trimester of pregnancy to at least one year of age (Blake *et al*., 2005). Dorne *et al*. (2005) reported that general hydrolysis activity was about 20 percent higher in children (> 6 months) than in adults (> 16 years), but they had no data for neonates.

Detoxification by Glutathione-S-transferase

Styrene-7,8-oxide may also be deactivated by conjugation to reduced GSH through the action of glutathione-S-transferase (GST) and then to the mercapturic acids (N-acetylcysteine derivatives) through γ-glutamyltransferase, dipeptidase, and N-acetylase. The GSH pathway for styrene-7,8-oxide detoxification has been demonstrated in rodents (Mendrala *et al.*, 1993; Linhart *et al.*, 1998) and to a minor extent in humans (Pacifici *et al.*, 1981; Pacifici *et al.*, 1987; Mendrala *et al.*, 1993). Earlier biomonitoring activities did not detect substantive levels of styrene-7,8-oxide-GSH adducts or styrene-7,8-oxide-mercapturic acids in exposed humans (Leibman, 1975; Bardodej and Bardodejova, 1970; Truchon *et al.*, 1990; Norstrom *et al.*, 1992). More sensitive analytical methods, however, have resulted in detection of low urinary levels of these metabolites (Hallier *et al.*, 1995; Maestri *et al.*, 1997; Ghittori *et al.*, 1997; de Palma *et al.*, 2001).

Although the levels of urinary GST-derived metabolites may be relatively low in exposed humans, GST activity toward styrene-7,8-oxide has been measured in human tissue. The activity exists in the cytosolic and microsomal fractions (Morgenstern and Depierre, 1988). However, most studies with styrene-7,8-oxide have used the cytosolic fraction. Pacifici *et al.* (1981) reported a rank order of cytosolic GST activity towards styrene-7,8-oxide as liver > lung ≈ kidney. In human liver, GST activity towards styrene-7,8-oxide has been associated with the isozyme GSTμ (Pacifici *et al.*, 1987). Mendrala *et al.* (1993) reported that prior inhalation exposure to styrene resulted in no change in rat or mouse liver or lung cytosolic GST activity towards styrene-7,8-oxide. In rat and mouse, the level of GST activity towards styrene-7,8-oxide is greater in the nasal olfactory epithelium than in the nasal respiratory epithelium (Green *et al.*, 2001).

In vitro kinetic analysis for GST activity towards styrene-7,8-oxide in liver provided K_m values of 0.17 mM for rat (Mendrala *et al.*, 1993), 0.25 mM for mouse (Mendrala *et al.*, 1993), and 4.7 mM for human (Pacifici *et al.*, 1987). The reported maximal velocities (V_{max}, nmol styrene-7,8-oxide hydrolyzed/min-mg protein) in liver are 39 for mouse (Mendrala *et al.*, 1993), 47 for rat (Mendrala *et al.*, 1993), and 22 for human (Pacifici *et al.*, 1987). The ratios of GST activity to epoxide hydrase activities in human liver, lung, and kidney ranged from two to three, whereas the ratios in the same organs of rat and mouse ranged from 17 to 78 (Pacifici *et al.*, 1981). Compared to humans, GST activity plays a more prominent role in the detoxification of styrene-7,8-oxide in the rat and mouse than epoxide hydrase activity.

The GSH pathway in rodents and in humans may have implications for styrene carcinogenicity by providing a pathway for detoxification and for competing toxicity. GSH levels in rat lung or liver tissue with styrene-induced non-neoplastic lesions appear to be decreased (Coccini *et al.*, 1997). The nephrotoxicity of styrene or styrene-7,8-oxide-GSH conjugates administered i.p. to rats occurs under conditions that suggest involvement of the GSH pathway (Chakrabarti and Tuchweber, 1987; Chakrabarti and Malick, 1991). These observations are relevant to styrene-7,8-oxide-mediated tumorigenicity because of potential competing biological effects due to the use of the presumed carcinogenic metabolite by the GSH pathway. At appropriate styrene-7,8-oxide levels, a shift could occur in the relative expressions of neoplastic and non-neoplastic effects. A decreased *in vitro* covalent binding of styrene-7,8-oxide to rat liver homogenate or microsomal fraction in the presence of increasing concentrations of GSH provides support for this hypothesis (Marniemi *et al.*, 1977).

Inter-individual variability among humans in GST activity towards styrene-7,8-oxide could also affect the carcinogenic outcome of styrene exposure. Pacifici *et al.* (1983) detected an age effect; cytosolic GST activity towards styrene-7,8-oxide was lower in human fetal liver than in human adult liver. Polymorphisms have been observed between the effects of styrene and GST expression in the human population, and they appear to be dependent on the measured endpoint. Vodicka *et al.* (2001b) reported a higher *hprt* mutation frequency in the lymphocytes of styrene-exposed hand-lamination workers than controls among those with a *GSTP1* polymorphism. Ollikainen *et al.* (1998) reported that lymphocytes obtained from humans who lacked the *GSTT1* gene exhibited a greater increase in styrene-induced SCE *in vitro* than in the cells obtained from donors with the gene. (All donors possessed the *GSTM1* gene.) The results suggested that for blood and blood forming organs a polymorphism in the detoxifying GST could affect *in situ* responses to the reactive styrene metabolite styrene-7,8-oxide.

De Palma *et al.* (2001) investigated GST polymorphisms in styrene-exposed workers and their association with excretion of urinary metabolites. The urinary levels of mercapturic acids, initiated by the conjugation of GSH to styrene-7,8-oxide, were low in individuals who were null for *GSTM1*, compared to those who were positive for this genotype. Polymorphisms in the *GSTT1* or *GSTP1* genotypes did not lead to the same decrease, and the excretion of urinary metabolites derived from the epoxide hydrase-dependent hydrolysis of styrene-7,8-oxide was not changed. The results suggested an interplay of the two styrene-7,8-oxide detoxification pathways and a polymorphism, which results in

a decrease in the nephrotoxic styrene-7,8-oxide-derived mercapturic acids and may lead to higher levels of a carcinogenic metabolite.

Pacifici and Rane (1982) detected general glutathione transferase activity toward [7-^{3}H]styrene 7,8-oxide in the cytosolic fraction of human fetal liver, lungs, kidneys, adrenal glands, gut, and placenta. Strange *et al*. (1985) studied the expression of the specific isozymes GST1, GST2, and GST3 in fetal, neonatal, and infant tissues. (The paper uses an outdated nomenclature, but indicates that some glutathione transferase activity is present early in the fetus.) GST1 was first expressed at 30 weeks of gestation (third trimester of pregnancy) and then steadily increased. Adult levels were reached in late infancy. GST2 was always expressed in liver and adrenal tissue but was only weakly expressed in spleen, cardiac muscle, and diaphragm. In kidney GST2 was not usually expressed until nearly 1 year after birth. The GST3 isozymes were present in all fetal, neonatal, and infant tissues; their expression in liver decreased after 30 weeks of gestation. More recent studies have also found some glutathione transferase activity throughout development (Mathew *et al*., 1992; Mera *et al*., 1994).

Although most of the studies on GSH-related styrene metabolism involve GST-catalyzed styrene-7,8-oxide conjugation, other GSH pathways are possible. A role for a free radical mechanism that does not involve styrene-7,8-oxide was suggested by Stock *et al.* (1986), who described the formation of the conjugate in isolated rat seminal vesicles in the presence of horseradish peroxidase or prostaglandin H synthase activity. The conjugate formed in the presence of oxygen was chemically identical to the conjugate formed from styrene-7,8-oxide in the presence of GST, but, under aerobic conditions, it proceeded through a series of free radical reactions on styrene. The reaction also proceeded anaerobically to different products. In tissues where there is a high level of peroxidase or prostaglandin synthase, this GSH mechanism could be important in styrene metabolism. However, the apparent minor role of GSH in the human metabolism of styrene suggests that this mechanism may not be relevant for understanding human styrene carcinogenicity.

Urinary Metabolites

In humans, styrene metabolism culminates in large amounts of urinary MA and PGA, end-products of the styrene-7,8-oxide hydrolysis pathway (Astrand *et al.*, 1974**;** Wigaeus *et al.*, 1983**;** Wieczorek and Piotrowski, 1985**;** Johanson *et al.*, 2000; Wenker *et al.*, 2001b). Specific yields (percent of pulmonary uptake/retention) vary among the studies and range from a low of 10 to 35 percent (Johanson *et al.*, 2000) to a high of 90 percent of absorbed dose (Bardodej and Bardodejova, 1970). These urinary metabolites are derived from styrene glycol through enzyme-mediated oxidative reactions. Urinary MA and PGA are biomarkers of styrene exposure. The relationship between the urinary levels of MA and/or PGA and air styrene has led to the development of biomonitoring protocols for styrene exposed workers (ACGIH, 1997). Symanski *et al.* (2001) recently demonstrated that in the reinforced plastics industry the levels of MA and PGA are higher among laminators than among assemblers, finishers, maintenance workers, or managers.

Rodents excrete MA and PGA (Truchon *et al.*, 1990; Elovaara *et al.*, 1991), but the rodent GSH pathway also plays an important role in styrene metabolism (see above).

The further metabolism of MA to hippuric acid appears to be of minor importance in humans (Leibman, 1975; Bardodej and Bardodejova, 1970). Ikeda *et al.* (1974) suggested that the apparent low yield of styrene-associated hippuric acid in humans might result from the presence of high background levels and wide physiological variation. The high levels in unexposed humans is a result of the non-specificity of hippuric acid as a styrene metabolite; e.g., it is also formed during the oxidation of odd-numbered fatty acids (Voet and Voet, 1990).

Metabolism of styrene may also result in formation of phenylacetaldehyde by two reactions: direct oxidation of styrene-7,8-oxide or oxidation of the vinyl side-chain of styrene to yield phenylethanol and thence phenylacetaldehyde (Sumner *et al.*, 2001). Barely detectable levels of phenylethanol were reported in early studies (Leibman, 1975). Current methodologies utilizing ^{13}C-NMR measurement detected phenylacetaldehyde in the urine of humans and rodents (Johanson *et al.*, 2000; Sumner *et al.*, 2001). The proportion of phenylacetaldehyde or its metabolites was reported as 16 to 27 percent in B6C3F$_1$ and CD-1 mice, three to five percent in rats (Sumner *et al.*, 2001), and less than five percent in humans (Johanson *et al.*, 2000).

Concurrent exposure to ethanol (beer, wine, mixed drinks) and styrene leads to decreased urinary MA levels and increased blood styrene glycol levels in humans (Wilson *et al.*, 1983; Berode *et al.*, 1986). MA clearance is delayed rather than decreased, and an accurate estimate of urinary MA can be obtained from more delayed measurements (ACGIH, 1997). In the context of the current discussion, an accurate assessment of MA excretion is needed to define an accurate dose-response relationship.

Excretion

Urinary excretion of metabolic end products is the major styrene elimination pathway in humans, and as noted above, MA and PGA are the major urinary metabolites. Sensitive analytical methods have detected GSH-related urinary metabolites (Maestri *et al.*, 1997; Ghittori *et al.*, 1997; de Palma *et al.*, 2001), but in humans the GSH pathway is less important as an excretion pathway (Pacifici *et al.*, 1981, 1987; Mendrala *et al.*, 1993).

Animal Data

Johanson *et al.* (2000a) exposed rats and mice for two hours to 50 ppm air styrene and reported that the label in urine and feces represented 76 percent of the pulmonary uptake in rats and 69 percent in mice.

The time course for excretion of the radioactive label from styrene was measured by Sumner *et al.* (1997) in F344 rats, B6C3F$_1$ mice, and CD1 mice after a one or three-day inhalation exposure to 250 ppm [7-^{14}C]-styrene. Following the one-day exposure, the label in F344 rats and CD1 mice declined by 93 and 87 percent between 12 and 24 hours, whereas that from the B6C3F$_1$ mice decreased by only 43 percent. Compared to the one-day exposure, the decrement in the urinary label (about 90 percent) between 12 and 24 hours following a three-day exposure was similar for the F344 rats and CD1 mice. The rate of decline of the urinary styrene label in the three-day exposed B6C3F$_1$ mice differed from that among the one day exposed mice (about 90 and 54 percent, respectively).

In the same study, rats (F344) and mice ($B6C3F_1$ and CD1) excreted the radioactive label from inhaled [7-^{14}C]-styrene in urine and feces (Sumner *et al.*, 1997). Immediately after a six-hour nose-only inhalation exposure, 37 percent appeared in the urine of the rat, whereas only 4 percent appeared in the feces. The remainder was found in the carcass and pelt (46 and 14 percent, respectively). Forty-eight hours later, 79 percent of the label had appeared in urine and 18 percent in the feces, while only one and two percent appeared in the carcass and pelt, respectively. In the CD1 mice, 17 percent of the labeled styrene appeared in the urine immediately after exposure. (No feces were present in the nose-only tubes.) The levels in the carcass and pelt were 51 and 32 percent, respectively. Forty-eight hours after exposure ended, 82 percent of the label had appeared in the urine and 6 percent in the feces. The carcass and pelt contained two and 10 percent, respectively. A major difference was observed in mice in that the pelt always contained a high level of the radioactivity: 78 percent immediately following exposure and 34 percent 48 hours post-exposure. Immediately following exposure the carcass contained 18 percent of the label, whereas only 4 percent was in the urine. Forty-eight hours after the inhalation exposure ceased for the $B6C3F_1$ mice, 60 percent appeared in the urine and 4 percent in the feces. The carcass and pelt contained 2 and 34 percent, respectively. Tissue analyses were conducted 48 hours after cessation of exposure and only 0.1 percent was observed for each of the rat and two mouse strains.

Sumner *et al.* (1997) reported no excretion of the labeled styrene as CO_2. Bergman (1979) reported a recovery of about 7 percent of the styrene dose as CO_2.

Human Data

Astrand *et al.* (1974) estimated that urinary MA represented about 50 percent of the styrene taken up by the lung among humans exposed to 50 to 150 ppm styrene vapors. Men exposed to 69 ppm styrene for two hours under light exercise excreted about 34 percent of the pulmonary uptake of styrene as MA in the urine (Wigaeus *et al.*, 1983). At 50 ppm styrene with light exercise for two hours, urinary MA represented 14 percent (range, 6 to 29 percent) of the styrene taken up by the lung (Johanson *et al.*, 2000).

Engstrom *et al.* (1976) studied excretion of MA in styrene-exposed workers. Urinary MA levels appeared to reach a plateau by 100 ppm styrene. The authors noted that, based on urinary MA levels, styrene was not completely eliminated during the 16-hours between daily work exposures. The excretion of MA among workers exposed to 23 ppm styrene in air occurred in two phases with reported $t_{1/2}$ values of 9 and 17 hours (statistically different, $p<0.05$). The first phase $t_{1/2}$ for MA excretion among workers exposed to 248 ppm styrene in air was six hours and was statistically different from the MA half-life at 23 ppm.

The urinary excretion of MA and PGA was also studied in humans exposed to air styrene levels of 50 to 200 ppm for four to eight hours by Guillemin and Bauer (1979). Total excretion of the metabolites by four days following exposure was 86 percent of the estimated retained styrene dose. Excretion of MA proceeded in two phases with $t_{1/2}$ values of four and 25 hours. The $t_{1/2}$ for PGA was ten hours. The ratio of MA/PGA was 4 at 0.5 hours post-exposure and 0.6 at about 20 hours post-exposure.

Excretion kinetics of the urinary styrene metabolites, MA and PGA, were investigated in humans (n = 5) not occupationally exposed to styrene (Wieczorek and Piotrowski, 1988).

The subjects inhaled 5, 24, or 47 ppm styrene for up to eight hours. Both metabolites appeared in urine within the first two hours, and the excretion characteristics were similar and biphasic. The $t_{1/2}$ values were 3 and 32 hours for MA and PGA.

Perbellini *et al.* (1988) reported that the urinary metabolites MA and PGA were not totally cleared by humans between end-of-shift and next-morning measurements. For MA, 19 to 23 percent of the end-of-shift amount was present the following morning. For PGA, 31 to 41 percent of the amount measured at end-of-shift on Friday was present on the following Monday morning before the start of the shift. The results suggested that human exposure to styrene does not cease upon removal from the source. Incomplete removal of styrene following daily exposures is supported by the results of Symanski *et al.* (2001) that showed increased levels of MA plus PGA in late week urine samples compared to samples taken early in the week.

Engstrom *et al.* (1976) reported that the $t_{1/2}$ for MA excretion among workers exposed to 23 and 248 ppm styrene in air was statistically different (nine and six hours, respectively, $p<0.05$), concluding that metabolism was saturated at 248 ppm. Lof and Johanson (1993) interpreted urinary excretion data to suggest saturation between 100 and 200 ppm in a two hour styrene inhalation study, but noted a change to almost 400 ppm styrene when the post-exposure interval was extended from five to 24 hours. The authors suggested that the metabolism had only been delayed. Guillemin and Bauer (1979) reported that among humans exposed to 50 to 200 pm styrene for four to eight hours, urinary metabolite excretion did not appear to plateau. The authors suggested that with up to an eight-hour inhalation interval between 50 and 200 ppm styrene, equilibrium had not been reached. Guillemin *et al.* (1982) reported that among workers exposed to two to 199 ppm styrene in air, a linear relationship was observed between urinary metabolites and cumulative exposure (ppm-hr). According to the PBPK model of Ramsey and Andersen (1984), the blood concentrations of styrene in humans following a one hour exposure to 51, 216, or 376 ppm styrene in air were consistent with saturation around 200 ppm. Ramsey and Andersen (1984) concluded that the shapes of blood styrene time curves in rats (area under the curve, AUC) were consistent with saturation of metabolism around 200 ppm styrene. Above this level, the removal of styrene after cessation of exposure changed from a perfusion-limited metabolism to ventilation-limited metabolism until the styrene concentration in blood was low enough to permit perfusion-controlled removal.

Wenker *et al.* (2001b) reported an apparent blood styrene clearance of 1.4 L/min and terminal elimination half-lives of 69 and 65 minutes among 20 men who were exposed to either 24 or 84 ppm styrene for one hour under light exercise (50 W). The authors tested the hypothesis that these styrene parameters, which they considered oxidation parameters, may be associated with CYP-dependent metabolism of styrene, either through the metabolism of model substrates or the presence of specific genotypes of *CYP2E1*. Neither the level of metabolism (defined as slow or fast metabolizers) nor the presence of different CYP2E1 genotypes correlated with the styrene oxidation parameters. Three possible explanations were suggested: 1) an invalid use of the chosen kinetic parameters to reflect styrene oxidation, 2) an invalid use of the model substrates to reflect styrene metabolism, and/or 3) a rate limiting effect of blood flow to the liver on styrene metabolism. Wenker *et al.* (2001b) suggested that, among the three explanations, the blood-flow limitation of metabolism may be a major contributor. Data on the level of

blood styrene oxide and the rate of enzyme-mediated styrene metabolism (as opposed to model substrates) would help to resolve this issue.

Styrene elimination from blood of human males (n = 8) exposed to 69 ppm styrene in air displayed biphasic kinetics over four hours (Wigaeus *et al.*, 1983). The rapid distribution phase half-life ($t_{1/2}$) was calculated as 1.9 minutes and the elimination phase $t_{1/2}$ was calculated as 41 minutes. Among humans exposed for two hours to 69 ppm of styrene vapors, non-conjugated styrene glycol in the arterial blood increased continuously during exposure, and decreased after exposure with a $t_{1/2}$ of 72 minutes (Wigaeus *et al.*, 1983).

TOXICOLOGY

Genetic Toxicity

Genetic toxicology studies on styrene have been grouped into three categories: human, rodent, and bacterial/non-mammalian. The large literature on *in vivo* findings in humans exposed to styrene is emphasized; other studies are discussed in the text or in tabular form. Results in humans are summarized in Tables 8 and 9. Table 8, adapted from IARC (1994a), includes *in vivo* human genotoxicity studies published up to 1993, while Table 9 summarizes human *in vivo* studies published from 1993 to 2004. Results from *in vitro* studies in humans and from *in vivo* and *in vitro* rodent studies are summarized in Table 10, and results from bacterial and non-mammalian systems in Table 11.

The underlying assumption in interpreting genetic toxicity test results is that damage to genetic material at the DNA or chromatid/ chromosome level has the potential to be expressed as a neoplastic event and that damage-related neoplastic events occur in all species. *In vivo* studies have direct relevance to humans, while *in vitro* tests have the advantage of yielding results in a timely manner and can serve as an indicator of potential carcinogenic hazards. As pointed out by Weisburger and Williams (1991), there is no one *in vitro* test that is superior to others. Differences in results can be expected to occur because of mechanistic considerations and species differences in metabolism and response. While a positive response may be predictive of carcinogenicity, a negative response may not always be predictive of non-carcinogenicity by a genotoxic mechanism (IARC, 1999). Each assay has its own basis, and results obtained from a battery of genotoxicity assays may or may not be concordant.

Genetic alterations in humans exposed to toxicants are routinely analyzed in tissue samples (e.g., blood, buccal cavity cells, exfoliated bladder cells) and referred to as "human *in vivo*" studies. *In vitro* human testing can be accomplished by treatment of cultured human cells with test compounds and analysis of genotoxicity endpoints. Laboratory animals, most commonly rodents, can be studied *in vivo* by collection and analysis of blood, bone marrow, or other tissues. For *in vitro* studies, rodent-derived tissue cell culture lines with stable, well-defined karyotypes and short generation times are often used. In addition, primary cell cultures may be used.

Cytogenetic Alterations

Tests for cytogenetic alterations detect genetic changes at the level of the chromatid or chromosome, rather than the DNA molecule. Common cytogenetic test systems detect

chromosome aberrations (CA), sister chromatid exchanges (SCE), and micronuclei (MN). Chromosome changes may be monitored as structural CA, a measure of DNA breakage due to errors in DNA repair or replication (IARC, 1999), or as aneuploidy, a measure of numerical changes. CA are manifested by changes in the size or structure of chromosomes and include chromatid breaks, chromatid exchanges, acentric fragments, dicentric chromosomes, ring chromosomes, inversions, and translocations. CA due to chromosome loss or fragmentation could happen during chromosome sample preparation and, thus, the potential contribution of artifact must be considered.

SCE can be monitored by differential staining to identify regions of DNA exchanged between chromatids due to damage-induced recombination. Unlike the random breakage observed in CA, the breakage that initiates the process of sister chromatid exchange is followed by a reciprocal reunion that restores the normal size and morphology of the chromosome. SCE do not result in gross structural losses or alterations in chromosomes and, therefore, require a more sensitive assay technique than CA. The level of SCE is routinely expressed as the number of SCE/cell. Because SCE are analyzed for intact chromosomes, this endpoint of genotoxicity is less susceptible to technical artifacts than CA (Anderson *et al*., 1993; IARC, 1999).

Micronuclei (MN) are formed when damage to chromosomes disrupts the normal process of chromosome segregation, which prevents damaged chromosomes from being incorporated into the daughter nucleus (IARC, 1999). MN can contain chromosomal fragments or whole chromosomes; chromosome fragments in MN are often used as simple indicators of chromosomal damage. MN assays can be conducted on cells harvested from exposed individuals or animals or on cultured cells. The resulting small extra-nuclear MN structures are easily visualized by light microscopy.

Human Studies of Cytogenetic Alterations *In Vivo*

IARC (1994a) reviewed more than 20 studies on cytogenetic alterations in occupationally exposed humans (Table 8). The mean air styrene levels in the studies ranged from 2 to 138 ppm, and the mean exposure durations ranged from three to 19 years. Cytogenicity studies of styrene exposed workers have also been reviewed by other authors (Barale, 1991; Scott and Preston, 1994; Bonassi *et al.*, 1996).

Bonassi *et al*. (1996) conducted a meta-analysis of 25 reports published between 1977 and 1995 on CA, MN, and SCEs to compare studies with varying numbers of test subjects and exposure ranges. Styrene exposure was categorized as low or high, based on a median air concentration of 30 ppm. The median exposure duration was nine years, and seven studies reported a mean exposure greater than ten years. To estimate the association between exposure to styrene and each cytogenetic endpoint, Bonassi *et al.* calculated the ratio of the mean frequency of the effect in the exposed group compared to the control group. A ratio was calculated for each genotoxicity endpoint and for both the low and high styrene exposure groups, weighted to account for random effects and sampling error. Workers exposed to air styrene levels greater than 30 ppm had a significant increase in CA compared to non-exposed workers (weighted ratio of 2.18; 95 percent confidence interval: 1.52-3.13). The apparent absence of increased CA among workers exposed to less than 30 ppm styrene compared to controls was interpreted by the authors as reflecting the limited statistical power of many of the studies to detect a small

response. No significant increases in SCE or MN were detected, and the authors commented on the limited statistical power of many of the studies.

Table 8. Cytogenetic Alterations *(In Vivo)* in Human Lymphocytes from Individuals Occupationally Exposed to Styrene[1]

Number Exposed	Number Referents	Length of Exposure (yrs)		Styrene in Air (ppm)		Cytogenetic Observations			Reference
		Range	Mean	Range	Mean	CA	MN	SCE	
10	3	1-15	3.2	≤ 300		+			Meretoja *et al.*, 1978a
16	6	1-15	6.3	≤ 300		+		-	
5	20	14-25		≤ 10		-			Fleig & Thiess, 1978
12	20	3-39		0-47	2	-			
14	20	2-24		50-300		(+)			
12	12	3-34		0-9		-			Thiess & Fleig, 1978
6	6	0.5-10		14-192	39	+			Högstedt *et al.*, 1979
24	24	4-27		0.7-170	58.1	-			Thiess *et al.*, 1980
36	37	0.3-12		1-382	138	+			Andersson *et al.*, 1980
20	21	0.3-12		1-382	138			(+)	
16	13	0.6-9.3		1-211	70	-			Watanabe *et al.*, 1981
18	6	0.2-30		40-50		(+)		-	Watanabe *et al.*, 1983
38	20	1-23		1-36	13		+		Högstedt *et al.*, 1983
25	22	1-22	9.4	7- >96		+		(+)	Camurri *et al.*, 1983
43	33	1-22		7- 96		+			Camurri *et al.*, 1984
18	9			2-44	13.2	-		-	Hansteen *et al.*, 1984
15	13	1-26			24	-			Nordenson & Beckman, 1984
12	12	1-26			24		+		Nordenson & Beckman, 1984
36	19	1-11		1-236	36			+	Pohlová & Srám, 1985
22	22	1-11		9-132					
21	21	1-25		8-63	24	-	-	-	Mäki-Paakkanen, 1987
32	32		18.8	27-55		+			Forni *et al.*, 1988
8	8		4.5	9-44		-			
11	11		10	28-140	61	-			Jablonicá *et al.*, 1988
11	15	0.1-25.4	8.1	1-39	13	-			Hagmar *et al.*, 1989
20	22	0.1-25.4	8.1	1-39	13		-		
7	8 (smokers)		8.6	1.7-131	50			-	Kelsey *et al.*, 1990
13	12 (non-smokers)		7.2	5.8-130	55			-	

Number Exposed	Number Referents	Length of Exposure (yrs)		Styrene in Air (ppm)		Cytogenetic Observations			Reference
		Range	Mean	Range	Mean	CA	MN	SCE	
11	11 (smokers)		6.4			-	-	-	Mäki-Paakkanen *et al.*, 1991
6	6 (non-smokers)		7.2			?	-	-	
17	17 (all)		6.7		70	-	-	-	
10	9		2.7	1-44	11.2		+		Brenner *et al.*, 1991
15					≤ 1.5				Yager *et al.*, 1990; 1993
16				0.2-55.3	2-25		-	+	
17					> 25				
50	54			5-182	43	-	-	-	Sorsa *et al.*, 1991
25	54			1-133	11	-	-	-	
7	7	1-18		5-24	12	-	-		Tomanin *et al.*, 1992
11	11	1.5-15		27-204	45	+	-		
28	20	1-26	8	29-42	37			+	Hallier *et al.*, 1994
52	24		2.9	2.2-111	31		-	-	Van Hummelen *et al.*, 1994
18	18	10-22	14.3		21	+	-		Anwar and Shamy, 1995
23	51			20-320		+		+	Artuso *et al.*, 1995
23	51			0.5-28		(+)		(+)	
12[3]	57					+			Kolstad *et al.*, 1996
53	41		9.9		30.3		-	+	Karakaya *et al.*, 1997
44	19		10-15	6-47		+			Somorovska *et al.*, 1999
79	90	0.2-25		0.03-0.3		+[5]			Lazutka *et al.*, 1999
97	90	0.2-21		1-1.4		+[5]			
14	7	0 – 35	8.7		<23	+			Oberheitmann *et al.*, 2001
14	30	7->15		17-19			+	+	Laffon *et al.*, 2002a
28	28	1-30	12	2-91	27		-	+	Teixeira *et al.*, 2004

+ = positive; (+) = weakly positive; - = negative; ? = inconclusive; blank = not tested.

[1] Adapted from Table 14 of IARC (1994a).

[2] Studies after 1993 added by OEHHA.

[3] Styrene exposed workers with myeloid leukemia (n=19) that underwent chromosome analysis.

[4] Results from chromosome based challenge assay.

[5] Workers also exposed to phenol and formaldehyde

Yager *et al.* (1993) demonstrated an increase in SCE with exposure among styrene workers (n = 48; 22 females, 26 males; average employment duration = six years, range: 0.5-27 years) in a boat manufacturing facility. Styrene exposure was estimated by repeated sampling of personal air. Three exposure groups were defined as having eight hour TWA styrene exposures of less than 1.4 ppm (low), 1.4 to 28 ppm (medium), and greater than 28 ppm (high). The authors reported a statistically significant increasing trend in lymphocyte SCE with increasing air styrene concentration, whether expressed as environmental ($r = 0.4$, $p = 0.008$) or exhaled breath ($r = 0.5$, $p = 0.001$) levels. The mean SCE/cell ($\pm$ SE) for each of the exposure groups (combined smokers and non-smokers) was 6.13 ± 0.18 (low), 6.48 ±0.21 (medium), and 6.85 ± 0.26 (high).

Yager *et al.* (1993) categorized styrene exposure according to smoking status to separate the contribution of cigarette smoking from that of styrene exposure to the observed SCE. Smoking was found to contribute 62 percent to the variability in SCE level, while styrene contributed 25 percent. The 13 percent contribution of a combined effect of smoking and styrene to the variability was not significant and, therefore, the effect of styrene or smoking to the generation of SCE could be considered independent events.

Studies on cytogenetic alterations among styrene-exposed workers published from 1993 to 2004, after the reviews of IARC and Scott and Preston (1994), are summarized in Table 9 and reviewed below. Additional recent reviews of styrene genotoxicity are Speit and Henderson (2005), Henderson and Speit (2005), and Nestmann *et al*. (2005).

Anwar and Shamy (1995) reported increased CA among male workers exposed to styrene in a reinforced plastics factory (n = 18) compared to male workers (n = 18) from an administrative section of the factory. The styrene group was exposed to a mean of 21 ppm styrene (based on urinary mandelic acid) for an average of 14 years. Among the CA (gaps, breaks, acentric fragments, and dicentrics), chromatid breaks were the major contributor to the observed CA. There was no correlation between the frequency of CA and duration of styrene exposure. The authors reported no increased level of MN among the styrene-exposed workers compared to the non-exposed controls.

Table 9. Human *in vivo* Genotoxicity of Styrene[1] (Studies Published from 1993 to 2004)

	Number Exposed	Exposure Level (ppm)	Exposure Duration (years)	Genotoxic Endpoint						Reference
				CA	SCE	MN	DNA Strand Break	Gene Mutation	DNA Adducts	
Boat manufacture	48	15 (R, 0.23 to 55)							+	Horvath *et al.*, 1994
Styrene production	25	<0.01 to 0.83	18						-	Holz *et al.*, 1995
Laminators	9, 11	38 to 16	11				+	+	+(l), -(g)[2]	Vodicka *et al.*, 1995; 1999
Reinforced plastics	23M, 24F	36 to 37						(+)		Bigbee *et al.*, 1996
Boat manufacture	17		<6 to >7						+[4]	Marczynski *et al.*, 1997
Plastics lamination	44	6 to 47	10 to 14				+	?		Somorovska *et al.*, 1999; Koskinen *et al.*, 2001
Laminators	9	14	8						+	Koskinen *et al.*, 2001
Reinforced plastics	14	17 to 19	17				+			Laffon *et al.*, 2002b
Reinforced plastics	86	19 ± 13	<4	-		+	?			Vodicka *et al.*, 2004

Abbreviations: M = male; F = female; + = positive; (+) = weakly positive;- = negative; ? = inconclusive; blank = no information.
[1] Data on exposure characteristics were taken directly from the published reports.
[2] (l) = lymphocytes; (g) = granulocytes. The study is based on the same cohort that was evaluated at different times.
[3] Styrene exposed workers with myeloid leukemia that underwent chromosome analysis.
[4] Adduct is 8-hydroxydeoxyguanine.
[5] CA results from chromosome based challenge assay.

Artuso *et al.* (1995) compared CA and SCE in peripheral blood lymphocytes from 46 male fiber-reinforced boat builders to those in 51 males who lived in the same area as the factory but had no history of occupational exposure to genotoxic chemicals. The exposed and referent groups were matched for age and smoking habits. The average age among the groups was 28 years for the boat builders and 30 years for controls. Information on duration of employment was not given. The exposed group was divided into low (n = 23; 2-120 mg/m^3 or 0.5-28 ppm) and high (n = 26; 86-1389 mg/m^3 or 20-326 ppm) styrene exposure categories. Increased levels of CA (chromatid, chromosome, and total aberrations) were detected in the high styrene exposure group as compared to controls ($p < 0.01$). At the high dose, the number of CA (aberrations/100 metaphases ± SE) were reported as 1.01 ± 0.20 for chromatid, 2.99 ± 0.34 for chromosome, and 4.0 ± 0.40 for total aberrations, compared to control values of 0.41 ± 0.09, 1.73 ± 0.24, and 2.13 ± 0.27, respectively. A positive trend with increasing styrene exposure was observed for each type of CA (chromatid, $p < 0.004$, chromosome, $p< 0.006$, and total aberrations, $p<0.0002$, respectively). Exposure to styrene was judged to be a risk factor for CA. CA were statistically significantly increased in the high exposure group: relative rate = 2.4 (95 percent CI, 1.3–4.7). Increased levels of SCE were observed with a positive trend with increasing styrene exposure ($p < 0.02$). Pair-wise comparison with controls was significant ($p < 0.05$) for the high and low exposure groups in a set of samples from 32 workers and 21 controls that was processed by one of the two laboratories contracted for analysis. The second laboratory processed a second set of samples from 13 workers and 13 controls; no significant differences were observed. The relative rates of SCE in the first group of samples were 1.22 (low exposure) and 1.26 (high exposure). There was a non-significant increase in SCE related to the level of cigarette usage. No association between alcohol ingestion or diagnostic X-rays and SCE levels was detected.

Karakaya *et al.* (1997) studied the genotoxic potential of styrene in male furniture workers (n = 53) who were exposed during handling of unsaturated polyester resins during hand lamination. The controls were unexposed males (n = 41) from a local university. The mean styrene level during lamination was 152 ppm, and during the 10-minute interval after lamination it was 30 ppm. The average exposure duration was 10 years and about 30 percent of each group smoked. The genotoxicity endpoints were SCE and MN in peripheral blood lymphocytes. No difference in MN was observed between the exposed workers and controls. The mean SCE level (SCE/cell) was higher in the exposed group than in the unexposed group: 5.15 ± 1.44(SD) vs. 3.66 ± 0.52 among non-smokers ($p < 0.01$), and 6.75 ± 1.34 vs. 5.88 ± 0.76 among smokers ($p < 0.01$). There was a non-significant trend towards increased SCE and MN with increasing duration of exposure ($p > 0.05$).

Styrene exposed workers (n = 44) in a plastics lamination plant exhibited increased levels of lymphocyte CA compared to unexposed factory controls (n = 10) (Somorovska *et al.*, 1999). Styrene exposure was defined by work activity: 1) controls, clerks (no styrene exposure); 2) low, maintenance (6 ppm); 3) medium, sprayers (13 ppm); and 4) high, hand laminators (47 ppm). Workplace air measurements did not detect toluene or acetone as potential confounders. The average employment duration was 10 (low), 15 (medium), and 14 (high) years for styrene exposure groups. Compared to the control group, the frequency of CA (aberrations/100 metaphases) in each of the styrene exposure groups was significantly higher ($p< 0.01$ or 0.001) than in the control group (control, 1.4;

low, 3.3; medium, 2.5; and high, 3.8, respectively). When the three exposed groups were pooled, the authors observed a higher frequency of CA in female workers than male workers (3.5 compared to 2.8 percent, respectively). Smoking was not related to the CA endpoint. The authors reported significant associations between CA and air styrene level ($r = 0.43$, $p = 0.001$), blood styrene ($r = 0.41$, $p = 0.001$), exhaled styrene ($r = 0.50$, $p < 0.001$), and exposure duration ($r = 0.6$, $p < 0.001$)

The study of Somorovska *et al.* (1999) was expanded to inquire into the relationship between the observed CA and genetic polymorphisms (Vodicka *et al.*, 2001b). Polymorphisms were evaluated for the enzymes cytochrome P_{450} CYP1A1 and CYP2E1 (bioactivation), epoxide hydrase (detoxification by hydrolysis), and glutathione-S-transferases GST M1, P1, and T1 (detoxification by conjugation) in lymphocyte DNA. The authors determined that for the total set of exposed and unexposed workers, CA correlated with air styrene level ($r = 0.4$, $p = 0.0007$), blood styrene level ($r = 0.5$, $p < 0.0001$), and employment duration ($r = 0.5$, $p < 0.0001$). Among the exposed population, CA correlated with blood styrene levels ($r = 0.5$, $p = 0.02$), but not with any of the investigated genetic polymorphisms.

Lazutka *et al.* (1999) tested workers in a carpet factory ($n = 79$) and in a plastic-ware plant ($n = 97$) for CA in peripheral blood lymphocytes. The air styrene level was 0.03 to 0.3 ppm in the carpet factory and 1.0 to 1.4 ppm in the plastic-ware factory. Phenol and formaldehyde were also present in both factories. CA (number of CA/100 metaphase cells) were observed in both groups of workers and their levels were significantly increased ($p < 0.05$) at all exposure durations compared to non-exposed controls ($n = 90$). The average CA level for carpet workers was 3.79 ± 0.32 (SE) and for plastic-ware workers was 4.17 ± 0.29 compared to 1.68 ± 0.13 for controls. There was no relationship between the CA level and the exposure duration. Although styrene may play a role in development of CA, a role for phenol or formaldehyde could not be ruled out.

Oberheitmann *et al.* (2001) used the challenge assay technique on blood lymphocytes obtained from styrene exposed boat builders to assess styrene-related CA. Blood preparations from styrene-exposed workers were subjected to X-irradiation, and then chromosome spreads were prepared and analyzed by fluorescence *in situ* hybridization (FISH) to detect CA. X-ray treatment induces additional damage in the lymphocytes from styrene-exposed workers that might be mis-repaired leading to CA. The use of fluorescent probes specific to chromosomes 1, 4, and 12 enhanced the ability to detect styrene-induced CA resulting from interchromosomal exchanges. The authors evaluated styrene-exposed (<100 mg/m^3) boat laminators ($n = 14$) and workers at the same plant who were not directly exposed to styrene ($n = 2$) and control workers ($n = 7$) from a different industry. In the absence of prior X-irradiation, the level of CA (chromosomal exchange CA/100 metaphases) in the lymphocytes of the styrene-exposed workers (0.22; 95 percent CI, 0.13-0.36) was higher than that in the controls (0.14; CI, 0.05-0.31), but the difference was not statistically significant. When lymphocytes from styrene-exposed workers and controls were subjected to X-ray treatment prior to analysis, CA detected in cells from the styrene-exposed workers (16.19; CI, 15.00-17.40) compared to controls (13.26; CI, 10.53-16.50, $p < 0.038$) increased with cumulative exposure to styrene (up to 35 years, $p = 0.015$, $R^2 = 0.4$) but not with blood styrene concentration. The data indicate an effect of chronic styrene exposure as opposed to acute styrene exposure.

SCE and MN were evaluated in whole blood of male workers (n = 14), employed seven to > 15 years at a fiberglass-reinforced plastics factory by Laffon *et al.* (2002a). The control group was 30 male university staff not exposed to chemicals or radiation. Styrene exposure was estimated from the urinary styrene metabolite, mandelic acid. The frequencies of SCE (number/cell) and MN (number/100 cells) in lymphocytes of exposed workers were greater than in the controls. For SCE the level was 3.51 ± 0.06 (SE) for exposed workers compared to 2.55 ± 0.05 for controls ($p < 0.01$). For MN the level was 24.63 ± 1.49 for workers and 13.91 ± 0.81 for controls ($p < 0.01$). The increase was detected at two exposure durations (seven to 15 and > 15 years). Distributions of the SCE and MN frequencies among the styrene-exposed workers were shifted to higher values relative to frequencies in the control group. The increased SCE distributions among styrene-exposed workers appeared to be associated with the presence of a small number of cells containing a high frequency of SCE (high frequency cells). High frequency cells were defined as cells whose SCE (or MN) frequency exceeded the 95th percentile of the SCE (or MN) distribution in a pooled data set from control individuals. In contrast, the distribution of increased MN frequencies for styrene-exposed workers compared to controls was not due to high frequency cells. Although SCE and MN frequencies increased with age, the effect was not statistically significant. An effect of cigarette smoking was detected only among the styrene-exposed group. The authors reported a positive correlation between SCE and MN ($r = 0.43$, $p = 0.01$).

Kolstad *et al.* (1996) studied the relationship between myeloid leukemia (ML) and CA among styrene workers. There were few patients (seven chronic myeloid leukemia (CML), 12 acute myeloid leukemia (AML)) available for study. Among seven males with CML, six were styrene-exposed workers and one was a control worker. Karyotype analysis revealed a 9;22 translocation (Philadelphia chromosome) in five of the six styrene-exposed CML patients; this karyotype change was not observed in the non-styrene exposed CML patient. Among the 12 AML patients, nine were styrene workers and three were controls; the authors reported no significant cytogenetic patterns. Kolstad *et al.* **(**1996) also compared the ML patients (CML plus AML) with the probability of styrene exposure. The odds ratios revealed non-significant increased risks for: (1) styrene-exposed workers, (2) styrene workers employed during the 1960s when workplace styrene air concentrations were high, and (3) styrene workers with more than ten years after first exposure. Increased risk was not observed with increasing styrene exposure nor with length of employment beyond one year. The study was limited by the small numbers of ML patients with chromosome analyses, lack of specific exposure data, incomplete case ascertainment, and lack of information on possible confounding factors. However, the authors concluded that styrene may play a role in clastogenic event(s) among ML patients in the reinforced plastics industry.

Human Studies of Cytogenetic Alterations *In Vitro*

Studies on cytogenetic alterations induced by styrene in human lymphocyte cultures, published between 1978 and 1993, were reviewed by IARC (1994a) (Table 10A). Of the eight reviewed studies, none used an exogenous metabolic system, and all were positive. It has been suggested that lymphocytes are metabolically competent to activate styrene (Belvedere and Tursi, 1981). Additional *in vitro* lymphocyte studies reviewed by Scott and Preston (1994) showed that styrene-induced cytogenetic alterations in human

lymphocyte cultures were enhanced by added metabolic activation; the enhancement was consistent with a role for oxidative metabolism through the cytochrome P_{450}-dependent monooxygenase system.

Table 10A. Cytogenetic Alterations (*In Vitro*) in Human Lymphocytes Exposed to Styrene[1]

Endpoint	Result	Reference
SCE	+	Norppa *et al.*, 1980a
	+	Norppa and Vainio, 1983
	+ [2]	Norppa *et al.*, 1983
	+	Chakrabarti *et al.*, 1993
	+	Lee and Norppa, 1995
	+	Bernardini *et al.*, 2002
MN	+	Linnainmaa *et al.*, 1978a
CA	+	Linnainmaa *et al.*, 1978a
	+	Pohlova *et al.*, 1984
	+	Jantunen *et al.*, 1986

[1] Without addition of activation system. Whole-blood lymphocytes were used except for Pohlova *et al*., 1985.
[2] Purified lymphocytes showed weaker response.

Styrene may be more genotoxic in whole blood than in purified human lymphocyte cultures. This is consistent with the studies of Norppa and Tursi (1984), in which the presence of erythrocytes (2×10^8/mL) in lymphocyte cultures led to an increased level of SCE compared to purified lymphocyte cultures alone ($<2 \times 10^4$ erythrocytes/mL). The presence of erythrocytes increased the SCE/cell approximately three-fold over a styrene concentration range of 2 to 4 mM. This observation is consistent with the proposed role for hemoglobin in the oxidative metabolism of styrene.

Human lymphocytes exposed to styrene in whole blood cultures exhibited increased SCE levels and a delay in cell cycle kinetics under conditions where viability was not affected (Chakrabarti *et al.*, 1993). The styrene concentrations ranged from 10 to 500 μM and the study was carried out in the absence of exogenous metabolic activation. The increase in SCE and decrease in cell cycle kinetics was linear up to 200 μM styrene, after which saturation was observed. SCE/cell increased from 13.0 to 22.6 over the styrene dose range tested, compared to 6.3 for control cultures ($p < 0.01$ for each dose). The authors concluded that styrene is genotoxic in human lymphocytes.

Lee and Norppa (1995) reported styrene-induced SCE in human lymphocytes in whole blood cultures at styrene concentrations of 0.5 and 1 mM. The levels of styrene induced SCE/cell in two experiments were 15.94 ± 4.6 (SD) and 14.16 ± 5.31 at 0.5 mM, and 21.22 ± 4.58 and 19.60 ± 5.33 at 1 mM, compared to 10.38 ± 3.14 and 9.82 ± 2.91 for

controls ($p < 0.001$ at each concentration). The authors concluded that styrene is genotoxic towards human lymphocytes.

In the study described by Chakrabarti *et al.* (1993), styrene-7,8-oxide, a styrene metabolite with putative genotoxic properties, increased as a function of styrene concentration, and saturation was apparent by 200 μM styrene. The level of styrene-7,8-oxide, as measured by styrene glycol in cultures of human lymphocytes, increased from approximately 0.5 to 4 nmol/10^6 cells over a styrene concentration range of 25 to 500 μM. The authors concluded that genotoxicity of styrene in human lymphocytes might proceed through this metabolite, although they also suggested a role for an active oxygen species formed from the action of hemoglobin. A role for styrene-7,8-oxide in styrene genotoxicity has also been proposed by Bastlova *et al.* (1995) and by Shield and Sanderson (2001), who reported mutations at the *hgprt* locus in styrene-7,8-oxide-treated human lymphocytes. Similarly, SCE were observed in styrene-7,8-oxide-treated human lymphocytes (Lee and Norppa, 1995; Uuskula *et al.*, 1995; Ollikainen *et al.*, 1998). Exposure of isolated human lymphocytes to styrene-7,8-oxide resulted in DNA strand breaks as measured by the comet assay (Bastlova *et al.*, 1995; Laffon *et al.*, 2001).

Rodent Studies of Cytogenetic Alterations *In Vivo*

The results of *in vivo* genotoxicity studies published from 1983 to 1993 were reviewed by IARC (1994a) and are shown in Table 10B. Rats, mice, and hamsters were exposed by i.p. injection, diet, or inhalation. Eight of eight studies in which SCE were measured were positive. Fewer studies were positive for MN (2/7) or CA (1/8). In addition, the review by Scott and Preston (1994) reported induction of aneuploidy among rats exposed to styrene by inhalation.

In studies in female mice and rats by Kligerman *et al.* (1993), a tissue specific dose dependence was observed for SCE. Female $B6C3F_1$ mice were exposed by inhalation to 125, 250, or 500 ppm styrene for six hours per day for two weeks. They exhibited statistically significant ($p < 0.05$) increases in SCE/cell in blood lymphocytes at all exposure levels (10.4, 11.4, and 11.7, respectively, compared to 9.5 for controls), in lung (11.3 for mid- and high-dose groups compared to 9.0 for controls), and in spleen in the mid- and high-dose groups (11.6 and 12.8 compared to 10.5 for controls). The authors reported no significant increases in CA or MN. Among female F344 rats exposed by inhalation to 125, 250, or 500 ppm styrene for the same period of time, a dose dependence of lymphocyte SCE/cell was also observed. The increased levels were significant ($p < 0.05$) in the 250 ppm and 500 ppm groups; the numbers of SCE/cell were 11.6 and 14.3, respectively, compared to 11.3 for controls. For rats exposed to styrene, no increase in CA or MN was observed.

Table 10B. SCE, CA, and MN in Cells of Animals Exposed *In Vivo*

Endpoint	Result	Reference
SCE, mouse bone-marrow and liver cells	+	Conner *et al.*, 1979
SCE, mouse bone-marrow, liver and alveolar macrophages	+	Conner *et al.*, 1980
SCE, mouse bone-marrow cells	+	Sharief *et al.*, 1986
SCE, mouse lymphocytes	+	Kligerman *et al.*, 1992
SCE, mouse lung cells	+	Kligerman *et al.*, 1992
SCE, mouse splenocytes	(+)	Simula & Priestly, 1992
SCE, rat splenocytes	+	Simula & Priestly, 1992
SCE, rat peripheral lymphocytes	+	Kligerman *et al.*, 1993
CA, mouse lung cells	-	Kligerman *et al.*, 1992
CA, rats peripheral lymphocytes	-	Kligerman *et al.*, 1993
CA, rat bone-marrow cells	+	Meretoja *et al.*, 1978b
CA, Chinese hamster bone-marrow cells	-	Norppa *et al.*, 1980b
CA, mouse bone-marrow cells	-	Sbrana *et al.*, 1983
CA, rat bone-marrow cells	-	Sinha *et al.*, 1983
CA, mouse bone-marrow cells	-	Sharief *et al.*, 1986
CA, mouse splenocytes	-	Kligerman *et al.*, 1992
MN, mouse bone-marrow cells	+	Norppa, 1981
MN, mouse splenocytes	-	Kligerman *et al.*, 1992
MN, mouse erythrocytes	-	Kligerman *et al.*, 1992
MN, mouse bone-marrow cells	(+)	Simula & Priestly, 1992
MN, rat bone-marrow cells	-	Simula & Priestly, 1992
MN, rat peripheral lymphocytes	-	Kligerman *et al.*, 1993
MN, Chinese hamster bone-marrow cells	-	Penttilä *et al.*, 1980

Symbols: + = positive; (+) = weakly positive; - = negative; 0 = not tested

Frequencies of MN were measured in the bone marrow of male NMRI mice exposed by inhalation to a dose of 0, 176, or 352 ppm styrene for six hours/day for 1, 3, 7, or 21 days (Vodicka *et al.*, 2001a). Mice exposed to the higher level of styrene in air for seven days exhibited an MN frequency (10.4 per 1000 polychromatic erythrocytes) about twice that found in control mice ($p = 0.014$). Significant increases were not detected at other times or at the low dose. The lack of response at 21 days may reflect the inhibition of cell proliferation by styrene due to cytotoxic effects and/or the elimination of chromosome fragments by natural cell turnover (half-life about 48 hours). Simple regression analysis failed to detect a significant correlation between MN frequency and blood or air styrene

levels, but multiple regression analysis revealed a significant correlation for both exposure parameters (blood styrene level $p = 0.04$ and air styrene level $p = 0.01$; $r^2 = 0.5$). Engelhardt *et al*. (2003) extended these findings. Male NMRI mice were exposed by whole body inhalation to styrene concentrations of 750 mg/m^3 (176 ppm) and 1,500 mg/m^3 (352 ppm) styrene for 6 h/day on 1, 3, 7, 14, and 21 consecutive days. Animals were killed immediately after exposure. Bone marrow was sampled for MN induction. This experiment showed no evidence of clastogenicity at either concentration or at any exposure interval.

Mammalian Studies *In Vitro*

IARC (1994a) reviewed reports of styrene-induced SCE and CA in cultures of Chinese hamster lung cells and ovary cells (Table 10C) in the presence, but not absence, of erythrocytes. This observation is consistent with the role of erythrocytes in the oxidative metabolism of styrene (Norppa and Tursi, 1984).

Table 10C. SCE and CA in Cells of Animals Exposed *In Vitro*

Endpoint	-S9	+S9	Reference
SCE, Chinese hamster ovary cells	-	+	de Raat, 1978
SCE, Chinese hamster ovary cells	-	+ [3]	Norppa *et al.*, 1985
SCE, rat lymphocytes	+	0	Norppa *et al.*, 1985
CA, Chinese hamster lung cells	-	(+)	Matsuoka *et al.*, 1979
CA, Chinese hamster lung cells	(+)	0	Ishidate & Yoshikawa, 1980

Symbols: + = positive; (+) = weakly positive; - = negative; 0 = not tested.
[3] Activation by erythrocytes

Other *In Vitro* Cytogenetic Studies

Cytogenetic changes caused by styrene have also been observed in several other test systems, as summarized in Table 10D.

Table 10D. Other Cytogenetic Alterations due to Styrene

Endpoint	Result	Reference
Allium cepa, chromosomal aberrations	+	Linnainmaa *et al.* (1978a,b)
Saccharomyces cerevisiae, gene conversion	+	Del Carratore *et al.* (1983)
Saccharomyces cerevisiae, homozygosis	+	Del Carratore *et al.* (1983)
Drosophila melanogaster, aneuploidy	-	Penttilä *et al.* (1980)

Symbols: + = positive; - = negative.

DNA Strand Breaks

Styrene induced DNA damage in mammalian cells has been studied by observing single-strand breaks following treatment with alkali, which disrupts base-pairing and causes the damaged DNA to become unwound. Single strands can then be monitored by chromatography (Erixon and Ahnstrom, 1979; Solveig-Walles and Orsen, 1983) or electrophoresis (Hellman *et al.*, 1995). A common method of visualizing DNA damage that results in single-strand breaks or alkali-labile sites is the "comet assay" in which treated cells are embedded in agarose at an alkaline pH and exposed to an electric field. Small fragments of DNA can flow out of the immobilized cell forming a tail or "comet" that can be stained and visualized (Hellman *et al.*, 1995). Measurements of tail length, tail intensity (or percent DNA in the tail), and tail moment (tail length x tail intensity) allow the number and the size of the breaks to be estimated (Hellman *et al.*, 1995; Laffon *et al.*, 2002a, McGregor and Anderson, 1999). While most authors refer to the damaged DNA thus observed as single-stranded breaks, IARC (1994a) noted that alkaline techniques may not distinguish between single-strand DNA breaks and alkali-labile sites. Alkali-labile sites may be derived from adducts that lead to apurinic or apyrimidinic sites (see Vodicka *et al.*, 2001a; Laffon *et al.*, 2002a); these sites tend to be repaired at slower rates compared to DNA single-stranded breaks (Chovanec *et al.*, 1998). Chovanec *et al.* (1998) commented that apurinic and apyrimidinic sites often convert into single-stranded breaks. Hence, the two phenomena (DNA alkali-labile sites and single-strand breaks) may be considered to exist on a continuum, and in this document they will be referred to as DNA single-strand breaks.

Human Studies of DNA Strand Breaks *In Vivo*

DNA single-strand breaks are monitored as alkali-sensitive disruptions in the base-pairing that causes the DNA to unwind, thus permitting the breaks to be observed by physical techniques (IARC, 1999). DNA damage, detected by chromatography of alkaline-treated DNA obtained from the blood of workers (n = 90) exposed to styrene in unsaturated polyester resins, was reported by Walles *et al*. (1988). Styrene exposure was monitored by end-of-shift blood styrene glycol and urinary mandelic acid from which an air styrene concentration of 50 to 100 ppm was estimated. Controls (n = 8) were office workers outside of the factory. Duration of employment ranged from 0.5 to 15 years. Duration of cigarette smoking ranged from 0 to 30 years for styrene workers and 0 to 25 years for the control office workers. The smoking rate (cigarettes per day) ranged from zero to 25 for styrene-exposed workers and 0 to 40 among the controls. DNA damage was expressed as the fraction of DNA (in double-stranded form) that remained bound to filters. Among the styrene-exposed workers the fraction of double-stranded DNA was 0.74 and among the control office workers the fraction of double-stranded DNA was 0.81. The difference was marginal by a two-tailed test ($p = 0.06$) and significant by a one-tailed test ($p < 0.05$). The level of single-strand breaks correlated with the level of urinary mandelic acid ($r = 0.6$) and blood styrene glycol ($r = 0.6$) (p-values not presented). Employment duration or cumulative cigarette smoking did not explain the variation in single-strand break levels.

In a different set of workers, Walles *et al.* (1993) reported single-strand breaks in DNA from leukocytes of styrene exposed workers from a plastics factory (incidence, 17/23).

Duration of exposure was 0 to 25 years. Smokers comprised 10/23 workers, and 6/23 workers used snuff. The mean individual (breathing zone) air styrene level was 7 ppm (range, 0.04 to 20 ppm). Other metrics of styrene exposure were individual end-of-shift blood styrene, urinary styrene, and urinary mandelic acid. The level of end-of-shift single-strand breaks for smokers and non-smokers was related to the eight-hour TWA. Significant exposure dependence was observed between DNA single-strand breaks and each of the following exposure metrics: blood styrene, urinary styrene, urinary metabolite, whole day TWA, last four hours TWA, exposure duration above 50 ppm, and maximum styrene exposure. There was no dose-dependence between DNA single-strand breaks and the last 30-minute TWA (the end of shift measurement). Similarly, there was no correlation between DNA single-strand breaks and exposure measured before a shift. The authors reported no relationship between DNA single-strand breaks and age, exposure duration, or use of snuff. A 2-fold increase in single-strand breaks following eight hours of exposure to 18 ppm styrene was calculated. Smoking 20 cigarettes per day corresponded to an end-of-shift increase in single-strand breaks of about 50 percent. The lack of a correlation between the end-of-shift increase and the next-morning blood samples reflected rapid repair kinetics in leukocytes. IARC (1994a**)** suggested that the method used by Walles *et al.* (1993) did not distinguish DNA damage caused by alkali-labile sites or single-strand breaks.

Vodicka *et al.* (1995) reported increased levels of DNA strand breaks among laminators (n = 7 females, 2 males) compared to factory controls (n = 4 females, 3 males). The damage was observed in the comet assay and differences were noted for amount of damaged DNA (T, percent DNA in tail), length of the tail (TL, μm) and tail moment (T x TL/100). The values for workers compared to controls were: T = 10.50 ± 5.78 percent (SD) compared to 1.0 ± 2.76 percent ($p < 0.007$), TL = 2.5 ± 1.49 μm vs. 1.00 ± 3.2 μm ($p = 0.032$), and TM = 5.50 ± 3.04 vs. 1.00 ± 3.41 ($p = 0.02$). The extent of DNA damage in the same group of workers was monitored one month later when the data were compared to non-factory laboratory controls (Vodicka *et al.*, 1999). DNA damage was greater in the laminators than the controls ($p < 0.001$). In the latter report, the authors reported correlations between air styrene levels or styrene exposure duration and DNA strand breaks in one group of styrene workers (air styrene: $r = 0.5$, $p = 0.006$ to 0.01; exposure duration: $r = 0.4$, $p = 0.02$ to 0.04). However, for an analysis of samples collected over a three-year interval, the correlations were weaker or non-existent.

In a study by Somorovska *et al.* (1999), DNA damage in lymphocytes, observed in the comet assay, was about two-fold greater in the high-styrene-exposed laminators and medium-exposed sprayers (~30 percent T), compared to the non-exposed control group (~15 percent T) ($p < 0.001$). The extent of damage did not differ between the two exposure groups and was not affected by smoking status. The distribution of damage (from undamaged to maximally damaged) differed between the controls and styrene-exposed groups, such that the majority of cells in the control group were undamaged, whereas there was an increase in the proportion of damaged cells compared to undamaged cells among the styrene-exposed workers.

DNA damage, measured by the comet assay (tail length), was evaluated in the whole blood of male workers (n = 14, employment duration seven to > 15 years) at a fiberglass-reinforced plastics factory by Laffon *et al.* (2002a). The control group was 30 male

university staff not exposed to chemicals or radiation. Styrene exposure was estimated from the urinary MA. The mean tail length in blood of exposed workers [48.68 ± 0.33 μm (SE)] was greater than in controls (43.34 ± 0.31 μm, $p \leq 0.01$). The increase was detected at two exposure durations (seven to 15 and > 15 years). An effect of cigarette smoking was detected, but only among the styrene exposed group; no effect of smoking was observed in the control group.

Godderis *et al*. (2004) studied 44 workers exposed to an estimated styrene level of 9.5 ± 9.6 ppm and 44 matched controls to examine the influence of genetic polymorphisms in biotransformation (GSTT1) and DNA repair enzymes (XRCC1 and XRCC3) on the levels of N-terminal hemoglobin adducts and genotoxicity biomarkers. XRCC1 (X-ray repair complementing defective repair in Chinese hamster cells) is involved in base excision repair and rejoining single strand breaks; XRCC3 is involved in repair of cross-links and chromosomal double strand breaks. Results from the Comet assay showed that subjects with high levels of N-terminal valine adducts had higher levels of DNA damage. Frequencies of micronucleated mononucleated (MNMC) and binucleated (MNBC) lymphocytes and micronucleated nasal epithelial cells differed significantly between styrene-exposed and control workers. In the whole group (styrene-exposed plus controls), higher frequencies of MNMC were found in individuals possessing the XRCC3 Met(241) allele; those with the XRCC1 Gln(399) allele showed higher frequencies of MNMC and MNBC. In vitro DNA repair capacity, as measured by residual DNA strand breaks in peripheral blood leukocytes after a styrene oxide (SO) challenge, was also influenced by styrene exposure. After 1 hour of repair, lower levels of residual DNA damage were found in individuals possessing GSTT1 ($P = 0.043$). After 24 hour of repair, lower residual DNA damage was found in individuals homozygous for XRCC1 Arg(194) ($P = 0.013$). Multivariate regression analysis indicated that the duration of exposure, smoking habits and polymorphisms of XRCC1 at codon 399 were important variables affecting the genotoxic responses. The authors conclude that DNA damage occurs in workers exposed to low styrene levels, and that characterizing genotypes of metabolizing and DNA-repair genes is important for the assessment of individual genotoxic risk from styrene exposure.

Rodent Studies of DNA Strand Breaks *In Vivo*

Solveig-Walles and Orsen (1983) observed DNA damage in several organs of NMRI mice exposed to styrene by i.p. injection. DNA damage was expressed as the fraction of single stranded DNA. Within 24 hours after i.p. exposure to 8 mmol/kg, single-strand breaks (SSBs) were observed in liver, lung, kidney, and brain. The fraction of SSBs was highest in kidney (0.38) and liver (0.26) four hours after i.p. injection; the levels decreased over the next 20 hours. Lung and brain SSB levels at four hours were 0.06 and 0.08, respectively, and increased slowly over the next 20 hours to 0.07 and 0.10, respectively. When the mice were treated with a range of doses (1.7 to 10.1 mg/kg), a dose-dependent increase in DNA SSBs was observed in kidney at the four hour time point (0.06 increasing to 0.14).

Solveig-Walles and Orsen (1983) also documented the levels of single-strand DNA breaks induced in mice receiving i.p. injections of styrene-7,8-oxide. Lung, liver, brain, testes, and kidney were analyzed following administration of 1.8-7.0 mmol/kg. One hour

after administration, a dose response relationship of increasing SSBs in kidney DNA was observed ranging from 0.05 up to 0.21 for doses of 1.8 and 7.0 mmol/kg, respectively. Kinetics of induction of SSBs over a 24-hour period by styrene oxide in lung, liver, brain, and testes demonstrated a rapid increase in SSBs in liver (0.14), lung (0.18), and brain (0.22) within one hour of administration. Single-strand breaks then decreased steadily for the remainder of the 24-hour observation period to about 60 percent of the initial levels.

DNA damage, as SSBs (or alkali-labile sites), was reported in lymphocytes, bone marrow cells, liver cells, and kidney cells in female C57BL/6 mice (n = 6 per dose and exposure time), four or 16 hours after they received a single i.p. injection of 100, 250, 350, or 500 mg styrene/kg (Vaghef and Hellman, 1998). Negative controls received corn oil and positive controls received cyclophosphamide. DNA damage was evaluated by the comet assay. The rank order of damage four hours post-exposure was lymphocytes > liver cells > kidney cells > bone marrow cells. Similar responses to styrene-7,8-oxide (at lower doses) were observed, and the rank order was different (lymphocyte = liver > bone marrow > kidney). The dose-response curves for styrene and for styrene-7,8-oxide exhibited sublinear kinetics, which was interpreted to reflect saturation of a detoxification pathway, perhaps mediated by glutathione, of the active metabolite (presumably styrene-7,8-oxide). DNA damage in the styrene-treated mice decreased 16 hours post-exposure for lymphocytes and liver (250 and 500 mg/kg) and remained similar in kidney (250 and 500 mg/kg). In bone marrow the styrene-induced DNA damage appeared to increase after 16 hours at the high dose. The different responses of specific cell types might reflect differences in metabolic capacities, sensitivities to induction or repair of DNA damage, and accumulation level of toxicant.

DNA strand breaks (or alkali-sensitive sites) and oxidized pyrimidines and apurinic/apyrimidinic sites (endonuclease III sensitive) were measured by the comet assay in peripheral lymphocytes, bone marrow cells, and liver cells of male NMRI mice exposed to styrene by inhalation at 0, 176, or 352 ppm styrene for six hours/day for one, three, seven, or 21 days (Vodicka *et al.*, 2001a). In bone marrow there were significant increases in the fraction of endonuclease sensitive sites at both air styrene levels (0.5 and 0.4, respectively, compared to 0.1 for controls, p = 0.05) following 21 days of exposure; no other significant increases were detected in this tissue. In lymphocytes, there was a significant increase in DNA strand breaks after exposure to the high styrene level for seven days. No strand breaks or endonuclease III sensitive sites were detected in liver cells (predominantly hepatocytes) in either treatment group at any time point. The authors interpreted the different tissue responses of DNA strand alterations as reflecting differences in metabolic parameters among these tissues.

Mammalian Studies I*n Vitro*

IARC (1994a) reported DNA strand breaks in an *in vitro* study with rat hepatocytes (Sinha *et al.*, 1983). Scott and Preston (1994) noted the importance of exogenous metabolic activation, which was consistent with a role for the cytochrome P_{450}-dependent monooxygenase enzymes.

Gene Mutation

The interaction of styrene or its metabolites with DNA can result in damage that becomes fixed as a mutation, which can cause a detectable change in phenotype. Two classes of gene mutations can be detected by mammalian or bacterial assay systems: forward mutations and reversion mutations. Forward mutations alter a wild-type gene, resulting in its inactivation, which leads to a detectable loss of gene function. Reversion mutations restore a gene function and thus bring about the return of the wild-type phenotype.

Forward-acting gene mutations that result in alteration of gene function in exposed individuals or cultured cells have been used to study styrene genotoxicity. The hypoxanthine-guanine phosphoribosyltransferase (*hprt*; also abbreviated as *hgprt*) gene codes for the enzyme, hypoxanthine-guanine phosphoribosyltransferase. The enzyme "salvages" the free purines, guanine and hypoxanthine, by catalytically reacting them with phosphoribosylpyrophosphate (PRPP) to form the DNA precursors guanosine monophosphate (GMP) and inosine monophosphate (IMP), respectively. The enzyme also catalyzes the reaction of several cytotoxic guanine and hypoxanthine analogs with PRPP. This gene is located on the X chromosome; only one copy is present in the cells of males and only one copy is activated in cells of females. Therefore, mutations that inactivate this gene cannot be compensated in males and are not compensated by the homologous gene on the silent (inactive) X chromosome in females. The *hprt* assay is based on the lethality of "toxic" nucleotides towards unmutated cells, those with an active *hprt* gene (Purves *et al*., 1995). Mutated cells, which lack the *hprt* gene function, are unable to catalyze the reaction of the "toxic" nucleotides with PRPP and therefore survive. Van Zeeland and Vrieling (1999) noted that for the *hprt* gene the mutational spectrum is dominated by GC to AT transitions. Bastlova and Podlutsky (1996) reported the same transition spectrum for the *hprt* gene in styrene-7,8-oxide treated human T-lymphocytes, and, in addition, they reported mutations that involve AT sites. Mutation frequencies, such as that for the *hprt* gene, are usually expressed as the number of mutations/10^6 cells.

A second assay that has been used to study forward mutations is the glycophorin A (GPA) assay. This assay is based on allele loss at the *GPA* locus, an autosomal gene that codes for a major sialoglycoprotein on the surface of erythrocytes (Grant and Bigbee, 1993). The GPA protein carries the antigenic determinants of the MN blood group, a genetic trait that is carried by half of the human population and, as such, restricts the applicability of this assay. Erythrocytes carrying mutated GPA proteins collected from MN heterozygous individuals are detected by means of fluorescent antibodies against M and N proteins. Currently used methodologies are sufficiently sensitive to allow the detection of rare variants in heterozygous persons. The allelic loss (or inactivation) is thought to occur in the relatively long-lived nucleated hematopoietic precursor cells of the bone marrow. Bigbee *et al*. (1996) suggested that the assay has the potential to reflect cumulative exposure rather than acute exposure. GPA mutations are expressed as number of mutations/10^6 cells.

Reverse mutations are often detected in assays that utilize microorganisms differing from wild-type members of the species by a specific nutritional requirement. A reversion mutation in the target gene allows the microorganism to regain the ability to grow independently of the specific nutrient. Ames and colleagues developed a number of

strains of the bacteria *Salmonella typhimurium* that are dependent on the presence of the amino acid histidine in the culture medium (Maron and Ames, 1983). Following treatment with a test substance, the frequency of histidine-independent *Salmonella* colonies that arise in a histidine-requiring strain can be measured. Strains of *Salmonella* have been developed that detect point mutations, frame-shift mutations, and mutations due to oxidative damage. *S. typhimurium* have been used to detect mutations resulting from the interaction of styrene with DNA.

A limitation of bacterial mutagenesis assays is that many organic chemicals, including styrene, may require prior metabolism to a genotoxic metabolite. Mammalian extracts, usually derived from rodent liver, are included in the test protocol to ensure that the necessary metabolic activation reaction occurs. Although many enzymes may be capable of catalyzing the formation of DNA reactive species, the mutagenicity assay protocol usually emphasizes the expression of the cytochrome P450-dependent monooxygenase complex. Metabolic activation is extensively used in *S. typhimurium* mutagenicity protocols. Interpretation of such data should be carried out with caution because the liver extracts may contain substances that detoxify (e.g., reduced glutathione, epoxide hydrase) as well as activate the test substance (cytochrome P450 system).

Despite the useful information made available by bacterial mutagenesis studies, the organization of the genome and the processing of genetic information are different between prokaryotes and eukaryotes. Mutagenicity assays that utilize "lower" eukaryotic systems also have been used to test styrene. These include mutation assays in yeast (*Saccharomyces*), the fruit fly (*Drosophila*), and the onion plant (*Allium cepa*).

Human Studies of Gene Mutation *In Vivo*

Vodicka *et al.* (1995; 1999) studied the mutation frequencies at the *hgprt* gene locus in T-lymphocytes among styrene-exposed workers. The subjects in Vodicka *et al.* (1999) were: (1) hand-lamination workers, (2) non-styrene exposed factory controls, and (3) laboratory controls from a local research institute. Average factory employment duration was 11 years (range = 2 to 17) for the laminators and 12 years (range = 2 to 22) for the factory controls. Sample collection periods were: A) late 1992, B) one day prior to a two week summer vacation in 1993, C) first day following return from vacation in 1993, D) one month after return from summer vacation in 1993, E) early 1994, and F) early 1995. Between group A and group F, the average air styrene level decreased from 38 to 16 ppm ($p = 0.08$). The study on *hgprt* mutation frequencies in the workers in groups B through E was described in Vodicka *et al.* (1995). When factory controls were used, there were increased mutation frequencies in the laminators in groups B, C, and E that were not significant ($p = 0.2$-0.9). For group D, the mutation frequency in laminators was less than that in the factory controls. When groups B through F were compared with laboratory controls, the laminators in groups E and F demonstrated increased mutation frequencies of 18 compared to 12 per million cells for group E and controls, respectively ($p = 0.02$), and 22 compared to 14 per million cells for group F and controls, respectively ($p = 0.04$); the results were not altered by cigarette smoking (Vodicka *et al.*, 1999).

In both sets of control workers, Vodicka *et al.* (1999) reported no detectable level of air styrene or urinary styrene metabolites. Among the lamination workers in groups B, D, and E, the blood styrene levels ranged from 0.7 to 0.9 mg/L and for factory controls the

range was 0 to 0.08 mg/L (p = 0.001 to 0.006). The lack of difference in the *hgprt* mutation frequencies between laminators and factory controls in groups B, C, and E could be due to a low styrene exposure of the administrative staff in the factory as evidenced by the presence of DNA adducts in their blood.

Vodicka *et al.* (1999) reported a positive correlation between air styrene (r = 0.4, p = 0.03) or exposure duration (r = 0.4, p = 0.05) and *hgprt* mutations among the styrene workers in group F. When data from all samplings were combined, the correlation for exposure duration was marginal (r = 0.4, p = 0.07) and that for air styrene level was not significant.

Laminators and administrative controls from the same factory (n = 18 and 19, respectively) were evaluated for *hgprt* mutations by Vodicka *et al.* (2001b). The mutation frequency among all exposed workers was $20/10^6$ cells (n = 18), which was not a significant increase compared to $13/10^6$ cells among 19 factory controls. When two outliers with extremely high mutation frequencies were omitted, the average *hgprt* mutation frequency among the laminators was $12/10^6$ cells (n = 16). The *hgprt* mutation frequency was not affected by smoking. When taking into account the entire group exposed to styrene, a correlation was found between *hgprt* mutation frequency and blood styrene levels (r = 0.5, p = 0.002). A marginal correlation was observed between years of employment and *hgprt* mutation frequency (r = 0.3, p = 0.07).

The mutation frequencies of two classes of peripheral blood glycophorin A (GPA) variants, ϕ/N and N/N (number of variants per 10^6 cells), were assessed in two separate studies on styrene exposed boat builders (Compton-Quintana *et al.*, 1993; Bigbee *et al.*, 1996). Approximately half of the population carries an M/N genotype; mutations in the M gene can be detected as a loss leading to φ/N or a recombination event resulting in N/N genotypes. The workers were categorized into four exposure groups according to TWA air styrene levels (Compton-Quintana *et al.*, 1993, also reviewed by IARC, 1994a). The exposure labels were: (0) never exposed to styrene, (1) previously, but not currently exposed to styrene, (3) average TWA/8-hr = 13 ppm, and (4) average TWA/8-hr = 40 ppm. (There was no group with the designation "(2).") Two combined groups were also evaluated: "0+1" combined average TWA/8-hr = 1.2 ppm, and "3+4" combined TWA/8-hr = 32 ppm. Job classification was not an adequate surrogate of styrene exposure. Although a control population consisting of unexposed factory workers in the same plant was originally part of the study, the authors reported measurable breathing zone styrene levels of 0.2 to 4 ppm for the control group work areas. Data were expressed as the frequency of ϕ/N or N/N variants (number per 10^6 cells) and two different GPA assays were used. With data from one GPA assay, the median ϕ/N frequency in combined group "(3)+(4)" was statistically greater than the ϕ/N frequency in combined group "(0)+(1)" (10 and 7, respectively, p = 0.03). The median ϕ/N frequency in group 4 (40 ppm) was 11 compared to 8 in group (0) (never exposed) (p = 0.09). Technical difficulties prevented an analysis of the N/N frequencies. Using a different GPA assay the authors reported median frequencies of the ϕ/N variant as 7 for combined group "(3)+(4)" compared to 5 for combined group "(0)+(1)" (p = 0.09), and 8 for group (4) compared to 4 for group (0) (p = 0.06). Compton-Quintana *et al.* (1993) noted the higher numbers of smokers and the small numbers of subjects in the more highly exposed groups and concluded that comparisons could not be made.

The frequencies of the GPA ϕ/N and N/N variants (number of variants/10^6 cells) were studied in styrene workers from ten reinforced plastics plants by Bigbee *et al.* (1996). The workers were matched for age, gender, and active smoking status with control individuals from a research institute and a university. The worker population consisted of 23 males and 24 females exposed to an average air styrene level (TWA/8-hr) of 37 ppm. The frequencies of ϕ/N variants were not different between exposed workers and controls. The frequencies of the N/N variants were increased among laminators (6.8 vs. 5.0 per 10^6 cells for controls, $p = 0.038$) and all workers (6.3 vs. 5.0 per 10^6 cells for controls, $p = 0.058$). When the workers were categorized into low and high styrene exposure, statistically significant differences compared to the controls were observed in the frequency of N/N variants for air styrene levels ≥ 20 ppm (7.2 vs. 5.2 per 10^6 cells, $p = 0.036$). Marginally significant differences in N/N variant frequencies were observed for the urinary metabolites MA and PGA, at levels ≥ 1.2 mmol/L (6.9 vs. 5.2 per 10^6 cells, $p = 0.067$). The authors reported an increased effect of styrene exposure on N/N variant frequencies in women workers compared to men. Where levels of airborne styrene-7,8-oxide were available, no clear association between the GPA variant cell frequencies and airborne styrene oxide was observed. Cigarette smoking was a confounder for the frequencies of the ϕ/N and N/N variants in the Bigbee *et al.* (1996) study. However, the authors reported no interactions between styrene air levels and cigarette usage.

Mammalian Studies – Gene Mutation

The IARC (1994a) review noted mutations at the *hgprt* locus in Chinese hamster lung V79 cells in one study (Beije & Jenssen, 1982), provided that exogenous metabolic activation was supplied. Negative results were found in another Chinese hamster lung cell study (Loprieno *et al.*, 1976).

Bacterial and Other Non-mammalian Studies – Gene Mutation

IARC (1994a) reviewed genotoxicity studies in bacteria, yeast, flies, and plants published between 1977 and 1983 (Table 11). The most widely studied bacterial system was *S. typhimurium*. In strains that detect mutagens by a frame-shift mechanism (TA1537, TA1538, and TA98), there was no evidence of styrene-induced mutations in the absence (20 studies) or presence (23 studies) of metabolic activation. Among the strains that detect mutagens by a base-substitution mechanism (TA100, TA1530, and TA1535), 6/19 studies were positive in the presence of metabolic activation, whereas in the absence of metabolic activation 1/19 was positive and 1/19 was weakly positive. In two studies, mutagenicity of styrene towards *Escherichia coli* (PQ37) was either inconclusive or negative in the absence of metabolic activation; responses in the presence of metabolic activation were not tested. No report on the response of *S. typhimurium* TA102, a strain that is sensitive to oxidative mutagens, was found.

The results reported by IARC (1994a) strongly suggest a requirement for metabolic activation of styrene to a genotoxic metabolite. Such bioactivation is generally provided by rodent liver extracts that contain enzymes of the cytochrome P_{450}-dependent pathway. The liver extracts also contain detoxifying components, such as reduced glutathione, reduced glutathione transferases, and epoxide hydrase. Hence, the formation of

mutagenic metabolites depends on the balance of the activating and deactivating enzymes present in the test system.

Table 11. Gene Mutation in Non-mammalian Test Systems[1]

Test System	Results[2]		Reference
	-S9	+S9	
SOS Repair			
Escherichia coli	?	0	Glośnicka & Dziadziuszko, 1986
	-	0	Brams *et al.*, 1987
Reverse Mutation *(base pair)*			
Salmonella typhimurium TA 100	(+)	(+)	Vainio *et al.*, 1976
	-	-	de Meester *et al.*, 1977
	-	-	Stoltz & Withey, 1977
	0	-	Watabe *et al.*, 1978
	-	-	Busk, 1979
	-	-	De Flora, 1979
	-	-	Florin *et al.*, 1980
	-	+	de Meester *et al.*, 1981
	-	-	Brams *et al.*, 1987
Salmonella typhimurium TA 1530	+	+	de Meester *et al.*, 1981
Salmonella typhimurium TA 1535	-	+	Vainio *et al.*, 1976
	-	+	de Meester *et al.*, 1977
	-	-	Stoltz & Withey, 1977
	0	-	Watabe *et al.*, 1978
	-	-	Busk, 1979
	-	-	De Flora, 1979
	-	-	Florin *et al.*, 1980
	0	+	Poncelet *et al.*, 1980
	-	+	de Meester *et al.*, 1981
Reverse Mutation *(frameshifts)*			
Salmonella typhimurium TA 1537	-	-	Stoltz & Withey, 1977
	0	-	Watabe *et al.*, 1978
	-	-	Busk, 1979
	-	-	Florin *et al.*, 1980
	-	-	de Meester *et al.*, 1981
	-	-	Vainio *et al.*, 1976
	-	-	de Meester *et al.*, 1977
Salmonella typhimurium TA 1538	-	-	Vainio *et al.*, 1976
	-	-	de Meester *et al.*, 1977
	-	-	Stoltz & Withey, 1977
	0	-	Watabe *et al.*, 1978

Test System	Results[2]		Reference
	-S9	+S9	
SOS Repair			
Escherichia coli	?	0	Glośnicka & Dziadziuszko, 1986
	-	0	Brams *et al.*, 1987
	-	-	Busk, 1979
	-	-	De Flora, 1979
	-	-	de Meester *et al.*, 1981
Salmonella typhimurium TA 98	-	-	Brams *et al.*, 1987
	-	-	Vainio *et al.*, 1976
	-	-	de Meester *et al.*, 1977
	-	-	Stoltz & Withey, 1977
	0	-	Watabe *et al.*, 1978
	-	-	Busk, 1979
	-	-	De Flora, 1979
	-	-	Florin *et al.*, 1980
	-	-	de Meester *et al.*, 1981
Saccharomyces cerevisiae	+	0	Del Carratore *et al.*, 1983
Forward Mutation			
Schizosaccharomyces pombe	-	-	Loprieno *et al.*, 1976
	0		Bauer *et al.*, 1980
Miscellaneous			
Saccharomyces cerevisiae, gene conversion	+	0	Del Carratore *et al.* (1983)
Saccharomyces cerevisiae, homozygosis	+	0	Del Carratore *et al.* (1983)
Drosophila melanogaster, sex-linked recessive lethal mutations	+	0	Donner *et al.* (1979)

[1] Adapted from Table 15 of IARC, 1994a.
[2] Symbols: + = positive; (+) = weakly positive; - = negative; 0 = not tested.

Styrene-7,8-oxide is presumed to be the metabolite that is responsible for mutagenicity towards *S. typhimurium* and its mutagenicity has been documented (IARC, 1994b). In addition, Watabe *et al.* (1982) reported the mutagenicity of another hypothesized styrene metabolite, styrene-3,4-oxide. This highly unstable chemical exhibited an enhanced mutagenicity towards *S. typhimurium* strain TA100 (base substitution sensitive) compared to styrene-7,8-oxide under conditions where cytotoxicity was minimized. No mutagenicity was observed in *S. typhimurium* strain TA98 (frameshift sensitive). Although styrene-3,4-oxide itself has not been reported in human or experimental animal tissues, its hydrolysis product, 4-vinylphenol, has been observed at low levels in the urine of styrene exposed humans and rats.

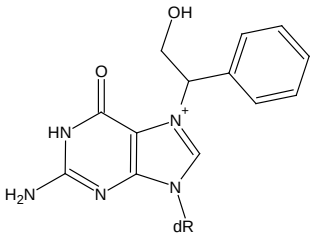
As reviewed in IARC (1994a), in *Drosophila melanogaster*, styrene exposure resulted in sex-linked recessive lethal mutations and not in aneuploidy. The yeast strain *Saccharomyces cerevisiae* exhibited gene conversion, homozygosis (homozygosity), and reverse mutation in response to styrene, and chromosomal aberrations were observed in *Allium cepa*, an onion-like plant (IARC, 1994a).

DNA Adducts

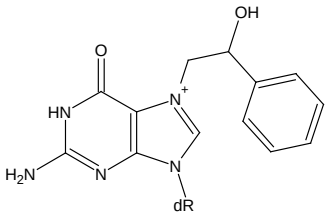
DNA adducts are another potential class of markers for DNA damage from styrene exposure. DNA adducts may result in base changes, strand breaks, and changes in gene expression, ultimately leading to tumors. A common adduct, 8-OHdG, is formed by reaction of deoxyguanine with an active oxygen species generated during the metabolism of styrene. Although 8-OHdG is a non-specific marker for oxidative DNA damage, it correlates with genotoxicity. Zhang *et al.* (2000) noted an increased level of *in vitro* transformation in Syrian hamster embryo cells as a function of the level of 8-OHdG.

Styrene-derived DNA adducts are formed by the covalent binding of styrene or a metabolite to a component of a DNA nucleotide, for example, to the adenine or guanine ring. Adduction to the DNA bases occurs through covalent binding to a ring nitrogen (N), an exocyclic N, or an exocyclic oxygen (O). The ring N number appears as normal script and the exocyclic N or O number appears as a superscript. Specific SO-DNA adducts are expressed in terms of the nucleic acid base (adenine, guanine, cytosine, or thymine), although the individual species detected in hydrolyzed DNA may be the deoxyriboside, the nucleotide, or the bisphosphate of a particular nucleic acid base. Covalent adducts formed between styrene and DNA nucleotides have been detected in the tissues of styrene exposed humans and rodents. The styrene-derived DNA adducts styrene-7,8-oxide-N^7-guanine (N^7-SO-guanine), N^2-SO-guanine, O^6-SO-guanine, N^1-SO-adenine, N^3-SO-adenine, and N^6-SO-adenine have been measured in test tube reactions of purified DNA with styrene. Adducts of cytosine and thymine have also been detected in test tube studies. The styrene molecule itself can react from two positions: α-adducts form from the aliphatic carbon proximal to the aromatic ring, and β-adducts link through the carbon atom distal to the aromatic ring (Reddy, 1993; Kato *et al.*, 1995; Koskinen and Plna, 2000); α and β adducts for guanine are shown in Figure 2.

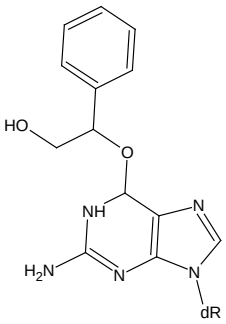
Styrene-derived DNA adducts can be detected by chromatographic analysis of nucleic acids obtained from hydrolyzed DNA isolated from styrene-exposed subjects. Another, more sensitive method is analysis of radioactivity (^{14}C or ^{3}H) in DNA following the administration of radiolabeled styrene; this approach is often used in rodent studies. Also, ^{32}P-postlabeling analysis has been widely used to identify DNA adducts in styrene-exposed humans and laboratory animals. In this procedure DNA from styrene-exposed subjects is digested to its constituent nucleotides, and each is labeled using ^{32}P and specific bacterial enzymes. Normal DNA bases are chromatographically removed, leaving only the nucleotides that contain styrene-derived adducts, which are then identified and quantitated.



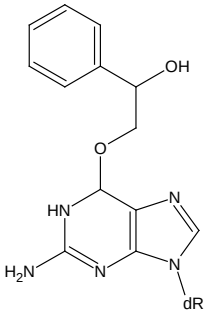
α–N7–SO–guanosine



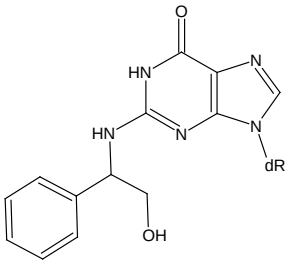
β–N7–SO–guanosine



α–O^6–SO–guanosine



β–O^6–SO–guanosine



α–N^2–SO–guanosine

Figure 2. Some Styrene-derived DNA Adducts for Guanine

The detection of styrene-derived DNA adducts takes into account all absorption routes and exposures from all sources. Van Zeeland and Vrieling (1999) and Koskinen and Plna (2000) noted that, in general, O^6-alkyl nucleotides have high mutagenic activity, whereas the N-alkylated nucleotides are significantly less mutagenic. However, styrene-derived DNA adducts formed by N-alkylation may lead to base-pair changes during DNA replication through the generation of unstable abasic sites or as a result of ring opening (Solomon, 1999; van Zeeland and Vrieling, 1999; Koskinen and Plna, 2000). Chovanec *et al.* (1998) noted that, in general, DNA repair of unstable abasic sites proceeds more slowly than DNA repair of alkylated nucleotides.

Human Studies of DNA Adducts

The DNA adduct, 8-hydroxydeoxyguanosine (8-OHdG), an indicator of non-specific oxidative DNA damage, was increased in the white blood cells of styrene exposed boat builders (n = 17) compared to age matched controls (n = 67) with no history of styrene exposure (Marczynski *et al.*, 1997). Oxidative DNA damage was expressed as the ratio of 8-OHdG to unchanged deoxyguanine (dG). The styrene-exposed boat builders exhibited an 8-OHdG/dG ratio of 2.2 compared to 1.5 for the non-exposed controls ($p < 0.001$). There were no age or smoking associated differences in the 8-OHdG/dG ratio. Exposure to styrene results in increased oxidative DNA damage.

Vodicka *et al.* (1993) studied styrene lamination workers exposed to 87 ppm styrene (n = 10, Group 1) for 12 years or to 49 ppm styrene for six years (n = 13, Group 2). Controls consisted of agricultural workers (n = 3, Group 1 controls) or non-agricultural workers (sample size not given, Group 2 controls) with similar age and smoking histories. The O^6-SO-guanine adduct was quantified in the peripheral blood lymphocytes of all groups. O^6-SO-guanine adduct levels were 4.7 ± 1.9 (SD)/10^8 nucleotides in Group 1 workers (87 ppm styrene) as compared with $0.3 \pm 0.3/10^8$ nucleotides in Group 1 controls (agricultural controls)($p < 0.001$). O^6-SO-guanine adduct levels were $7.3 \pm 4.9/10^8$ nucleotides in Group 2 workers (49 ppm styrene) as compared with $1.1 \pm 1.3/10^8$ nucleotides in Group 2 controls (non-agricultural workers).

The levels of O^6-SO-guanine in the lymphocytes of styrene hand-lamination workers over a 2.5-year period were described by Vodicka *et al.* (1994; 1995; 1999). The subjects were styrene hand-lamination workers (n = 11), non-styrene exposed factory controls (n = 7), and laboratory controls (n = 10) from a local research institute. Sampling occurred at six times (A through F), from late 1992 (A) to early 1995 (F). Average factory employment duration was reported as 11 (range, 2 to 17) years for the laminators and 12 (range, 2 to 22) years for the factory controls. The laboratory controls were used for the two last sampling times (E and F) because of an earlier observation of measurable levels of the O^6-SO-guanine adducts among the factory controls (Vodicka *et al.*, 1995). The average air styrene level among the laminators decreased from 38 (group A) to 16 ppm (group F) ($p = 0.08$) during the 2.5-year interval, whereas urinary MA decreased by approximately 30 percent. Both sets of control workers had no detectable air styrene or urinary MA. Among the lamination workers in groups B, D, and E, the blood styrene levels ranged from 0.7 to 0.9 mg/L, while for the factory controls the range was zero to 0.08 mg/L ($p = 0.001$ to 0.006).

Vodicka *et al.* (1999) reported that O^6-SO-guanine levels among styrene hand-lamination workers ranged from $4.8 \pm 2.8 /10^8$ to $7.2 \pm 4.9 /10^8$ nucleotides during the 2.5-year interval (groups A and F), while for the plant controls (groups B-E) the levels ranged from $0.8 \pm 0.4 /10^8$ to $1.4 \pm 0.8 /10^8$ nucleotides. The range for laboratory controls was $0.5 \pm 0.4 /10^8$ to $0.8 \pm 0.8 /10^8$ nucleotides from 1994 to 1995 (groups E and F). The authors reported statistically significant increases in O^6-SO-guanine adduct levels for laminators compared to the factory controls in groups B ($p = 0.006$), C ($p = 0.001$), D ($p = 0.002$), and E ($p = 0.002$). The differences between the laminators and laboratory controls in groups E and F were also significant ($p = 0.002$ and $p = 0.008$, respectively). In group E, the difference between factory and laboratory controls was marginally

significant (p = 0.06). The authors did not detect a decrease in O^6-SO-guanine in workers after a two-week vacation (comparing Groups B and C). No significant differences in adduct levels were observed in the styrene exposed workers across the six sampling times during the 2.5-year period. Vodicka *et al.* (1994, 1999) suggested that the lack of O^6-SO-guanine decrease following the two-week vacation and the lack of accumulation of the adduct over the 2.5-year interval might reflect a balance between formation and removal of the adduct. No statistically significant differences in adduct levels were observed within individuals over the various sampling times. A significant inter-individual difference (p = 0.03) among lymphocyte adduct levels was detected among all individuals. This could be attributed to individual differences in exposure, individual metabolic responses, and individual DNA repair capacities.

In group F, Vodicka *et al.* (1999) reported a positive correlation between exposure duration and lymphocyte O^6-SO-guanine (r = 0.6, p = 0.006) and a non-significant correlation between levels of the adduct and air styrene concentration. When all sample groups (A-F) were evaluated, positive correlations were observed between exposure duration and lymphocyte O^6-SO-guanine (r = 0.7, p <0.001) as well as between levels of air and blood styrene (r = 0.8, p <0.001).

Horvath *et al.* (1994) measured levels of N^2-SO-guanine and O^6-SO-guanine (two adducts shown to form in calf-thymus DNA treated with styrene-7,8-oxide) in the lymphocytes of workers exposed to styrene (n = 48) in a boat manufacturing facility. Styrene exposure was estimated with personal samplers and ranged from 0.23 to 55 (average = 15) ppm. The ranges of adduct levels were 0.6 to $100/10^8$ nucleotides (mean, 15.8 ± 3.22 (SE)$/10^8$) for N^2-SO-guanine and 0.1 to $70.9/10^8$ (mean, $14.2 \pm 2.3/10^8$ nucleotides) for O^6-SO-guanine. The relationships between air styrene level and adduct level were significant for N^2-SO-guanine (r = 0.2, p = 0.05) and for O^6-SO-guanine (r = 0.3, p = 0.012).

Koskinen *et al.* (2001a) detected N^1-SO-adenine adducts (α- and β-isomers) in the lymphocytes of three out of nine hand lamination workers who had been exposed to 18 ppm styrene for about eight years. The mean adduct level in these three workers was 0.79 ± 0.14 adducts$/10^9$ nucleotides. No adducts were observed in 11 non-factory controls. The low levels of the N^1-SO-adenine adduct in the styrene-exposed workers were detected by ^{32}P-postlabeling modified to overcome the propensity of N^1-SO-adenine to undergo deamination. The presence of N^1-SO-adenine adducts in the workers may be relevant for chronic exposure studies, since the half-life of the N^1-SO-adenine adduct is longer (19 days) than that of the N^7-SO-guanine adduct (one to five days).

Rodent Studies of DNA Adducts

Rodent DNA adduct studies have utilized either analysis of ^{14}C or ^{3}H in DNA following exposure to radiolabeled styrene or ^{32}P-postlabelling analysis. SO-DNA adducts have been observed in rats and mice exposed to styrene by different routes of exposure for a few hours to three weeks.

Rats (individually) and mice (groups of four) were exposed by inhalation in a small chamber (2 L desiccator) to 7-[^{3}H] styrene (Cantoreggi and Lutz, 1993). After 4.5 to 6 hours (rats) and 6 to 9 hours (mice), styrene doses of 23 to 39 and 85 to 110 mg/kg,

respectively, had been metabolized. DNA adducts were quantified by total radioactivity in the nucleotides obtained by hydrolysis of the DNA in liver and lung, and adduct levels were expressed as the covalent binding index (CBI), defined as μmol adduct/mole DNA nucleotide/mmole styrene/kg. The authors accounted for lability of the styrene tritium label, which is exchanged into water during styrene metabolism and may then be incorporated into the deoxyribose of a nucleic acid base.

Four male rats exposed to 2 to 29 mg styrene/kg and four female rats exposed to 32 to 39 mg styrene/kg exhibited CBIs in liver that ranged from < 0.1 to < 0.2. For two male rats the CBI in lung were < 0.1 and < 0.3 and lung adduct levels in the female rats were < 0.08 and < 0.23 for two rats and 0.07 for the other two female rats. In the livers of four male mice exposed to doses of 85 to 92 mg styrene/kg, the adduct CBIs ranged from 0.05 to 0.08, and in the livers of four female mice the range was 0.09 to 0.18. There was insufficient mouse lung tissue for DNA analysis. The difference between the CBIs in male and female mouse liver was not statistically significant. Based on the CBIs for the adducts in rat liver and lung and in mouse liver, the DNA-binding potency of styrene is so low that significant tumor induction in a standard assay for carcinogenicity is unlikely to be due to DNA adduct formation alone.

Boogaard *et al.* (2000b) measured DNA adducts in the liver and lung of male Sprague-Dawley rats and male CD1 mice that were acutely exposed by inhalation for six hours to 160 ppm [^{14}C] styrene (uniformly ring labeled). The adducts were quantified following neutron thermal hydrolysis (NTH) that detected some purine adducts such as N^7-SO-guanine and N^3-SO-adenine, and enzymatic (P1 nuclease and phosphatases) hydrolysis of the remaining DNA backbone by which additional adducts (e.g., N^1-SO-adenine and N^6-SO-adenine and pyrimidine adducts) were detected. Among the identified purine adducts were the two isomers of N^7-SO-guanine, and unidentified purine adducts of styrene glycol and phenylethanol. Other adducts were also present but they were not identified. N^2-SO-guanine, O^6-SO-guanine, and N^6-SO-adenine were not detected.

In rats studied by Boogaard *et al.* (2000b), the major liver adduct resulting from acute styrene exposure was N^7-SO-guanine (3 adducts/10^8 nucleotides). The level of N^7-SO-guanine in rat lung DNA was ~1 adduct/10^8 nucleotides (see Figure 5 of Boogaard *et al.*, 2000). A styrene glycol-derived adduct was detected in rat liver. The N^7-SO-guanine adduct level in rat alveolar Type II cells (65 percent purity) was about 1 adduct/10^8 nucleotides. There were also unidentified adducts in the alveolar Type II cells at levels similar to that of N^7-SO-guanine. Smaller amounts of adducts were observed in alveolar non-Type II cells.

For mice, N^7-SO-guanine in liver represented a minor adduct (≤ 0.5 adduct/10^8) resulting from acute styrene exposure (see Figure 4 of Boogaard *et al.*, 2000b). Other adducts were present at higher levels in mouse liver DNA; one of them was identified as N^7-SO-guanine, a phenylethanol-derived adduct (3 adducts/10^8 nucleotides). N^7-SO-guanine and unidentified adducts were each observed in mouse lung at levels of ≤ 1 adducts/10^8 nucleotides. In mice the level of N^7-SO-guanine adduct in bronchiolar Clara cells was similar to total lung levels.

Boogaard *et al.* (2000b) calculated CBIs from the radioactivity associated with the total adducts (the sum of NTH and enzymatic hydrolysis) in liver and lung, following acute

styrene exposure. CBIs in liver were 0.19 for rat and 0.25 for mouse. CBIs in lung were 0.17 for rat and 0.24 for mouse. The CBIs calculated by Boogaard *et al.* (2000) are higher than those calculated by Cantoreggi and Lutz (1993). One reason could be the use of a highly and uniformly ring-labeled styrene preparation in which the ^{14}C-label is stable compared to the tritium label in the former study. Similarly to Cantoreggi and Lutz (1993), Boogaard *et al.* (2000b) concluded from the CBI values that DNA adduct formation should not play an important role in styrene tumorigenicity in chronically exposed mice.

DNA adducts were detected in the lungs of male NMRI mice exposed by inhalation to 0, 750, or 1500 mg/m^3 (0, 176, or 352 ppm) styrene for six hours/day for one, three, seven, or 21 days using the ^{32}P-postlabelling method (Koskinen *et al.*, 2001b; Vodicka *et al.*, 2001a). The number of mice analyzed from each dose level and exposure duration varied with the adducts analyzed and ranged from one to six. The major adducts identified in the lungs of the exposed mice were βN^7-SO-guanine and βN^1-SO-adenine; no adducts were detected in the lungs of the control mice. The levels of βN^7-SO-guanine at 21 days were 6.5 per 10^8 nucleotides at 176 ppm (n = 2) and 23 per 10^8 nucleotides at 352 ppm (n = 6). At the same time, the levels of βN^1-SO-adenine were 0.14 per 10^8 nucleotides at 176 ppm (n = 3) and 0.60 per 10^8 at 352 ppm (n = 4). Vodicka *et al.* (2001a) measured the blood styrene levels in the exposed mice at one, three, seven, and 21 days; the levels were 0.99 ± 0.51 (S.E.) to 2.04 ± 0.55 mg/L for 176 ppm air styrene and 2.48 ± 1.06 to 7.44 ± 3.21 mg/L for 352 ppm. The authors found that blood levels correlated with N^7-SO-guanine ($r = 0.87$, $p < 0.001$) and N^1-SO-adenine ($r = 0.79$, $p = 0.002$). There was also a correlation between N7-SO-guanine and N^1-SO-adenine ($r = 0.70$, $p < 0.001$). Among the mice exposed to the high styrene dose, there appeared to be an increase in the level of both adducts with increasing duration of exposure (i.e., three to 21 days).

O^6-SO-guanine levels were measured in liver samples (flash frozen in liquid N_2) from female and male CRL (CD) rats (n = 5 per sex per dose group) exposed to styrene by inhalation for two years at doses of 0, 50, 200, 500, or 1,000 ppm (Otteneder *et al.*, 2002). The median levels of O^6-SO-guanine (summing the α and β isomers reported separately) in the livers of the rats exposed to 1,000 ppm air styrene six hr/day, five days/week for two years were $9/10^7$ DNA nucleotides in males and $8/10^7$ nucleotides in females. Liver adduct levels were below the limit of detection for rats in the lower dose groups. Similarly treated lungs were evaluated for the same adduct in tissues obtained from five female CRL (CD) rats exposed to 0 or 500 ppm styrene for two weeks and from female CRL (CD) mice exposed to 0, 40, or 160 ppm for two weeks. The βO^6-SO-guanine isomer was not detected in any of the lung samples from the styrene exposed rats or mice. The levels of the αO^6-SO-guanine isomer in the lungs from the rats and mice exposed for two weeks were not statistically significantly different from controls; rat lung values were less than $1/10^7$ (0 ppm) and $1.9/10^7$ (500 ppm) and mice lung values were $1.8/10^7$ (0 ppm), $2.8/10^7$ (40 ppm), and $1.6/10^7$ (160 ppm) with a detection level of 1 per 10^7 nucleotides.

DNA adducts were observed in the livers of mice exposed to 7-[^{14}C]-styrene by i.p. injection (Nordqvist *et al.*, 1985). N^7-SO-guanine was detected by ultraviolet analysis of chromatographically separated constituents of hydrolyzed DNA. Two hours after exposure, the level of N^7-SO-guanine in liver DNA was 17 ± 5 nmol/g DNA in the mice

administered 1.1 mmol 7-[^{14}C]-styrene/kg, and 31 ± 6 nmol/g DNA in mice administered 4.9 mmol 7-[^{14}C]-styrene/kg (Nordqvist *et al.*, 1985). The authors reported that the levels of this adduct represented about 10 percent of the total radioactivity in the DNA samples. The non-linear increase in adduct levels between the two doses suggested to the authors the presence of saturation of metabolic activation. In mice exposed to 1.1 mmol styrene-7,8-oxide/kg by i.p. injection, liver levels of N^7-SO-guanine were 8 nmol/g DNA.

Male NMRI mice (n = 7 per group) were exposed to styrene by a single i.p. injection of 0, 0.28, 0.51, 1.12, 2.04, 3.23, or 4.35 mmol styrene/kg (Pauwels *et al.*, 1996). Three hours post-injection, DNA adducts were measured in lung, liver, and spleen. The adducts were identified as N^7-SO-guanine and O^6-SO-guanine. The CBI for N^7-SO-guanine was 0.21 (lung), 0.18 (liver), and 0.24 (spleen) μmol per mol DNA nucleotide/mmol styrene per kg bw. The CBI for O^6-SO-guanine was 0.18 (lung), 0.10 (liver), and 0.05 (spleen) μmol per mol DNA nucleotide/mmol styrene per kg. The level of O^6-SO-guanine (fmol/mg DNA) was greater in lung (294 to 1,223) than in liver (156 to 799) or spleen (61 to 833) at all styrene doses (control levels in these organs were 47, 45, and 26 fmol/mg DNA, respectively), whereas the level of N^7-SO-guanine in lung was greater than in the other two tissues at the highest four doses (1,388 to 2,056 fmol/mg DNA in lung, 654 to 1,541 in liver, and 800 to 1,188 in spleen). At the lower two doses, the N^7-SO-guanine levels were 97 to 398 fmol/mg DNA for lung, 170 to 588 for liver, and 422 to 709 for spleen. Control levels were 33, 12, and 58 fmol/mg DNA in lung, liver, and spleen. For both adducts, the increase in adduct level with dose was supralinear. The authors suggested on the basis of pharmacokinetic modeling that styrene metabolism to a reactive metabolite, e.g., styrene-7,8-oxide, was not completed at the higher doses during the short three-hour exposure interval employed in this study.

Other Genotoxicity

Styrene did not cause transformation of cultured mouse cells *in vitro* (Male *et al*., 1985).

Genetic Toxicity Studies - Summary and Conclusions

There have been consistent reports of genotoxic damage in lymphocytes of styrene exposed reinforced plastics workers that manifests as CA (Anwar and Sharmy, 1995; Artuso *et al.*, 1995; Kolstad *et al.*, 1996; Somorovska *et al.*, 1999, Koskinen *et al.*, 2001, Oberheitmann *et al.*, 2001), SCE (Karakaya *et al.*, 1997; Laffon *et al.*, 2002), MN (Laffon *et al.*, 2002), single-strand breaks (SSBs)/DNA damage (Vodicka *et al.*, 1995; Vodicka *et al.*, 1999; Somorovska *et al.*, 1999; Koskinen *et al.*, 2001; Laffon *et al.*, 2002), mutations in the *hprt* gene or Glycophorin A locus (Vodicka *et al.*, 1995; Vodicka *et al.*, 1999; Bigbee *et al.*, 1996; Compton-Quintana *et al.*, 1993), and formation of styrene-derived DNA adducts (Horvath *et al.*, 1994, Vodicka *et al.*, 1995; Vodicka *et al.*, 1999; Marczynski *et al.*, 1997; Koskinen *et al.*, 2001) and the non-specific DNA adduct 8-OHdG (Marczynski *et al.*, 1997). The studies of Vodicka *et al.* (1995, 1999) demonstrated concordance among DNA strand breaks, *hprt* mutations, and styrene-derived DNA adducts in occupationally-exposed workers. Since several cancer studies in humans occupationally-exposed to styrene have observed an increased risk of lympho-hematopoietic cancers with increased exposure to styrene (see section on carcinogenicity in humans), these findings of genotoxicity in the lymphocytes of styrene-exposed

workers are particularly relevant. The observation by Kolstad *et al.* (1996) of specific chromosome translocations (Philadelphia chromosome) in myeloid leukemia patients with a history of occupational exposure to styrene further suggests an association between hematopoietic tumors and styrene genotoxicity.

Consistency of results across genotoxicity biomarkers was demonstrated by Laffon *et al.* (2001b) who reported strong correlations between SCE, MN, and DNA strand breaks in reinforced plastics workers and by Brenner *et al.* (1991), who reported correlations among a number of independently measured genotoxicity endpoints, namely MN, single strand breaks, other measurements of DNA damage, and styrene-hemoglobin adducts, among styrene-exposed boat builders.

There are supporting data for a genotoxic mechanism of action for styrene from *in vitro* analysis of human lymphocytes in culture and from rodent studies *in vivo* and *in vitro*. Chakrabarti *et al.* (1993) and Lee and Norppa (1995) demonstrated that styrene induces SCE in cultured human lymphocytes. In addition, genotoxicity of the styrene metabolite styrene-7,8-oxide was demonstrated by SCE (Lee and Norppa, 1995; Uuskula *et al.*, 1995; Ollikainen *et al.*, 1998), *hprt* mutations (Shield and Sunderson, 2001), and DNA strand breaks by the comet assay (Bastlova *et al.*, 1995; Laffon *et al.*, 2001) in lymphocytes.

This extensive body of evidence suggests that styrene is genotoxic in humans and experimental animals and that a primary mechanism of genotoxicity involves metabolic conversion of styrene to the epoxide, styrene-7,8-oxide. Styrene-3,4-oxide and other metabolites capable of inducing oxidative DNA damage may also contribute to styrene's genotoxicity. Increases in several different genotoxicity endpoints including cytogenetic alterations (chromatid and chromosome aberrations, SCE, MN), single-strand DNA breaks/alkali labile DNA damage, gene mutations, and styrene-derived DNA adducts have been documented in styrene-exposed workers in the reinforced plastics industry, in boat manufacturing, in carpet manufacturing, and in the plastics lamination industry. Similar findings have been reported in cultured human lymphocytes and in experimental animals *in vivo* and *in vitro*. In addition, lower eukaryotes such as yeast and *Drosophila* exhibit genotoxicity in response to styrene exposure, and styrene is mutagenic in *S. typhimurium* strains sensitive to base substitution mechanisms. Although negative and equivocal results have been obtained in some studies, the weight of evidence strongly suggests that styrene is genotoxic in humans, rodents, and non-mammalian species. This conclusion is strengthened by the dose-response relationships observed in exposed workers and experimental animals, and by the consistency of results across multiple exposure routes in rodents. In addition, evidence has been documented for mutagenicity of DNA adducts in styrene-exposed workers and for genotoxicity of styrene metabolites such as styrene-7,8-oxide and styrene-3,4-oxide. Rueff *et al.* (2009), in reviewing the genetic effects observed in humans from occupational exposure to styrene, point out that genetic polymorphisms in humans make it difficult to develop clear-cut exposure/response associations. They suggest that more information on the polymorphisms of enzymes related to the occurrence of sister chromatid exchanges, chromosomal aberrations, and micronuclei would enhance the ability to correlate styrene exposure with genotoxic effects.

Evidence of styrene genotoxicity is consistent across a wide variety of species including humans (described above), experimental animals, lower eukaryotes, and bacteria. Rodent studies *in vivo* by Kligerman *et al.* (1993) demonstrated SCE in blood lymphocytes, lung, and spleen in mice and blood in rats. Vodicka *et al.* (2001a) demonstrated MN in bone marrow from mice exposed to styrene by inhalation. They further demonstrated DNA damage by the comet assay in the lymphocytes, bone marrow, and liver of styrene-exposed mice. DNA strand breaks in mice exposed to styrene by ip injection were also reported by Solveig-Walles and Orsen (1983) and Vaghef and Hellman (1998) in the liver, lung, kidney, brain, and bone marrow. The use of mammalian tissue culture cells *in vitro* demonstrated SCE, CA, and *hprt* mutations in Chinese hamster lung and ovary cells (IARC 1994a). Styrene-induced gene conversion, recombination, and reverse mutation in *S. cerevisiae* were noted by IARC (1994a). CAs were also induced in a study in the plant *Allium cepa* (IARC, 1994a). A number of studies have demonstrated the mutagenic activity of styrene in *S. typhimurium* strains sensitive to base substitutions, using protocols that included metabolic activation with rat liver extracts (IARC, 1994a).

Dose Response for Genotoxicity

A dose response relationship between increasing styrene exposure and increased genotoxicity has been clearly established in studies of occupationally exposed workers and in experimental animals. Yager *et al.* (1993) reported increasing SCE/cell with increasing air styrene levels in boat manufacturing workers exposed to low (1.4 ppm), medium (1.4 to 28 ppm), and high (>28 ppm) styrene. In a study of workers in a reinforced plastics factory, Artuso *et al.* (1995) demonstrated increasing levels of CA with increasing exposure to air styrene over a range of 0 to 326 ppm. For the three CA endpoints evaluated, chromatid aberrations, chromosome aberrations, and total CA, increased aberrations were observed as a function of dose, with positive trend tests. Somorovska *et al.* (1999) reported increasing CA with increased styrene exposure in plastics lamination workers exposed to air styrene levels ranging from 0 to 47 ppm. In this study, significant relationships were also reported for CA and exhaled air and blood styrene levels. Increasing styrene-derived DNA adducts associated with increasing exposure to styrene in air were described by Horvath *et al.* (1994) in workers in a boat manufacturing facility. In this study, the air styrene concentrations ranged from 1 to 235 mg/m^3 and significant linear relationships were found between styrene exposure and two different styrene-derived DNA adducts. Walles *et al.* (1993) reported a dose response for DNA strand breaks and air styrene levels, blood and urinary styrene, and urinary styrene metabolites. Vodicka *et al.* (1999) reported dose response relationships between DNA adducts and air styrene or exposure duration among styrene-exposed workers.

Dose response relationships for styrene exposure in mice have been reported for SCE (Kligerman *et al.*, 1993), DNA single strand breaks in mice (Solveig-Walles and Orsen 1983; Vaghef and Helman, 1998), and styrene derived DNA adducts (Pauwels *et al.*, 1996). Kligerman *et al.* (1993) demonstrated tissue specific dose dependence for SCE in blood, lung, and spleen in mice exposed to 0 to 500 ppm styrene by inhalation. Statistically significant increases in SCE/cell were documented for all doses and organs examined. In female rats SCE/cell increased with increasing styrene concentrations of greater than 250 ppm. Solveig-Walles and Orsen (1983) reported increasing DNA single strand breaks in relation to increasing styrene administered i.p. to mice. A dose

dependent increase was most apparent in kidney for doses of 1.7 to 10.1 mg/kg. Vaghef and Hellman (1998) also demonstrated a dose response for mice injected i.p. with 0 to 500 mg/kg styrene, although the response exhibited sublinear kinetics. Among mice exposed to styrene by inhalation for three weeks, there was a dose-response relationship between blood styrene levels and two DNA adducts (Vodicka *et al.*, 2001a). Pauwels *et al.* (1996) reported increasing levels of styrene-derived DNA adducts in mice given 0 to 4.35 mmol/kg styrene i.p. The dose response was not linear over the entire range tested, which may have been a consequence of the short exposure duration (three hours).

Species Comparison

Study of styrene genotoxicity in humans has been limited to occupational exposures by inhalation. Various routes of exposure, including inhalation, oral, and i.p. injection, have been characterized in mice, rats, and hamsters. Not all genotoxicity endpoints were assessed in each study. A number of studies have demonstrated SCE, but not CA or MN, in the lymphocytes, lung, liver, and bone marrow of mice and rats exposed to styrene by inhalation (Conner *et al.*, 1979, 1980; Kligerman *et al.*, 1993). Mice exposed by inhalation to styrene also exhibited MN and alkali sensitive sites in bone marrow (Vodicka *et al.*, 2001a). Meretoja *et al.* (1978) reported the presence of CA in rats exposed to styrene by inhalation. MN and DNA damage have also been reported in mice administered styrene by i.p. injection (Solveig-Walles and Orsen, 1983; Norppa, 1981; Vaghef and Hellman, 1998). Solveig-Walles and Orsen (1983) described single-strand breaks in DNA from kidney, lung, liver, testes, and brain of mice exposed to styrene by i.p. injection. Norppa (1981) reported MN in the bone marrow cells of mice administered styrene by i.p. injection. Solveig-Walles and Orsen (1983) demonstrated single-strand DNA breaks in the liver, lung, kidney, and brain of mice, and Vaghef and Hellman (1998) reported single-strand breaks in lymphocytes, bone marrow, liver, and kidney of mice.

Both inhalation and i.p. injection studies in mice and rats have demonstrated the formation of styrene-derived DNA adducts in lung, liver, and spleen (mice only). Specifically, administration of styrene by inhalation to mice and rats led to the formation of styrene-derived DNA adducts in the liver and lung in a number of studies (Cantoreggi and Lutz, 1993; Boogaard *et al.*, 2000; Otteneder *et al.*, 2002) and in lung (Koskinen *et al.*, 2001b, Vodicka *et al.*, 2001a). The observations of styrene-derived DNA adducts in mouse lung are highly relevant to the observed carcinogenicity of styrene in the mouse lung in long-term bioassays. Following i.p. administration of styrene, Norquist *et al.* (1985) demonstrated the presence of the styrene-derived DNA adduct N7-SO-guanine in mouse liver and Pauwels *et al.* (1996) reported formation of N7-SO-guanine in the lungs, liver, and spleen of mice. The few studies reviewed in Barale (1991), with an oral exposure, were negative for CA in bone marrow of mice (Loprieno *et al.*, 1978; Sbrana *et al.*, 1983). Inhalation and i.p. studies in hamsters have been negative for MN and CA (Penttila *et al.*, 1980; Norppa *et al.*, 1980).

In humans, peripheral blood lymphocytes from occupationally exposed workers have been found to exhibit CA, SCE, MN, DNA strand breaks, gene mutations, and styrene-derived DNA adducts (Horvath *et al.*, 1994; Anwar and Sharmy, 1995; Artuso *et al.*, 1995; Vodicka *et al.*, 1995, 1999; Bigbee *et al.*, 1996; Kolstad *et al.*, 1996; Karakaya *et al.*, 1997; Marczynski *et al.*, 1997; Somorovska *et al.*, 1999; Koskinen *et al.*, 2001;

Lazutaka *et al.*, 1999; Laffon *et al.*, 2002a,b). In experimental animals, genetic damage in the form of CA, SCE, MN, DNA single-strand breaks, and styrene-derived DNA adducts has been reported for lymphocytes, bone marrow, lung, liver, kidney, spleen, brain, and testes (Conner *et al.*, 1979, 1980; Meretoja *et al.*, 1978; Solveig and Orsen, 1983; Norppa, 1981; Kligerman *et al.*, 1993; Vodicka *et al.*, 2001a, Solveig-Walles and Orsen 1983; Vaghef and Hellman, 1998; Cantoreggi and Lutz, 1993; Boogaard *et al.*, 2000; Koskinen *et al.*, 2001b; Otteneder *et al.*, 2002; Norquist *et al.*, 1985; Pauwels *et al.*, 1996). A number of studies examined multiple tissues for DNA damage by styrene via inhalation or i.p. injection in mice. Solveig-Walles and Orsen (1983) reported that DNA strand breaks in mice given styrene i.p. developed more quickly in liver and kidney, with peak occurrence at four hours, compared to lung and brain, which exhibited peak amounts of DNA damage at 24 hours. In a similar study by Vaghef and Hellman (1998) lymphocytes and liver accumulated DNA damage fastest; the damage peaked at four hours and then decreased over 16 hours. In this study, kidney and bone marrow accumulated DNA damage more slowly, which also decreased much slower, over 16 hours post-treatment. A longer-term study by Vodicka *et al.* (2001a) demonstrated that lymphocytes accumulated DNA damage over a period of seven days while in bone marrow the peak of DNA damage was apparent at 21 days. These differences in tissue responses may be due to a variety of factors including route of exposure, metabolic capacities, sensitivity to induction or repair of DNA damage, and the toxicant levels accumulating in the tissues.

Structure-activity

Further support for the carcinogenic properties of styrene comes from structure activity studies of the ability of styrene analogues to induce genotoxicity endpoints. Norppa and Vainio (1983) demonstrated that methyl-substituted styrene and styrene-7,8-oxide analogues can readily induce SCE in human whole blood lymphocyte cultures. Ethylbenzene and 2-phenylethanol, which lack a double bond in the side chain, were only weakly mutagenic in this assay. The weak effects of styrene analogues without a double bond in the side chain suggest that the metabolites responsible for SCE induction are derived from the conversion of the vinyl group to styrene-7,8-oxide. In classifying styrene-7,8-oxide as a group 2A carcinogen (probably carcinogenic to humans), IARC (1994b) considered its genotoxic properties as evidenced by *in vivo* and *in vitro* CA, MN, and SCE, *in vitro* gene mutation in bacterial and rodent cells, and covalent styrene-derived DNA adducts in humans and rodents. The presence of small amounts of 4-vinylphenol in the urine of styrene exposed humans and rats (Bakke and Scheline, 1970; Pantarotto *et al.*, 1978; Pfaffli *et al.*, 1981) indicates that small amounts of styrene-3,4-oxide, a highly mutagenic compound (Watabe *et al.*, 1978) are formed. The presence of 8-OHdG in styrene exposed workers (Marczynski *et al.*, 1997) suggests that oxidative damage resulting from styrene metabolism may play a role in the genotoxic process as evidenced by the genotoxicity of this adduct towards SHE cells (Zhang *et al.*, 2000) and its modulation of cytosine methylation (Turk *et al.*, 1995).

In a study sponsored by the Styrene Steering Committee (SSC) of the European Chemical Industry Council (CEFIC), Henderson and Speit (2005) reviewed the genotoxicity of styrene in humans. They downgraded nine studies (of a total of 15) considered by the original authors (and accepted by the peer reviewers of the studies and the editor of the

peer-reviewed journal) to be positive, from positive to inconclusive or negative. As a result of their reanalysis Henderson and Speit (2005) decided that the genotoxicity evidence was weak. OEHHA does not agree with the downgrading of these studies and the conclusion of Henderson and Speit.

Nestmann *et al*. (2005) concluded that styrene, through metabolism to styrene oxide, could be clastogenic in humans at workplace levels greater than 125 mg/m^3 (29 ppm). However, they also note that animals exposed only to styrene do not show clastogenic effects up to 1500 mg/m^3 (350 ppm). The greater genetic heterogeneity in the human population than in inbred laboratory animal strains could explain the finding of an apparent higher human sensitivity to clastogenesis.

Developmental Toxicity

Developmental Effects - Human Studies

Styrene effects on human development have not been well studied epidemiologically. We have grouped the existing studies of potential developmental effects into miscarriages, congenital malformations, and adverse birth weight effects. No studies of styrene and late fetal death or transplacental carcinogenesis were found in the published scientific literature. Styrene crosses the human placenta and has been found in fetal and umbilical cord blood at levels proportional to those in maternal blood (Dowty *et al.*, 1976). Exposure to styrene in the studies reviewed here has been occupational, with the highest exposures occurring in the reinforced plastics industry.

Miscarriages

Hemminki *et al.* (1980) reported the risk of spontaneous abortion among women union members who worked in the "styrene industry," described as "mainly reinforced plastics." Pregnancies and spontaneous abortions were identified by linkage to the Finnish hospital discharge registry for 1973 to 1976, and the risk among workers was compared to risk in the general population. Six spontaneous abortions occurred among the workers, yielding a risk of 15 percent, which is significantly greater than the risk of 5.5 percent in the general population (RR = 2.7, $p<0.01$). Hemminki *et al.* (1984) updated this study by extending the identification by three years and using two new reference groups: 1) all chemical union members (about 6 percent were in the styrene industry), and 2) styrene industry members both before they were members and after they ceased membership as another reference group. In contrast to the 1980 findings, spontaneous abortion risk in the styrene workers was less than that in the reference populations. A strength of the 1984 study is its use of employed reference groups, because, relative to the general population, they are more similar socioeconomically to the styrene workers, and their spontaneous abortion risks were calculated by the same methods. Study limitations include small numbers of workers, exposure to other chemicals in the reference groups, exposure misclassification (only some of the women in the styrene industry were exposed to styrene, according to the authors), no adjustment for potentially confounding variables such as age, smoking, and history of pregnancy loss, and potential bias from including multiple pregnancies/woman (due to lack of independence of events).

Harkonen and Holmberg (1982) compared the histories of spontaneous abortion in Finland among 67 female lamination workers exposed to styrene (95 pregnancies) to 67 age-matched solvent-unexposed women (102 pregnancies). Pregnancies and spontaneous abortions were identified in interviews as well as information on styrene exposure and potential confounding variables such as smoking and alcohol consumption. The authors did not report risks or relative risks. Based on numbers of spontaneous abortions (four in each group), OEHHA calculated that the risk in the exposed group was 25 percent compared to 18 percent in the unexposed group. The risk ratio was 1.2, which was not statistically significant. During pregnancy, the styrene-exposed women, in comparison to the styrene-unexposed women, more often smoked (44 percent compared to 23 percent of pregnancies) and more often consumed some alcohol (28 percent compared to 19 percent). Limitations of the study include small numbers of workers, a higher proportion of induced abortions among the exposed, no description of the reference group, no information on history of pregnancy loss (a potential confounding variable), and potential bias from including multiple pregnancies/woman (due to lack of independence of events).

Spontaneous abortion and maternal exposure to styrene among Finnish workers biologically monitored for solvent exposure were examined in a case-control study (Lindbohm *et al.*, 1990). The study methods were similar to those used in the analysis of paternal exposure by Taskinen *et al.* (1989) described below. Cases were spontaneous abortions in the years 1973 to 1983 (n = 73, of which three were exposed to styrene). Controls were live births matched on maternal age (n = 167, of which 17 were exposed to styrene). A government program had monitored the women's exposures via biological samples; for styrene, mandelic acid was measured in urine. Additional exposure and risk factor information was obtained via questionnaire. In estimating styrene exposure, the investigators considered (blind to case status) data on occupation, job description, solvent handling (they appear to have asked specifically about styrene), and urine levels of MA. No excess risk was found. Compared to women with no or low exposure to styrene during the first trimester of pregnancy, women with high exposure had an odds ratio (OR) of 0.3. The investigators speculated that the apparently protective effect of styrene may have been due to spontaneous abortions occurring so early in the pregnancy that they were not known to the women (subclinical). The investigators referenced a study by Mutti *et al.* (1984c), which showed that serum levels of prolactin increase with increasing styrene exposure. They hypothesized that excess prolactin, which has been associated with oligomenorrhea, amenorrhea, and infertility, might cause very early subclinical spontaneous abortion. Strengths of the study included relatively good exposure data, blind exposure assessment, and control of the potentially confounding variables smoking, alcohol, and history of pregnancy loss. Limitations included small numbers with styrene exposure, exposure of the controls to other solvents, and a lower questionnaire response rate for the cases (70 percent) compared to the controls (86 percent).

Congenital Malformations

Harkonen *et al.* (1984a) performed a cohort study of congenital malformation risk among children of men and women working as reinforced plastic workers at 160 workplaces in Finland. The study design was partly historical and partly prospective. A questionnaire, sent to the workplaces in 1976, identified currently employed workers. Births and congenital malformations among the children were identified by linking to national

registries over the period 1963 to 1979. The risk of congenital malformation among the children "born during styrene exposure" was then compared to the risk among children in the general population of Finland. Based on small numbers, no association with styrene was found among male workers (relative risk (RR) = 0.7, 95 percent CI 0.29 to 1.52, based upon seven observed malformations) or female workers (RR = 0.7, CI 0.02 to 3.73, only one malformation). Because the cohort study did not have detailed exposure information, the investigators also conducted a nested case-control study using only the men. For each child with a malformation, two births without malformation from the cohort were selected as controls. The authors reported no numbers for the results, but "the case-control analysis found no differences in the styrene exposure intensity of the case and control fathers." A limitation was that the methods for creating the cohort were poorly described; for example, it was not clear whether just current or current plus former workers were identified in the 1976 questionnaire survey.

Holmberg and colleagues in Finland published a series of studies of congenital malformations and maternal organic solvent exposure using the same data sources and similar methods. The first paper reported findings of a case-control study of organic solvent exposure and CNS malformations (Holmberg, 1979). Cases were 120 CNS malformations reported to the national registry over a two-year period starting in 1976, and controls were 120 maternal-age-matched deliveries immediately preceding the case delivery in the same district. First trimester organic solvent exposures, including styrene, were assessed with questionnaire interviews, during which the interviewer knew the case/control status. While not mentioned in the text, one of the tables shows an association with styrene: three of 120 cases and zero of 120 controls were exposed. The odds ratio was not calculable due to the zero value. Two of the three case exposures were at work from "plastics manufacturing" and the third exposure was at home from reinforced plastics lamination by the husband. A strength of the study was assistance from a chemist and an industrial hygienist in assessing exposure. Limitations included the interviewer not being blind to case/control status and small numbers. In 1980, Holmberg and Nurminen (1980) published the same CNS malformation results as in the Holmberg 1979 paper, except that only occupational exposures were considered (home exposures were ignored). Therefore, in the 1980 paper two (instead of three) of 120 cases and zero of 120 controls were considered exposed to styrene. With respect to styrene, comments here about Holmberg (1979) pertain equally to the 1980 paper.

The third paper in the series was a case-control study of organic solvent exposure and oral cleft malformations (Holmberg *et al.*, 1982). Cases were 378 oral cleft malformations reported to the national registry over a 2.5-year period starting in 1977, and controls were 378 maternal age-matched deliveries immediately preceding the case delivery in the same district. First trimester organic solvent exposures, including styrene, were assessed with questionnaire interviews, during which the two interviewers knew the case/control status. While styrene is not mentioned in the text, one of the tables reported results for styrene: only one of the 378 cases and only one of the 378 controls was exposed. Both exposures occurred at home from husbands' manufacturing of vehicle parts. A strength of the study was the assistance of a chemist and an industrial hygienist in assessing exposure. Limitations included the interviewers not being blind to case/control status and small numbers.

The last paper in the series on maternal organic solvent exposure and malformations was published in 1986 (Holmberg *et al.*, 1986). Four categories of malformations were studied: CNS, oral clefts, skeletal, and cardiovascular. Cases were all those reported to the national registry in Finland over specific time-periods (the periods varied by type of malformation and ranged from two to five years), and controls were the maternal-age-matched deliveries immediately preceding the case deliveries in the same districts. First trimester organic solvent exposures, including styrene, were assessed with questionnaire interviews, during which the two interviewers knew the case/control status. An improvement over previous papers in the series was that exposure level was considered,: five relative exposure categories (none, slight, noteworthy, considerable, and heavy) were assigned based on questionnaire review by two industrial hygiene and two occupational medicine staff blinded as to case/control status. In the analyses of specific solvents, including styrene, all four malformation types were combined into a single case group of 1,475 mothers, who were compared to all 1,475 controls. Exposure was divided into that at work and that outside work, and was counted if the level was "noteworthy" or higher.

For styrene exposures at work, 3 of the 1,475 cases and 3 of the 1,475 controls were exposed. For styrene exposures at home, 4 of the 1,475 cases and 2 of the 1,475 controls were exposed. Odds ratios were not calculated. Strengths of the study included estimation of styrene exposure levels and blinding of the staff who assigned the levels. Limitations included the interviewers not being blind to case/control status, small numbers of exposed subjects, and the apparent lack of peer review, since the paper was published in the proceedings of an international course on safety and health aspects of organic solvents held in Finland in 1985. This study has only limited use for evaluating health risks of styrene exposure.

Decreased Birth Weight

The birth weight of babies born to women working as reinforced plastics workers at 40 manufacturing facilities was studied using a historical cohort study design (Lemasters *et al.*, 1989). The investigators obtained data from company records and interviews with a potential cohort of 2,177 women. The interview and pregnancy sample selection process yielded final study cohorts of 229 exposed and 819 unexposed births. The investigators used three exposure indices: categorical (none, n = 819; low, n = 154; high, n = 75), cumulative ppm-days, and cumulative ppm-days for specific gestational time periods. They also examined a subcohort of 50 "highly exposed" laminators, gel-coaters, and sprayers, for whom the estimated mean exposure was 82 ppm. The non-exposed reference pregnancies were from "office and production staffs at the plastics companies and from other local companies in same communities" and from pre-exposure pregnancies in women who later contributed an exposed pregnancy. After adjusting for potentially confounding variables (including smoking and history of adverse reproductive outcomes), a small decrease in birth weight was found in the 75 "high" category pregnancies (average birth weight was 1 percent lower than in the nonexposed, with a 95 percent confidence interval of – 1.6 percent to + 0.2 percent). In the "highly exposed" subgroup (50 of the 75), average birth weight was estimated to be 4 percent lower than in the nonexposed (95 percent confidence interval of – 7.7 percent to + 0.6 percent, p = 0.08). Strengths of the study included good study design and analysis. The study has not been replicated.

Developmental Effects – Animal Studies

Developmental toxicity studies of styrene have been performed in rats, rabbits, mice, and hamsters, as discussed below. Rats have been exposed by the oral or the inhalation route, whereas other species were exposed by inhalation only.

As background information of general relevance to developmental toxicity, styrene and its metabolites have been shown to cross the placenta in gestation day 16 mice (Kishi *et al.*, 1989, 1992). There is also indirect evidence from studies of testicular effects in offspring that styrene is secreted in the milk of exposed lactating rats, and/or that styrene treatment affects lactation (Srivastava *et al.*, 1992a).

Non-neurotoxic Developmental Endpoints in Animals

Studies of the developmental toxicity of styrene that did not assess developmental neurotoxicity have been performed in rats, rabbits, mice, and hamsters (Ragule, 1974; Murray *et al.*, 1978; Kankaanpa *et al.*, 1980; Rosen *et al.*, 1988; Chernoff *et al.*, 1990; Srivastava *et al.*, 1990, 1992a; Beliles *et al.*, 1985).

In one study, groups of 23 pregnant white rats (strain unspecified) were exposed to 5 or 50 mg/m^3 (1.2 or 12 ppm) styrene for four hours/day throughout pregnancy (Ragule, 1974; Brown, 1991). Fifteen additional animals served as controls. Although not explicitly stated in the paper, it appears that some litters were evaluated prior to delivery, while other dams were allowed to deliver and nurse their litters. For the 50 mg/m^3 group, statistically significant increases in pre-implantation deaths (20.7 ± 3.8 percent versus 3.6 ± 3.1 percent in the control) and total embryo mortality (25.2 ± 7.2 percent versus 15.5 ± 4.4 percent in the control) were identified (no p values provided). Changes in these parameters were not noted in the lower concentration group. The number of stillborn pups per female showed a significant increase in treated animals relative to controls (0.2 ± 0.631 versus 0). There were also significant increases in pup mortality per litter during the second week of life for treated animals (1.66 ± 0.11 and 0.6 ± 0.4 for the high and low concentration groups, respectively, versus 0 in the controls). No treatment-induced changes were reported for offspring weight, size, or soft tissue pathology. "General toxic action" (presumably some sign of maternal toxicity) was noted at the high concentration of 50 mg/m^3.

In a second series of experiments (Ragule, 1974), pregnant female rats were divided into two treated and one control group of 20 animals each. The treated groups were exposed to styrene at concentrations of 1.5 or 5 mg/m^3 (0.35 or 1.2 ppm). Half of the animals in each treated group were exposed to styrene throughout pregnancy, the other half only for the first trimester of pregnancy. All animals were evaluated by necropsy on gestation day 21. Significant increases in resorption frequency were found in both test groups exposed throughout pregnancy (0.2 ± 0.214 and 1.3 ± 0.757, versus 0 in the controls). Significant increases in preimplantation mortality were reported for both concentrations of styrene and for both exposure scenarios (throughout pregnancy and first trimester only). With exposure to either styrene concentration throughout pregnancy, there were significant increases in postimplantation mortality relative to controls; this effect was not seen with first-trimester only exposure. The size and weight of offspring born to dams exposed to 5 mg/m^3 (1.2 ppm) styrene throughout pregnancy were reported to be significantly lower

than control values. These parameters were not affected in the 1.5 mg/m^3 group animals, or in animals exposed to either concentration of styrene for the first trimester only.

In a large study consisting of several experiments, styrene was given to rats orally or by inhalation, and to rabbits by inhalation (Murray *et al.*, 1978). Pregnant rats administered 2 mL/kg styrene (in peanut oil) by gavage were treated twice daily during gestation days 6 to 15 with total daily doses of 0, 180, or 300 mg/kg. For the inhalation experiments, pregnant rats and rabbits were exposed to 0 or 300 ppm styrene on gestation days 6 to 15 (rats), or days 6 to 18 (rabbits), for seven hours/day. In additional experiments, animals were exposed to styrene concentrations of 0 or 600 ppm. Dose-group sizes ranged from 23 to 29 pregnant animals for rats, and 16 to 20 for rabbits.

When exposed by either inhalation or the gavage route, pregnant rats gained significantly less weight than controls over gestation days six to nine. For treatment by the gavage route, there was an apparent dose-effect for this endpoint. A concentration-response relationship was not apparent for inhalation exposure, but both concentrations were not used in the same experiment. No differences in maternal weight gain between treated and control animals were seen for gestation day intervals 10 to 15 or 16 to 20; data were not presented for maternal weight gain over the entire gestation period.

For the rat experiments overall, for either route of exposure, there were no differences among groups in pregnancy rate, fetal viability, resorption frequency, or mean fetal body weight. Nor was prenatal exposure of rats to styrene, by either route, associated with any increased incidences of external or visceral malformations. Mean fetal crown rump length was also unchanged for exposed litters, with the exception of the group exposed to 300 ppm styrene by inhalation (significantly lower than the 44.8 ± 1.0 mm recorded for the concurrent controls at $p < 0.05$). In the companion experiment, which used the 600 ppm level of styrene, the mean crown-rump length was less than that recorded for the 300 ppm group (43.2 ± 1.7 mm, and 43.8 ± 1.5 mm, respectively). Mean crown-rump length for the 600 ppm group was also lower than that for concurrent controls (44.0 ± 1.4 mm), but the difference did not reach statistical significance.

These experiments provide lowest-observed-adverse-effect levels (LOAELs) for maternal toxicity in the rat of 180 mg/kg-day by the oral route and 300 ppm by inhalation. No-observed-adverse-effect levels (NOAELs) for maternal toxicity could not be established for either route of exposure. For developmental toxicity, the NOAEL for the oral route was 300 mg/kg-day, the highest dose tested (HDT), and the LOAEL for the inhalation route was 300 ppm; no NOAEL was identified (based on the significant decrease in crown-rump length).

Pregnant rabbits exposed to 300 or 600 ppm styrene by inhalation suffered no mortality, and displayed no signs of toxicity. There were no changes in maternal body weight gain. There were no significant differences among groups for numbers of litters, implantation frequency, fetal viability, resorption frequency, and fetal body weight or crown-rump length. No malformations were observed in any group. A significant ($p = 0.049$) increase in the frequency of unossified 5th vertebrae, an anatomical variation, was found in the 600 ppm group.

In a third study, groups of 13 to 15 pregnant BMR/T6T6 mice were exposed to 0 or 250 ppm styrene by inhalation on gestation days 6 to 16 for six hours/day (Kankaanpaa *et al.*,

1980). Animals were necropsied for evaluation of their litters on gestation day 16, immediately following the day's exposure session. Also, groups of 2 to 15 pregnant Chinese hamsters were exposed to 0, 300, 500, 750, or 1,000 ppm styrene for six hours daily on gestation days 6 to 18. Pregnant animals were necropsied on gestation day 18, following the day's exposure session.

No maternal toxicity data were reported for mice exposed to 250 ppm styrene. Treated and control litters did not differ significantly in the percentages of live fetuses, or in the number of live fetuses per litter. The percentage of dead or resorbed fetuses was increased in the treated group from 18.2 percent to 26.9 percent ($p < 0.10$), although this endpoint appears to have been evaluated on the basis of total fetuses, rather than on a per litter basis. The percentage of malformed fetuses was increased from 0.9 to 2.9 percent. These figures also appear to be based on fetal, rather than litter, incidence. The observed malformations were described as including: "exteriorization of liver in the control group and bone defects (rib fusion, extra ribs) in the exposed group."

No significant effects on the percentage of live fetuses, the number of live fetuses per litter, or the number of dead or resorbed fetuses, were found for hamsters exposed to 300, 500, or 750 ppm styrene by inhalation. No malformations were reported for any of the concentrations. At 1,000 ppm, the percentage of live fetuses was reduced from 73.8 percent to 34.0 percent, and the average number of fetuses per litter was reduced from 5.3 to 2.4. These changes were not reported as statistically significant, but it is not clear whether statistical analysis was performed. The percentage of dead or resorbed fetuses increased from 26.2 percent to 66.0 percent. This change was reported to be statistically significant ($p < 0.001$), but this seems to have been calculated on the basis of total fetuses, rather than on a litter basis. No maternal data for hamsters are provided in the paper, although the discussion described the extent of maternal toxicity as "small," and noted that other studies have found hamsters relatively tolerant to styrene. These experiments provided evidence of increased prenatal death at 250 ppm in mice and 1,000 ppm in hamsters. No effects were seen on the offspring of pregnant hamsters exposed to 300, 500, or 750 ppm styrene.

In an investigation of the relationship between maternal and developmental toxicity, styrene was tested in pregnant Sprague-Dawley rats (Rosen *et al.*, 1988; Chernoff *et al.*, 1990). Styrene was administered by gavage at 1,147 mg/kg-day. Three to five animals were necropsied on each of gestation days 8, 12, and 16 for weighing of maternal thymus, spleen, and adrenals. Maternal weight gains were also assessed at these times. The remaining 13 animals were killed on gestation day 20 for maternal and fetal evaluations.

None of the styrene-exposed dams died during the study. Maternal weight gain for styrene-exposed dams was lower than controls at each of the time-points measured. These differences were statistically significant ($p < 0.01$) for days 8 and 12 only. Organ weights were significantly reduced in treated animals only on gestation day 12. Only thymus and spleen weights were affected ($p < 0.01$, and $p < 0.001$, respectively). Day 12 adrenal weights were not affected. For 133 fetuses from 13 styrene-exposed litters, there were no significant effects on viability, fetal weights, the frequency of supernumerary ribs, effects on fourth or lateral ventricles, or on the right kidney. The left kidneys of styrene-treated fetuses had a significantly increased frequency of enlarged renal pelvis ($p < 0.05$). Fetal data were evaluated on an individual, rather than litter, basis.

Timed-pregnant rats (10-14/group) were gavaged with styrene at doses of 0, 250, or 400 mg/kg-day on gestation days 6 to 15 (Srivastava *et al.*, 1990). Dams were observed daily for clinical signs of toxicity, and weighed on gestation days 6, 10, 15, and 20. Animals were necropsied for fetal evaluations on gestation day 20. No deaths or behavioral abnormalities were observed among maternal animals. Body weights of the 250 mg/kg-day dams were not different from controls. At the higher dose of 400 mg/kg-day, however, maternal body weights on gestation day 20 were significantly ($p < 0.001$) lower than those of controls. The authors note that the significant decrease in maternal weights may have been accounted for by the extent of fetal resorptions and retarded fetal growth for litters in that group.

No gross or skeletal abnormalities were observed in fetuses of any group. The mean numbers of corpora lutea per litter did not differ among groups. The mean number of implantations per litter was 9.43 in controls, 9.8 at 250 mg/kg-day styrene, and 5.83 at 400 mg/kg-day styrene. The number of live fetuses/litter was 9.36 in controls, 9.8 at 250 mg/kg-day, and 4.83 at 400 mg/kg-day. No statistical analysis was reported for these endpoints. Fetal weights showed a significant ($p < 0.001$) decrease for the 400 mg/kg-day dose group. However, fetal weight data were not presented as litter means, but rather as mean values for "50 fetuses selected randomly from different litters in each group."

In a study of styrene effects on testicular development (Srivastava *et al.*, 1992a), twelve pregnant rats were divided into three groups of four. At parturition, eight male pups were randomly assigned to each dam, forming three groups of four dams with eight pups each. Lactating dams were gavaged with styrene at doses of 200 or 400 mg/kg-day; control animals were given the groundnut oil vehicle. Treatment was continued throughout lactation. Pups were weaned after 21 days and pooled within dose groups. At post-natal days 31, 61, and 91, six pups were randomly removed from each group and necropsied for evaluation of testes and epididymides.

During the treatment period, neither dams nor pups displayed any signs of styrene toxicity. There was no significant effect of styrene on body or testis weight at any dose. No histopathological differences were noted between treated and control animals. Epididymal spermatozoa counts were significantly depressed ($p < 0.05$) at 400 mg/kg-day at both 61 and 91 days of age.

Statistically significant ($p < 0.05$) changes were found in testicular activities of β-glucuronidase (β-Glu), acid phosphatase (AP), and γ-glutamyl transpeptidase (γ-GGT) for 31 and 61 day-old animals after exposure to the high dose of styrene. At the high dose, significant ($p < 0.05$) changes were seen at 31 and 61 days for sorbitol dehydrogenase (SDH), and at 61 days only for lactate dehydrogenase (LDH). No differences from controls were seen in the enzymes at the lower dose, or at other time points; and no changes were observed in glucose-6-phosphate dehydrogenase (G6PDH) at any dose or time point.

While Srivistava *et al*. (1992a) note that the functional significance of these enzymes to testicular development is not fully understood, they cite papers indicating that the appearance of certain enzymes (i.e., SDH, LDH, G6PDH, γ-GGT, β-Glu, and AP) in specified testicular cell types is a tool used to identify key stages of spermatogenesis. They cite the example of rising and falling levels of LDH and SDH reported to be

associated with maturation of the germinal epithelial layer of seminiferous tubules. On this basis, the authors suggest that the altered patterns of LDH and SDH activity observed in styrene-treated animals may indicate that styrene exposure causes degeneration of the germinal epithelium. Similarly, the authors suggest that the increases in γ-GGT and β-Glu seen in styrene-treated animals may have indicated interference with the normal physiology of Sertoli cells, in turn affecting development of spermatocytes and spermatids. Thus the authors concluded that their results, in particular the decreased epididymal sperm counts at the 400 mg/kg-day dose of styrene, suggest that styrene treatment of lactating mothers resulted in damage to the developing germ cells in the maturing testes of their male offspring.

In a study of the reproductive effects of styrene monomer (Beliles *et al.*, 1985), 50 male and 70 female rats per test group, and 76 male and 104 female controls, were placed on a two-year drinking water study. Concentrations of styrene monomer given in drinking water were 0, 125, or 250 ppm. The authors calculated approximate daily doses of 7.7 and 14 mg styrene/kg-day for low and high-dose males, respectively, and 12 and 21 mg styrene/kg-day for low and high-dose females, respectively. Ten males and 20 females randomly selected from each group were mated to produce the F1 generation. The F1 animals were eventually mated to produce a third generation of exposed animals.

Life-long ingestion of drinking water containing up to 250 ppm styrene did not significantly alter the physical appearance or longevity of the rats in this study, or result in "consistent or meaningful" differences between treated and control animals for body weight up until the last week of the study. There were no significant feed consumption differences among groups, although water consumption was affected by styrene. No changes in weights or histological appearance were noted for ovaries or testes of treated animals at necropsy. In the reproductive component of the study, mean body weight and food consumption of F_0 females were not affected by styrene treatment, nor were indices of male or female fertility, or gestational survival impaired by styrene exposure.

F_1 pup survival at postnatal day 21 was significantly ($p < 0.05$) diminished in 250 ppm-group animals, but the authors noted that all pup deaths between days 15 and 21 appeared to involve pups from only two litters. There were no treatment-related effects evident in pup liver or kidney weights, in organ-to-body-weight ratios, or upon histopathological examination of pup liver and kidney.

When mature F_1 animals were mated to produce the F_2 generation, pregnant dams showed no treatment-related changes in mean weight or food consumption. Neither male nor female fertility was affected, but pup viability at birth was significantly decreased with 250 ppm styrene ($p < 0.05$). Pup survival was significantly reduced for this group on postnatal days 1, 7, and 14 ($p < 0.05$ in each case), but the reduction was not significant on day 21. Pup organ weights, organ-to-body-weight ratios, histopathology, and cytogenetics performed on femoral bone marrow samples were all considered "unremarkable."

Average body weights and feed consumption for F_2 female breeders were also unaffected by styrene treatment. There was a non-significant reduction in the proportion of 250 ppm-group females producing offspring, but two of the five females failing to litter had been mated to the same male. At postnatal days 7 and 14, F_3 pups in the 250 ppm group

weighed less than controls ($p < 0.05$ for both times), but there was no difference on postnatal day 21. Pup organ weights, organ-to-body-weight ratios, histopathology, and cytogenetics revealed no consistent indications of dose-related differences among groups.

In summary, a number of studies were reviewed in which repeated doses of styrene were administered throughout gestation or during a restricted portion of gestation, and offspring were evaluated at or before the time of birth (Ragule, 1974; Murray *et al.*, 1978; Kankaanpa *et al.*, 1980; Rosen *et al.*, 1988; Chernoff *et al.*, 1990; Srivastava *et al.*, 1990).

Only two studies were identified which evaluated the effects of styrene on non-neurological developmental endpoints using a study design incorporating postnatal exposures and evaluations. One of these, Srivastava *et al.* (1992a), focused on development of the male rat reproductive system following lactational exposure to styrene. (These data are also discussed in the section on male reproductive toxicity.) The other, Beliles *et al.* (1985), was a chronic drinking water study conducted in rats, which included a multigeneration reproductive toxicity component. (These data are also discussed in the section on female reproductive toxicity.)

Supplementary information relevant to the potential developmental toxicity of styrene includes mechanistic studies (Daston *et al.*, 1991a; Taubeneck *et al.*, 1994), studies conducted *in vitro* (Daston *et al.*, 1991b), studies of chemicals related to styrene (i.e., styrene oxide; Sikov *et al.*, 1986), and studies of the distribution of styrene from the mother to her fetuses or nursing pups (Kishi *et al.*, 1989, 1992; Srivastava *et al.*, 1992a).

Effects in Offspring prior to Parturition

Taken as a whole, several studies (Ragule, 1974; Murray *et al.*, 1978; Kankaanpa *et al.*, 1980; Rosen *et al.*, 1988; Chernoff *et al.*, 1990; Srivastava *et al.*, 1990) provide evidence for adverse effects of styrene on endpoints including prenatal viability, growth, and morphological development in rats, mice, and hamsters. Taken individually, however, most of these studies have deficiencies in design and/or reporting that limit or prevent their use in deriving fetal and/or maternal NOAELs and LOAELs. Among these studies, the one most suitable for quantitative risk assessment is that of Murray *et al.* (1978).

Murray *et al.* (1978) found no significant effects on pregnant rabbits exposed to 0, 300, or 600 ppm styrene by inhalation. No LOAELs could be determined for maternal animals from these data. There were no significant differences between treated and control rabbits for numbers of litters, implantation frequency, fetal viability, resorption frequency, and fetal body weight or crown-rump length. No malformations were observed in any group. A significant ($p = 0.049$) increase in the frequency of unossified 5th vertebrae (a morphological variation) was found in litters of the 600 ppm group. This observation was noted in the text of the paper, but was not presented in the same tabular form as the other data.

In additional experiments reported in the same paper (Murray *et al.*, 1978), no effects were found on rat offspring exposed prenatally to twice-daily gavage doses of styrene totaling 0, 180, or 300 mg/kg-day. Maternal weight gain was significantly decreased during gestation days 6 to 9 (the first three days of the treatment period), but not during the later part of the treatment period (days 10 to 15) or subsequent to the cessation of treatment (gestation days 16 to 20). A similar pattern of maternal weight effects was

observed when pregnant rats were exposed to 0, 300, or 600 ppm styrene by inhalation. Considering these data, the LOAELs for maternal toxicity in the rat are 180 mg/kg-day by the oral route and 300 ppm by inhalation. No NOAELs for maternal toxicity could be established for either route of exposure.

For developmental toxicity, the oral route NOAEL was 300 mg/kg-day (the HDT), and the inhalation route LOAEL was 300 ppm; no NOAEL was identified (based on the significant decrease in crown-rump length). Interpreting a concentration effect from the Murray *et al.* (1978) inhalation data for rats is complicated by an experimental protocol in which the two concentration levels, 300 and 600 ppm, were not run concurrently. Each concentration was tested separately, and compared to its own control group. Mean crown-rump length at 600 ppm was lower than that for the concurrent controls, although the difference was not statistically significant. Mean crown rump-length at 600 ppm was also lower than that for the 300 ppm group (in the other experiment). The two control groups also differed from one another.

Postnatal Observations

Srivastava *et al.* (1992a) found significant reductions in epididymal sperm counts for exposed male offspring at 61 and 91 days of age. The LOAEL for this effect was 400 mg styrene/kg-day, with a NOAEL of 200 mg/kg-day. The same dose-relationship was found for significant reductions in testicular concentrations of certain enzymes. In the absence of data on styrene secretion in milk, the dose to pups in this study is unknown.

Beliles *et al.* (1985) conducted a chronic drinking water study of 0, 125, and 250 ppm styrene in rats, which included a multigeneration reproductive toxicity component. Approximate daily doses were 7.7 or 14 mg/kg for males, and 12 or 21 mg/kg for females. No adverse effects were identified in animals exposed for two years. No maternal toxicity was noted for three generations of breeding animals. No significant changes in fertility were noted. There were no treatment-related effects on absolute or relative pup organ weights, or on organ histology, for any of the three generations. There were significant effects on pup viability at birth for the F_2 high-dose group. Viability was also affected at various postnatal time-points for high-dose pups of the F_1 and F_2 generations. Body weights of high-dose pups were affected in the F_3 generation on postnatal days 7 and 21. Based on offspring viability data, a LOAEL of 250 ppm (or 21 mg/kg-day) can be set for developmental toxicity of styrene, with a corresponding NOAEL of 125 ppm (or 12 mg/kg).

Developmental Neurotoxicity

Neurotoxicity has been the basis of both occupational and environmental exposure limits for styrene including the ACGIH Threshold Limit Value (TLV), the U.S. EPA Reference Concentration (RfC) for air, and the OEHHA chronic Reference Exposure Level (REL) for air. Of five publications on the developmental neurotoxicity of prenatal exposure to styrene in rats, three appear to describe aspects of one study (Chen *et al.*, 1989; Kishi *et al.*, 1995; Kishi *et al.*, 1992). A second study has been described in the two other publications (Katakura *et al.*, 1999, 2001). These studies and others involving postnatal styrene exposure (Zaidi *et al.*, 1985; Shigeta *et al.*, 1989; Khanna *et al.*, 1991; Aikawa *et al.*, 2001) contribute to the body of information concerning the effects of styrene on the

developing nervous system. Several of these studies also provide information relevant to the potential of styrene to delay attainment of physical developmental landmarks.

Groups of pregnant Wistar rats were exposed to styrene by inhalation at 0, 60, or 297 ppm, 6 h/day, on each of gestation days 7 to 17 (Chen *et al.*, 1989). It is not stated how many litters were treated, or how the resulting offspring were later selected for inclusion in various behavior test protocols. Data tables show numbers of 4 to 29 animals per concentration (single sex at the lower n values, and both sexes at the higher end). No clinical evidence of toxicity was reported for maternal rats; there were "slight" effects on maternal weight with 297 ppm styrene. No marked differences were found between treated and control animals for gestation length, the number of implantation sites, or the number of fetuses.

Body weights of the 297 ppm group pups were significantly ($p < 0.01$) lower than those of controls at one to seven days postnatal age. At 21, 77, and 125 days, body weights of animals from both styrene-exposed groups were lower than controls (no data or level of significance provided). Developmental landmarks, such as eye-opening, incisor eruption, auditory startle response, and air righting reflex, were all significantly different in 297 ppm pups as opposed to controls (all at $p < 0.01$ or 0.05).

Both groups of styrene-exposed pups showed delays in responses on the grasping test, while the 297 ppm group animals also showed delays in response on the negative geotaxis and rotation tests. There were no clear differences between treated and control animals for the righting test or precipice avoidance test.

At the earlier time-points for the open field test, 297 ppm group animals displayed statistically significantly higher numbers of movements and frequencies of standing than did controls. The differences in "number of squares of movement" disappeared by the 127 to 128 days of age test time-point. A similar pattern of age and treatment-related findings was noted for the rotation rod test: treated animals, especially from the 297 ppm group, remained on the rod for shorter periods, but this difference disappeared by 120 days of age. At 40 to 50 days of age, 297 ppm group offspring showed significant increases in the frequency of activity over the first hour of testing ($p < 0.01$), as well as over the first 24 hours ($p < 0.05$) and over the dark part of the light-dark cycle ($p < 0.05$). In the first three of seven sessions after a continuous reinforcement procedure (CRF), treated animals showed significantly fewer bar presses than controls ($p < 0.05$ for 297 ppm and 60 ppm group animals during session 3; $p < 0.01$ for 60 ppm animals during session 1, and for both groups during session 2). For the fourth session, and thereafter, there were no significant differences between groups.

Brown *et al.* (1991) noted that the data in Chen *et al.* (1989) appear to originate from the same experiments as in Kishi *et al.* (1995; discussed below). In the Chen paper, the data appear to have been analyzed on an individual pup basis, rather than by litter.

Pregnant Wistar rats inhaled 0, 50, or 300 ppm styrene for six h/day during gestation days 7 to 21 (Kishi *et al.*, 1995). Fourteen pregnant animals served as controls, three were exposed to 50 ppm styrene, and seven to 300 ppm. Fewer litters were evaluated in the behavioral studies: five at 0 ppm, two at 50 ppm, and five at 300 ppm. This was said to be due to the limited number of inhalation chambers restricting the eventual number of

litters of the same age for testing in the behavioral batteries. On postnatal day one, all litters were culled to no more than six pups. Offspring were weaned on postnatal day 22.

None of the dams showed evidence of toxicity, and there were no significant differences among groups in maternal weight gain, gestation length, or number of live-born offspring per litter. Three pups from different groups were found dead prior to culling on postnatal day one, but this pup mortality was not considered treatment-related. Compared to untreated controls, the bodyweights of pups from the 300 ppm styrene group were not significantly altered on postnatal days 1 and 125, but were significantly reduced on postnatal days 21 and 77.

Delays in incisor eruption, eye-opening, auditory startle reflex, and righting reflex were observed in styrene-exposed pups; these delays appeared to be dose-related. Vaginal opening in females was the only developmental landmark which did not appear to have been affected. These differences were significant "when the calculations were based on the mean of the data obtained for all offspring within a litter," but significance levels are not clearly assigned to specific group comparisons in the tabulated data. A comparison based on data for individual offspring "implied that even the 50 ppm exposure group had delayed auditory startle reflex, and righting reflex compared to the control group, $F(2,66) = 42.3$ ($p < 0.05$), and $F(2,66) = 44.4$ ($p < 0.05$), respectively."

Preweaning behavior tests included surface righting, pivoting, bar holding, negative geotaxis, and cliff drop avoidance. Pivoting and bar holding were both significantly ($p < 0.0001$) delayed in offspring prenatally exposed to 300 ppm styrene. There was also a significant time-by-dose interaction in the neurobehavioral development of bar holding. Surface righting also showed a significant dose effect.

Postweaning assessment of neurological parameters included open-field activity, rotarod performance, spontaneous activity, operant behavior, postnatal sensitivity to barbiturates, and brain pathology. Open field behavior was evaluated at 30 to 31 days, 60 to 61 days, and 127 to 128 days. Compared to control animals, ambulation was significantly increased in high-concentration group males at 30 to 31 and 60 to 61 days ($p < 0.01$ at both time points). Rearing was significantly increased in males and females of the high-concentration group at both 30 to 31 and 60 to 61 days ($p < 0.01$ in all cases except for females on day 60 to 61; that p value was < 0.05). Ambulation and rearing were not affected in males or females on days 127 to 128.

Rota-rod duration times at 8, 12, and 16 rpm were significantly reduced for 300 ppm animals at 30 and 60 days postnatal age ($p < 0.01$ in all cases), but were not affected at 120 days. As there were no differences between the sexes within each group, data for males and females were analyzed together.

Males only were used for observations of 24-hour activity levels and for determination of rates on a continuous reinforcement schedule. No significant differences were observed between control and 300 ppm animals for exploratory activity during the first hour, or light phase activity. Significant differences between these groups were found, however, for total activity over 24 hours ($p < 0.05$), and dark phase activity ($p < 0.01$). A significant effect of prenatal exposure to 300 ppm styrene on the response rate to continuous reinforcement was found only in the third of seven sessions ($p < 0.01$). In this case, the response rate was decreased.

Barbiturate-induced sleep times did not differ among groups when tested at 150 days postnatal age. No overt microscopic abnormalities were detected in serial sections of brain, lung, liver, or kidney of maternal animals, or in offspring at 21 or 160 postnatal days of age.

Kishi *et al.* (1992) reported neurochemical data for animals exposed to styrene as part of the Kishi *et al.* (1995) study. Pregnant Wistar rats were exposed to 0, 50, or 300 ppm styrene by inhalation, for six h/day during gestation days 7 to 21. Fourteen pregnant animals served as controls, three were exposed to 50 ppm styrene, and seven to 300 ppm. Following parturition, some litters were retained for evaluation in the behavioral studies: five at 0 ppm, two at 50 ppm, and five at 300 ppm. Ten pups/group were necropsied for neurochemical analyses on postnatal day one. Pup brains were separated into cerebrum and cerebellar portions and assayed separately for norepinephrine (NA), 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA), 5-hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA), and 5-hydroxytryptamine (5-HT).

Basic maternal and litter data are the same as reported by Kishi *et al.* (1995). In Kishi *et al.* (1992), however, pup body weights at one-day postnatal age are reported for the full set of offspring (rather than just those used for the behavioral studies). Pups in the two treated groups weighed significantly less than control pups ($p < 0.01$ at both concentrations of styrene), but there was no apparent dose response.

There were no differences among groups of pups or dams in brain weight or the histological appearance of brain and other tissues. Protein contents of cerebrum and cerebellum did not differ among groups of one-day old pups. 5-HT, 5HIAA, and HVA were significantly ($p < 0.05$) reduced in pups prenatally exposed to 300 ppm styrene. DA was reduced in this group also (at a non-significant p value of < 0.1). 5HT was reduced in the cerebellum of rat pups prenatally exposed to 300 ppm styrene ($p < 0.1$). No significant changes were shown for DOPAC or NA in the cerebrum, or for 5HIAA, DA, DOPAC, HVA, or NA in the cerebellum.

Timed-pregnant Wistar rats were exposed to 0, 50, or 300 ppm styrene by inhalation during gestation days 6-20 (Katakura *et al.*, 1999). Two control groups were established: one fed *ad lib* (control-1), and the other pair-fed the amount consumed by the 300 ppm styrene exposed group (control-2). The 50 ppm group was also pair-fed to the 300 ppm group, to control for possible effects of feed consumption differences between groups. Food consumption during the treatment period was 18.0 ± 1.3 g/day for the *ad lib* control group and 15.5 ± 2.4 g/day for the groups pair-fed to the 300 ppm styrene group.

Ten to 14 litters per treatment group were evaluated at birth. There were no significant differences among groups in live-born offspring/litter, pup body weight, or cerebellar weight. Mean cerebrum weight was significantly ($p < 0.05$) decreased in the group of pups exposed prenatally to 300 ppm styrene compared to *ad lib*-fed controls. Brain, liver, lung, and kidney tissues from dams and pups at birth or 21 days of postnatal age, examined for microscopic pathology, had no lesions.

Brain tissues of newborn pups were assayed for 5-HT, 5-HIAA, DA, 3,4- DOPAC, 3-methoxytyramine (3-MT), HVA, and NA. Measurements were made on three or four pups/litter, from ten litters for each treatment group; data were analyzed on a per litter basis. Compared to *ad lib*-fed controls, 5-HT and HVA were significantly reduced in the

cerebra of newborn offspring exposed prenatally to 300 ppm styrene; HVA was also significantly decreased relative to pair-fed controls.

Pups evaluated on postnatal day 21, following prenatal exposure to 50 ppm styrene, had significantly increased 5-HT levels in the striatum, relative to either *ad lib* or pair fed controls. This increase was not seen in the 300 ppm styrene group, nor was it seen for 5-HT levels in the neocortex or hippocampus. In the 300 ppm styrene group, 5-HIAA was significantly decreased in the frontal neocortex relative to *ad lib*-fed controls and significantly reduced in the pups' hippocampi compared to either control group. The ratio of 5-HIAA/5-HT in the hippocampus was significantly decreased in both styrene-exposed groups. Striatal DA levels of 21 day-old offspring in the high concentration group were significantly increased over *ad lib* controls.

In neonates, DA levels did not differ between control and styrene-exposed groups, but HVA was significantly decreased in the 300 ppm styrene-exposed group. The authors suggest that HVA metabolism may have been reduced in these animals. Day 21 DA levels for this group were significantly increased relative to *ad lib* controls, an observation the authors suggested may have resulted from a "catch-up" phenomenon.

The authors hypothesize that styrene exposure during a critical period of prenatal development may decrease 5-HT in the fetal brain, in turn affecting later overall brain development. Exposure to the higher concentration of styrene was associated with decreased cerebrum weights, as well as decreased 5-HT. Pair-feeding to control for reduced feed intake of treated dams appears to have accounted for some, but not all, of the quantitative changes induced by styrene exposure.

Katakura *et al.* (2001) studied the effects of prenatal styrene exposure on postnatal development and brain serotonin and catecholamine levels in rats. The data appear to have originated from the experiment reported in Katakura *et al.* (1999). The earlier report emphasized neurotransmitter levels in different brain regions, while the 2001 report provides information on additional endpoints such as gestation length and age at attainment of physical and behavioral developmental landmarks. Maternal feed consumption during treatment was significantly ($p < 0.01$) reduced in animals exposed to 300 ppm styrene, with respect to *ad lib* fed controls. Maternal weight gain over the same period, however, was not significantly reduced in either styrene-exposed group.

Gestation length was significantly ($p < 0.05$) increased for the 300 ppm group compared to *ad lib*-fed controls (22.1 $\pm$ 0.6 and 21.6 $\pm$ 0.5 days, respectively), but did not differ significantly from the pair-fed groups. While the number of live-born offspring/litter did not differ among groups, the percentage of neonatal deaths was significantly higher in the 300 ppm group than for either *ad lib* or pair fed controls. The percentages of male pups over all litters were 49.2 $\pm$ 0.1 for *ad lib* fed controls, 48.0 $\pm$ 0.1 for pair-fed controls, 45.4 $\pm$ 0.1 at 50 ppm styrene (pair-fed to the high concentration group), and 41.6 $\pm$ 0.1 at 300 ppm styrene. These differences were not statistically significant.

When females and males were considered together, pup body weights on postnatal day 21 did not differ among groups. Incisor eruption (upper and lower), eye opening, and attainment of the air-righting reflex were all significantly delayed in high-concentration group pups relative to *ad lib* controls. Eruption of the upper incisors and attainment of the air-righting reflex were also significantly delayed in the high-concentration group

relative to pair-fed controls. The air-righting reflex was also delayed in the 50 ppm group relative to *ad lib* controls ($p < 0.05$). No differences were found among groups for age at ear unfolding or for attainment of the surface-righting reflex.

Zaidi *et al.* (1985) studied changes in dopamine receptor binding following prenatal, lactational, or prenatal plus lactational exposure to styrene in rats. For the prenatal-exposure-only experiments, styrene was given to the dams throughout gestation by gavage at 200 mg/kg-day. Treatment stopped at parturition. Pups were randomized within each group, and litters normalized to eight pups per dam. Dams were divided into four groups with three animals/group, consisting of dams with cross-fostered pups as follows: 1) vehicle dam, vehicle pups, 2) styrene dams, styrene pups, 3) vehicle dams, styrene pups, and 4) styrene dams, vehicle pups.

In another experiment, four pregnant rats were given 200 mg/kg styrene orally throughout gestation and lactation. After parturition, pups within each group (treated and control) were randomized, and adjusted to eight pups per dam. Another group of four dams was given styrene only during the lactation period, and a final group received styrene during pregnancy and lactation, but the dams were given pups to suckle which had not been exposed to styrene during gestation.

At two to three weeks of age, six pups of either sex from each group were terminated for dopamine receptor binding assays on their brain tissues. Behavioral studies were carried out on three-week old pups, using amphetamine-induced locomotor activity and apomorphine-induced stereotypy as parameters of dopamine receptor sensitivity.

Body weights and number of pups per litter were recorded at birth, and during the first three weeks of age. No significant differences were observed with styrene exposure, nor did styrene have any significant effect on protein content of the brain striatum in any of the treatment groups. Exposure to styrene during the gestation period alone did not affect ^{3}H-spiroperidol binding to dopamine receptors at either two or three weeks postnatal age. Pups exposed during gestation and lactation, or during the lactation period alone, did show significant ($p < 0.05$) increases in ^{3}H-spiroperidol binding to membrane fractions prepared from corpus striatal tissues. Animals exposed to styrene during gestation and lactation also showed significant increases over controls in amphetamine-induced locomotor activity and apomorphine-induced stereotypy at three weeks of age ($p < 0.05$).

Styrene exposure during lactation, or gestation and lactation combined, caused a significant increase in ^{3}H-spiroperidol binding at dopamine receptor sites. The maturation of dopamine receptors occurs during postnatal days 7 to 28 in rats. Scatchard analysis of the binding plot led to the further conclusion that the increased binding was due to an increase in the maximum number of binding sites, rather than to increased receptor affinity. The behavioral observations were also considered indicative of super-sensitivity of dopamine receptors following styrene exposure during the critical period.

Thirty-two male and 41 female THA-strain rats were exposed to styrene by inhalation from postnatal day 1 through day 48 (Shigeta *et al.*, 1989). At birth, litters were culled to eight offspring, with three to five pups of each sex. Prior to weaning, pups were exposed with their dams. Subsequent to weaning, males and females were caged separately in groups of four. Exposure concentrations were 0, 25, or 50 ppm, and animals were maintained in the exposure chambers seven hours/day, six days/week. The animals were

weighed on postnatal day one, and then weekly until the tenth week after delivery. Pinna detachment, incisor eruption, and eye opening were recorded as developmental landmarks. Behavioral tests consisted of the Sidman avoidance test at seven weeks and open-field activity at nine weeks of age.

Body weights of male and female rats exposed to both styrene concentrations were significantly lower than controls on postnatal day one. There were no differences between the higher and lower styrene concentration groups. It is not stated whether the animals were weighed before or after the exposure session. No differences were found between the groups of dams for weight gain or other parameters, nor were inter-group differences observed for suckling or nursing behavior.

At subsequent weekly weighings for weeks one to seven (data not provided for later weeks), there was a tendency for lower weights in treated animals (particularly for females, and with the higher styrene concentration in males). There were 12 control males, and 14 males in each treated group. Female control, low-, and high-concentration groups each consisted of 17 animals. For females exposed to 50 ppm, body weights were significantly ($p < 0.01$) lower than those of controls at all time points. For females exposed to 25 ppm styrene, body weights were significantly lower than controls for all weighings except week five. For males exposed to 50 ppm styrene, body weights were significantly lower than those of control animals for all weighings except weeks two and four. Males exposed to the lower styrene concentration were significantly lighter than controls at weeks one and seven. At six weeks for females, and at five and six weeks for males, body weights of the high concentration group were significantly lower than body weights of the low concentration group.

The timing of eye opening did not differ among groups, but both pinna detachment and incisor eruption showed effects of treatment. The numbers of animals/group were 29, 31, and 31 for the control, 25 ppm, and 50 ppm groups, respectively. Pinna detachment was significantly delayed in rat pups exposed to styrene at 25 ppm. Incisor eruption was significantly delayed in both the 25 and 50 ppm groups. There was no indication of increasing delay with increasing concentration for either of these endpoints.

Open-field activity at nine weeks of age was tested in 12 male and 17 female controls, and 14 males and 17 females in each treated group. Treated animals tended to display a longer latency to enter the field than did control animals, but this difference was significant only for females exposed to 50 ppm styrene. Fewer rearings were observed for treated than for control animals, but this difference was only significant for females exposed to 50 ppm styrene. The numbers of squares crossed was also reduced in treated animals, which was significant for both males and females at 50 ppm. There were no differences among groups for the numbers of fecal pellets or urine puddles produced.

Sidman shock-avoidance testing was conducted on the rats at seven weeks of age. Data on ten sessions for the first 30 minutes of the hour-long test were analyzed for acquisition speed. Data from ten sessions for the second 30 minutes were evaluated for acquisition level. The only significant differences occurred during the latter 30 minutes of testing. For males, there was a significant decrease in avoidance rate during the ninth session in the 50 ppm group. For females, significant decreases in avoidance rates were observed for the seventh and ninth sessions.

Shigeta *et al*. (1989) considered their results to provide evidence of styrene-induced neurobehavioral impairment and growth suppression in rats by postnatal exposure.

Khanna and coworkers (1991) studied the effects of prenatal exposure to styrene combined with protein deprivation. From gestation day one, two of 12 pregnant rats were fed either a normal diet (20 percent casein) or a low protein diet (8 percent casein). Beginning on gestation day six, and continuing throughout pregnancy and lactation, half the animals in each diet group were given styrene orally at a daily dose of 100 mg/kg. Dietary controls were pair-fed to styrene-treated animals. At parturition, each litter was randomly culled to eight pups.

For dams on a low-protein diet (without styrene exposure), pup body weight gain on postnatal day 21 showed a 27 percent decrease relative to normally-fed controls. Styrene-exposed pups fed a normal diet did not show any difference in weight gain from controls. Styrene-exposed pups on a low-protein diet, however, were significantly impaired for weight gain, even relative to low-protein controls. A similar pattern of occurrence and relative magnitude of adverse effects was observed for pup brain weight, as well as for appearance of physical and behavioral developmental landmarks. Eye opening, pinna detachment, maxillary incisor eruption, fur growth (dorsal and ventral), forward locomotion, forward locomotion with head up position, surface righting, air righting, and cliff avoidance were all significantly delayed in styrene-exposed pups on a low protein diet compared to non-exposed animals on a low protein diet. Only the eruption of mandibular incisors did not differ in timing between the two groups. In most cases (with the exception of forward locomotion with the head up position), these parameters were also significantly affected compared to untreated, normal diet control; styrene treatment made these effects significantly more severe. None of the evaluated parameters were affected by styrene exposure of animals fed a normal diet.

Six pups from each group were tested for amphetamine-induced locomotor activity on postnatal day 21. There were no significant differences among groups, apart from the styrene-treated, low-protein diet animals. The latter group showed 44 percent greater locomotor activity as compared to low-protein controls. Monoamine oxidase, Na^+, K^+-ATPase, and succinic dehydrogenase activities were all significantly reduced in styrene-treated animals on a low-protein diet. No changes from control values were found with the low protein diet alone, or with styrene treatment of animals on a normal diet. The same pattern of responsiveness was observed for substrate binding to dopamine receptors from the corpus striatum and to serotonin receptors from the frontal cortex. Binding per gram protein was significantly increased in the styrene-treated, low protein diet group ($p < 0.01$ for dopamine receptors, and $p < 0.05$ for serotonin receptors). Scatchard analysis of receptor binding characteristics indicated that the change in dopamine receptor binding was due to an increased number of binding sites. The increase in serotonin (5-HT) receptor binding was considered to be due to an increased number of binding sites and to a decreased affinity.

Styrene was given to pregnant rats of the THA ("Tokai High Avoider") strain (Aikawa *et al.*, 2001). This strain was bred over 48 generations to attain an electric shock avoidance rate of 95 percent or greater in a Sidman avoidance behavior learning test. For the present study, 10 week-old males and females were caged on a one-to-one basis, and mating confirmed by plug presence. Styrene, in an olive oil vehicle, was administered in

subcutaneously-implanted silicon capsules calibrated to provide doses of 0, 0.07, or 0.7 mg/day. (All data are reported in terms of this daily dose, and are not adjusted for body weight.) In the discussion portion of the paper, the authors express these doses as corresponding to average administration of 0, 0.23, or 2.3 mg/kg-day. The overall average dam weight on gestation day 0 was approximately 200 g. Dosing began on gestation day 9, and was continued through postnatal day 28.

Throughout gestation and lactation, the body weights of the dams were recorded at weekly intervals, and water consumption was recorded every two days. Pups and dams were weighed on postnatal day three, and litters were culled to eight pups (four male and four female) per dam. The number of dams treated is not reported, but appears to be three to four litters per group, based on the reported numbers of post-culling pups per group. Pups were weighed weekly and evaluated twice daily for attainment of physical developmental landmarks. On postnatal day 28, pups were weaned, separated by sex, and given an open-field test. Spontaneous motor activity was evaluated (Animex apparatus) on postnatal day 35, and Sidman avoidance-learning tests were performed on postnatal day 49. The animals were killed and necropsied on postnatal day 70.

No significant differences were found among groups for dam or pup weights, water consumption by dams, or attainment of developmental landmarks by pups. The authors noted that during the lactation period, offspring in the 0.7 mg/day styrene group tended to remain physically apart from one another, in contrast to control and 0.07 mg/day styrene pups. In a three-minute open field test, no clear differences were observed between groups for frequencies of specified behaviors including resting, square crossing, and rearing. No significant changes were found among groups of male rats for hourly spontaneous motor activity over 24 hours, while female rats showed significant differences between animals exposed to 0.7 mg styrene and control animals or animals exposed to 0.07 mg styrene. Both male and female rats exposed to 0.7 mg styrene showed significant changes in the shock counts during the first 30 minutes of a one hour Sidman avoidance test, but not during the latter 30 minutes of the same test session.

In summary, of the five publications on the developmental neurotoxicity of prenatal exposure to styrene in rats, three appear to describe aspects of one study (Chen *et al.*, 1989; Kishi *et al.*, 1995; Kishi *et al.*, 1992). A second study has been described in the two other publications (Katakura *et al.*, 1999, 2001). These studies, along with additional studies incorporating postnatal styrene exposure (Zaidi *et al.*, 1985; Shigeta *et al.*, 1989; Khanna *et al.*, 1991; Aikawa *et al.*, 2001), contribute to the weight of evidence indicating that styrene exposure can have adverse and lasting effects on the developing brain. These studies have also provided evidence of styrene-induced developmental delays.

Chen *et al.* (1989) and Kishi *et al.* (1995) describe physical and behavioral effects of prenatal styrene treatment, while Kishi *et al.* (1992) report on neurochemical parameters. The Chen *et al.* (1989) paper appears to have analyzed the data on the basis of individual pups. The lack of litter-based statistics limits the utility of this paper for risk assessment, although it appears that the data were later published in more detail in English (Kishi *et al.*, 1995). The data were reanalyzed from the later publication using litter-based statistics, insofar as possible given the limited number of dams treated/dose (14 at 0 ppm, 3 at 50 ppm, and 7 at 300 ppm). Only 12 litters, exposed at the same time, were used for neurobehavioral testing (5 at 0 ppm, 2 at 50 ppm, and 7 at 300 ppm). No significant

adverse effects on maternal animals were reported, giving a NOAEL of 300 ppm for maternal toxicity. For offspring exposed to 300 ppm, significant changes were observed in pre- and post-weaning behavioral tests, including pivoting, bar-holding, aspects of open-field activity, and rotarod duration times. These findings support a LOAEL of 300 ppm for developmental neurotoxicity of prenatal exposure to styrene in rats. Although some effects were reported at 50 ppm, there were only two litters, too few to properly assess the data as either a LOAEL or NOAEL.

Kishi *et al.* (1992) reported neurochemical data from the same study. However, body weights for PND-1 pups are reported for the larger group of all treated pups, rather than just those carried forward into the behavioral tests. Pup weights were significantly reduced at both concentrations of styrene, although there was no obvious concentration-response. Specific changes in brain neurochemistry were found for pups exposed prenatally to 300 ppm. Therefore, data from this study indicate a LOAEL of 300 ppm for neurochemical parameters, with a NOAEL of 50 ppm, and a LOAEL of 50 ppm for reduced fetal weight, with no NOAEL.

Katakura *et al.* (1999, 2001) compared data for styrene-treated animals to pair-fed as well as to *ad lib* fed controls. Feed consumption by dams exposed to 300 ppm styrene was reduced to 86 percent of *ad lib* fed control dams; rations available to the 50 ppm and pair-fed control groups were reduced accordingly. This procedure did not result in significant differences among groups for maternal weight gain during exposure, nor were significant differences between *ad lib* and pair-fed control groups found for any parameter evaluated. Thus, while nutritional factors may have had some influence on sensitivity to styrene, maternal under-nutrition alone would not appear to explain the developmental effects observed with prenatal styrene exposure. Compared to pair-fed controls, significant developmental delays, growth deficits, and increased neonatal mortality were observed for offspring of the high-concentration group. At 50 ppm styrene, there was a significant increase in 5-HT relative to either control group. Therefore, 50 ppm is the LOAEL, with no NOAEL established.

Zaidi *et al.* (1985) employed cross-fostering to separate the potential effects of pre- versus post-natal styrene exposure. No significant effects were found with oral exposure to 200 mg/kg-day styrene restricted to the prenatal period. With post- as well as pre-natal exposure, evidence was found for altered neurochemical and behavioral parameters, suggesting an effect of styrene on dopamine receptor binding. However, study design details, such as the use of a single dose of styrene, small dose-group sizes (3-4 dams/group), and the lack of information on maternal effects, limit the usefulness of these data for quantitative risk assessment.

An additional gavage study conducted in rats (Khanna *et al.*, 1991) evaluated the effects of gestational and lactational exposure to 100 mg/kg-day styrene in the presence or absence of protein deprivation. Offspring of rats fed a low protein diet showed sensitivity to adverse effects of styrene, including deficits in parameters of growth and attainment of developmental landmarks, changes in amphetamine-induced locomotor activity, and changes in binding of dopamine and serotonin receptors. None of these effects were observed in styrene-treated rats fed a normal diet. Khanna *et al.* (1994) looked at the results of gestational and lactational protein deprivation with styrene

treatment introduced only at postnatal day 36. A similar pattern (i.e., toxicity of styrene only in protein-deprived animals) was observed.

An inhalation study conducted in rats from postnatal days 1 to 48 (Shigeta *et al*, 1989) found significant effects of styrene exposure on postnatal growth and maturation, as well as on open field activity and shock avoidance learning. No effects were observed on maternal weight gain or nursing behavior; no LOAEL can be determined for maternal toxicity in this study. Effects on offspring were observed at both concentrations tested (25 and 50 ppm), with no clear effect of increasing concentration. The LOAEL for developmental effects was 25 ppm, with no NOAEL identified. Unweaned pups were exposed in the inhalation chambers along with their dams. This treatment protocol raises the possibility that pups were not only exposed via inhalation, but also via their dam's milk, and/or through ingestion of styrene condensed on the surface of their dam's fur and skin. Hence the delivered dose may have been both higher and more variable than that calculated by inhalation exposure alone.

Use of subcutaneous implants to deliver styrene to pregnant and lactating rats resulted in significant effects on spontaneous motor activity and shock avoidance of offspring (Aikawa *et al.*, 2001). Exposed pups did not differ from controls in weight gain or age at attainment of developmental landmarks. The LOAEL for effects on the developing nervous system was a daily total dose of 0.7 mg styrene, or an average of 2.3 mg/kg-day. The corresponding NOAEL was 0.07 mg/day, or 0.23 mg/kg-day. However, the report only describes the preparation and implantation of sealed silicon tubes. There is no information pertaining to calibration or verification of styrene doses actually attained.

Cruzan *et al*. (2005b) assessed adverse functional and/or morphological effects on the rat neurological system in the F2 generation following whole-body inhalation exposures to styrene of the F0 and F1 generation. Four groups of male and female rats (25/sex/group) were exposed to 0, 50, 150, and 500 ppm styrene for 6 hours/day for ≥ 70 consecutive days prior to mating for the F0 and F1 generations. Exposure continued for the F0 and F1 females throughout mating and through gestation day (gd) 20. On lactation day (ld) one through four, the F0 and F1 females were gavaged with styrene at 66, 117, and 300 mg/kg/day. Inhalation exposure resumed on ld five and continued through weaning on postnatal day (PND) 21. The neurological development of randomly selected F2 pups was assessed by functional observational battery (FOB), locomotor activity, acoustic startle response, learning and memory evaluations, brain weights and dimension measurements, and brain morphometric and histologic evaluation. Slight body weight changes and histopathologic changes in the nasal olfactory epithelium were found in F0 and F1 rats at 500 ppm and, to a lesser extent, at 150 ppm. Exposure-related reductions in mean body weight were found in F1 and F2 offspring of the 150 and 500 ppm groups. Slightly delayed development was observed in F2 offspring from the 500 ppm group, expressed as reduced body weight (through day 70) and slightly delayed acquisition of some physical landmarks of development. Swimming ability, determined by straight channel escape times measured on PND 24, was increased, and grip strength was decreased in both sexes on PND 45 and 60 at 500 ppm compared to controls. The authors stated that the exposure-related developmental and neuromotor changes in F2 pups from dams exposed to 500 ppm occurred in endpoints known to be both age- and

weight-sensitive. The authors considered 50 ppm styrene to be the NOAEL for growth of F2 offspring and 500 ppm to be the NOAEL for F2 developmental neurotoxicity.

Supplementary Information

Daston *et al.* (1991a) and Taubeneck *et al.* (1994) studied the relationships among chemical exposures, maternal and fetal zinc status, and resulting fetal effects. A single gavage dose of 300 mg/kg styrene to pregnant rats on gestation day 11 or 11.5 led to significant maternal weight loss and decreased feed consumption on the day following treatment. Maternal hepatic zinc concentration was also increased with styrene exposure, but embryonic zinc levels were not affected. Treatment had no effects on standard parameters of developmental toxicity.

Styrene was tested for potential to cause developmental toxicity in an *in vitro* assay system using cultured chick embryo neural retinal cells (Daston *et al.*, 1991b). Styrene concentrations at or above 5 mM were found to have no significant effects on cell aggregation, growth, or differentiation in this system. A concentration of 5 mM in culture media was considered to be "probably in excess of the *in vivo* systemic concentration at the LD_{50} (rat)."

Styrene 7,8-oxide at 300 ppm 7 h/day, 5 days/week, for three weeks was rapidly lethal to female rats; significant mortality (16 percent) also occurred at 100 ppm styrene oxide (Sikov *et al*., 1986). When surviving females were mated and continued on styrene oxide exposure (7 h/day, 7 days/week) through gestation day 18, significant effects were observed on adult mortality, weight gain, and liver and lung weights. Pregnancy rate was reduced. Fetal weight and length were significantly reduced with gestational exposure to 100 ppm styrene oxide, and the frequencies of sternebral and occipital ossification defects were significantly increased.

At 50 ppm styrene 7,8-oxide (7 h/day, 7 days/week, through gestation day 24) pregnant rabbits showed increased mortality, decreased body weight and feed consumption, and increased absolute and relative lung weights (Sikov *et al*., 1986). At 15 ppm, only feed consumption showed a significant effect. The percentages of litters having resorptions was significantly increased in both concentration groups ($p \leq 0.05$), with an apparent concentration-response. No effects were found on fetal weight, fetal length, placental weight, sex ratio, or soft tissue or skeletal anomalies or variations.

Developmental Toxicity Summary

Developmental toxicity was examined in epidemiological studies on spontaneous abortion, congenital malformation, and reduced birth weight. Of five studies on spontaneous abortion risk, one found an excess risk due to styrene exposure, but it was not confirmed in an updated evaluation (Harkonen and Holmberg, 1982; Hemminki *et al.*, 1980; Hemminki *et al.*, 1984; Taskinen *et al.*, 1989; Lindbohm *et al.*, 1990). The evidence for styrene related congenital malformation risk is limited due to small numbers in the studies (Holmberg, 1979; Holmberg and Nurminen, 1980; Holmberg *et al.*, 1986). In the single study on styrene-related decreased birth weight, Lemasters *et al.* (1989) reported a small statistically non-significant decrease (four percent) compared to unexposed controls.

Animal developmental toxicity studies have shown that styrene exposure of pregnant dams increases embryo mortality and fetal resorptions, decreases fetal weight, and decreases testicular function in male fetuses. Other studies have shown adverse effects of styrene on nervous system development in animals.

Reproductive Toxicity

Male Reproductive Effects - Human Studies

Styrene oxide, a primary metabolite of styrene, can form adducts with DNA. Reproductive toxicity effects have been studied in men in categories of semen quality (morphology, volume, motility) and concentration, and time to pregnancy and miscarriages when the father was exposed to styrene in the workplace.

Semen Quality and Quantity

Jelnes reported semen quality findings in a cross-sectional study of 25 reinforced plastics workers building windmills in Denmark (Jelnes, 1988). Three weeks after the facility closed, the author collected semen and blood samples and measured sperm morphology (percent normal shape), concentration, percent live, percent immotile, semen volume, and serum follicle stimulating and luteinizing hormones. Styrene and acetone in air had been measured by industrial hygiene surveys 10, 15, and 28 weeks prior to biological sample collection. The three surveys found median breathing zone concentrations of styrene of 68, 84, and 128 ppm, respectively. The reference group was 46 age-matched fertility clinic patients for whom it was not known yet whether the man or the woman was the source of the fertility problem. Only sperm morphology (percent normal shape) indicated a potentially adverse effect of styrene. The median percent of sperm with normal morphology in the exposed group was 47 percent, while in the unexposed group it was 60 percent ($p = 0.02$). A limitation of the study was a high potential for chance findings due to multiple comparisons (seven variables). The use of fertility clinic controls, some of whom probably had abnormal semen, may have introduced bias, which would make an adverse effect of styrene more difficult to find.

Semen quality was also studied by Kolstad *et al.* (1999b). With a participation rate of just 30 percent among newly employed men at four Danish reinforced-plastics companies, semen samples were collected from 34 workers at the time of initial employment and from 21 nonexposed farmers. A second semen sample was requested from all subjects after 6 months but, unfortunately, 11 of the 34 styrene-exposed workers dropped out of the study, in part because “at least some of the dropouts participated only to obtain a free infertility checkup….this clearly illustrates the potential for selection bias in cross-sectional studies.” The final participation rate was 18 percent, and potential selection bias is a major concern. Another concern is substantial uncontrolled differences in the exposed and unexposed subjects in their age (mean = 27.5 years among styrene workers vs. 39.0 among farmers) and smoking habits (61 percent of styrene workers were smokers vs. 23 percent of farmers). The investigators related intra-individual changes in conventional semen parameters and sperm-DNA denaturation patterns to the internal dose of styrene exposure as measured by postshift urinary mandelic acid. A statistically significant decline in sperm density was seen during styrene exposure, from 63.5 to 46.0

million sperm/mL, whereas no decline was seen in the nonexposed subjects. The total sperm count was almost halved from an initial value of 175 million sperm/ejaculate. No dose-response relationship was apparent when the sperm parameters were related to internal levels of exposure. While a declining sperm count following styrene exposure is suggested, the internal and external comparisons are inconsistent, which may be due to the high intra-individual variability of semen parameters and the limited study size. The authors concluded that "due to the small numbers these findings are only preliminary."

Sallmen *et al.* (1998) examined *time to pregnancy* among couples in which the husband was occupationally exposed to styrene at the beginning of the attempt to become pregnant. The reference group had couples in which the husband was not exposed to styrene. All of the men had participated in a Finnish government biological monitoring program for organic solvents. The men studied by Sallmen *et al.* were the same men used as cases and controls in the Taskinen *et al.* (1989) study of spontaneous abortion after paternal exposure. The fecundability ratio for exposed compared to unexposed was 1.1 for low to intermediate exposure and 1.0 for high exposure to styrene. One limitation of the study was potential bias since the percentage of pregnancies ending in spontaneous abortions was unusually high (23 percent compared to about 10 percent in the general population). The large proportion of spontaneous abortion was an artifact caused by the nature of the sampling scheme for the Taskinen *et al.* case-control study. The subjects were all pregnancies ending in spontaneous abortion (among the men biologically monitored) and a sample of live births that was three times larger than the case group. The potential for bias comes from the fact that time to pregnancy is known to be longer for pregnancies ending in spontaneous abortion. The investigators could have controlled the potential bias by including a variable for spontaneous abortion. To address the issue they did an analysis limited to just live births that showed little difference from the results using all pregnancies, but the analysis was for organic solvents as a whole, not styrene. Another limitation was possible exposure of the controls to other organic solvents.

Kolstad *et al.* (1999a) analyzed time to pregnancy for the youngest child of men employed in the reinforced plastics industry in Denmark, Holland, and Italy The cohort of 541 pregnancies was subdivided into those that occurred during employment in the reinforced plastics industry and those that occurred before such employment (the non-exposed reference group). The pregnancies were assigned to styrene exposure categories of none, low, medium, and high. The epidemiologic measure of association was the fecundity ratio, where fecundity is the probability of pregnancy and the ratio is the probability of pregnancy in the exposed divided by the probability in the non-exposed. The ratios were: low exposure 0.75 (95 percent CI = 0.51 – 1.09); medium exposure 0.82 (CI = 0.57 – 1.18); and high exposure 0.90 (CI = 0.57 – 1.40).

Strengths of the study included good sample size and control of potential confounders. A potential bias is that the study excluded men who attempted but never achieved pregnancy, and may have excluded those for whom it took >13 months (the paper says that the >13 month pregnancies were "censured," which could mean either excluded or censored in the survival analysis).

Kolstad *et al.* (2000) again analyzed male fecundity. Time to pregnancy was used to calculate odds of pregnancy between 220 styrene-exposed men and 382 unexposed referents who had fathered a child. While a slightly increased time to pregnancy was

observed in the styrene workers overall (fecundity ratio 0.79, not statistically significant), the time to pregnancy was not increased in workers with the highest exposures (ratio 1.09). The investigators concluded that it is unlikely that styrene exposure has a strong effect on male fecundity.

Paternal Exposure to Styrene (Miscarriages)

A Finnish study examined paternal styrene exposure and spontaneous abortion (Taskinen *et al.*, 1989). The authors conducted a case-control study that was nested within a cohort of pregnancies among the wives of men occupationally exposed to organic solvents from 1973 to 1983. Cases were spontaneous abortions (120) identified by linkage to the national Hospital Discharge Register and hospital polyclinic data, and controls were live births (251) matched on maternal age at the time of conception. If the wife had two or more spontaneous abortions, one was selected at random. A government program had monitored the men's exposures via biological samples; for styrene, mandelic acid was measured in urine. Additional exposure and risk factor information was obtained via questionnaire mailed to the subjects. In making exposure estimates for the men, the investigators considered (blind to case status) data on occupation, job description, solvent handling, and biological levels in the 80 days prior to conception, the period of spermatogenesis. No excess risk was found. In comparison to men not exposed to styrene during the 80 days, the ORs were as follows: low/rare OR = 1.0 (six cases exposed), intermediate OR = 0.9 (12 cases exposed), and high/frequent OR = 0.7 (17 cases exposed). Strengths of the study included relatively good exposure data, blind exposure assessment, control of some potentially confounding variables (e.g., paternal and maternal exposure to other solvents and history of pregnancy loss), and reasonable sample size. Limitations included exposure of the controls to other solvents, and lack of control for smoking and alcohol.

Lindbohm *et al.* (1991) published a paper on the effects of paternal occupational exposure (of all kinds) on spontaneous abortions. A nationwide database on pregnancy outcomes was used, along with census data on occupation and socioeconomic status, to evaluate the potential contribution of paternal occupation to risk for spontaneous abortion. A medical diagnosis of spontaneous abortion had been made in 99 of 186 pregnancies included. In 10 percent of pregnancies, the husband was considered to have been occupationally exposed to one or more mutagens; the rate of spontaneous abortion was unaffected (OR = 1.0). Of the 25 specific mutagenic chemicals considered, paternal exposures to four were associated with an increased risk for spontaneous abortion: ethylene oxide, rubber chemicals, solvents used in refineries, and solvents used in the manufacture of rubber products. For workers considered to have moderate or high exposures to solvents used in the plastics industry, and to styrene itself, the odds ratios were 1.0 and 0.9, respectively (24 spontaneous abortions out of 285 pregnancies, and 9 out of 120, respectively). A limitation of the study was misclassification of exposure due to use of census occupation and industry statements to infer exposure; random misclassification would have biased the ratio toward 1.0.

Male Reproductive Effects – Animal Studies

Studies pertinent to evaluating the potential reproductive toxicity of styrene include a multi-generation reproductive toxicity conducted in rats (Beliles *et al.*, 1985), studies of testicular toxicity in rats and mice (Srivastava *et al.*, 1989, 1992a, 1992b; Takao *et al.*, 2000), and a study of ovarian toxicity in rats (Bakhtizina *et al.*, 1983). In addition, the study design and results of a multi-generation reproductive toxicity study conducted by the drinking water route in rats (Beliles *et al.*, 1985) are described and discussed in the previous section on developmental toxicity.

Adult male rats (225 $\pm$ 10 g) were randomly divided into three groups of six each (Srivastava *et al.* 1989). Group I was given 200 mg/kg styrene by gavage in 0.2 mL groundnut oil. Group II was given the same treatment, but with a dose of 400 mg /kg styrene. Group III served as vehicle-only controls. Treatment was given six days/week for 60 days. Animals were weighed at weekly intervals, and the reproductive organs were evaluated 24 hours following the last dose of styrene. No animals died during the exposure period. The authors state that there were no statistically significant changes in body weight, testes weights, or epididymal weights; these data were not presented.

At the 400 mg/kg dose, statistically significant ($p < 0.05$) changes were found in the testicular activities of β-glucuronidase (β-Glu), acid phosphatase (AP), γ-glutamyl transpeptidase (γ-GGT), sorbitol dehydrogenase (SDH), glucose-6-phosphate dehydrogenase (G6PDH), and lactate dehydrogenase (LDH). No differences from controls were seen in the levels of these enzymes at the lower dose. A significant ($p < 0.05$) decrease in total epididymal sperm count was observed at 400 mg/kg only. Animals in this dose group also displayed abnormalities of testicular histopathology.

Under the conditions of this study, the LOAEL for reproductive toxicity in male rats was 400 mg styrene/kg-day (based on reduced epididymal sperm counts and altered histopathology); the corresponding NOAEL was 200 mg/kg-day.

A study of the effects of lactational exposure to styrene on the developing rat testis (Srivastava *et al.*, 1992a) is discussed in the previous section on developmental toxicity. Srivastava *et al.* (1992b) studied the effect of styrene on testicular enzymes of growing rats. In this study, three litters of seven pups each per dose group were gavaged with styrene at 0, 100, or 200 mg/kg-day in 0.2 mL groundnut oil. Treatment commenced at one day of age, and was continued six days/week for 60 days. Twenty-four hours following the last styrene dose, six randomly-selected animals/group were necropsied.

According to the text of the paper, styrene-exposed pups did not show overt clinical symptoms of toxicity, nor were there treatment effects on weekly body weight. These data were not presented in tabular form. Absolute and relative testes weights were significantly reduced in the 200 mg/kg group, relative to control animals ($p < 0.05$, in both cases). Total epididymal sperm counts were also significantly reduced in the 200 mg/kg group ($p < 0.05$).

As in a previous paper by the same group (Srivastava *et al.*, 1992a), statistically significant ($p < 0.05$) changes were found in the activities of testicular β-glucuronidase (β-Glu), acid phosphatase (AP), and γ-glutamyl transpeptidase (γ-GGT) for 61 day-old animals after exposure to 200 mg styrene/kg. Significant changes were also seen in this

group for concentrations of sorbitol dehydrogenase (SDH), lactate dehydrogenase (LDH), and glucose-6-phosphate dehydrogenase (G6PDH). All changes were statistically significant at $p < 0.05$. The authors suggest that these alterations in enzyme activities might be involved in the ultimate pathogenic events responsible for the decreased numbers of sperm. Based on the significant decrements in testes weight and in epididymal sperm counts, the LOAEL for male reproductive toxicity in this study was 200 mg/kg-day, with a NOAEL of 100 mg/kg-day.

Five-week-old male C57BL/6 mice were exposed to 0, 5, or 50 μg/mL styrene in drinking water for four weeks (Takao *et al.*, 2000). Two control groups were given either plain water or 0.005 percent ethanol (vehicle controls). Seven animals were assigned to each treatment group. There were no differences for any measured parameter between water-only animals and vehicle controls. Average water intake, body weight, relative or absolute testis weight, and absolute spleen weight did not differ significantly among groups. Relative spleen weights were significantly ($p < 0.05$) increased over vehicle control values in both styrene-exposed groups, without a clear concentration-relationship. Histological examination of hematoxylin-eosin stained testis and spleen did not reveal differences among the four groups of animals.

At 5 μg/mL, styrene exposure did not affect plasma free testosterone levels. At the higher styrene concentration of 50 μg/mL, plasma free testosterone levels were significantly reduced as compared to vehicle controls ($p < 0.05$). Plasma corticosterone levels tended to be reduced in both styrene-exposed groups, but these differences did not reach statistical significance. Plasma luteinizing hormone levels did not show significant differences from control values with styrene exposure.

Male Sprague-Dawley rat pups were given 2 mM styrene in 25 μL sesame oil by subcutaneous (s.c.) injection on the day of birth (Kuwada *et al.*, 2002). Pups were necropsied for evaluation on postnatal day 21 (juvenile), 35 (pre-puberty), or 50 (puberty) ($n = 10$, 20, or 30, respectively). Testes weights of styrene-exposed animals were significantly decreased relative to vehicle controls ($p < 0.01$) on postnatal day 21, but not on days 35 or 50. At postnatal day 21, histological evaluation of pups exposed neonatally to styrene showed differences from control animals. Leydig cells in the testes of styrene-exposed pups were considered to be morphologically normal, but were "organized as a sheath around the seminiferous tubules instead of as the single isolated clusters of cells seen in the control testis." Steroidogenesis appeared to be reduced in testis of 21-day old exposed animals (no statistical analysis reported for these data), but steroidogenesis and spermatogenesis appeared normal at the age of 50 days.

In summary, in a series of studies on the male reproductive system, Srivastava and coworkers (1989, 1992a,b) gave styrene by the oral route at doses of 0, 200, or 400 mg/kg-day. Adults and young rats were treated by gavage; nursing pups were exposed via their dams' milk. All three of these studies found significant reductions in total epididymal sperm counts at 400 mg/kg, and no effects at 200 mg/kg-day. Abnormal testicular histopathology was also observed for the adult rats exposed to 400 mg/kg styrene, and decreased testicular weights in the young rats exposed directly by the oral route. Thus, based on these data, the LOAEL and NOAEL for reproductive toxicity in male rats were 400 and 200 mg/kg-day styrene, respectively.

Cruzan *et al*. (2005a) evaluated the effects of inhaled styrene on reproductive capability of rats. Four groups of male and female Crl:CD(SD)IGS BR rats (25/sex/group) were exposed to 0, 50, 150, or 500 ppm styrene for 6 hours/day for ≥ 70 consecutive days prior to mating for the F0 and F1 parental animals. Inhalation exposure of F0 and F1 females continued throughout mating and gestation through gestation day (gd) 20. Inhalation exposure of the F0 and F1 females was suspended from gd 21 through lactation day (ld) four. On ld one through four, the F0 and F1 females received styrene in olive oil via gavage at 66, 117, and 300 mg/kg-day. The oral doses were calculated to provide maternal blood peak concentrations similar to that of the inhalation exposure. Inhalation exposure of the F0 and F1 females was re-initiated on ld five.

Assessments included gonadal function, estrous cyclicity, mating behavior, conception rate, gestation, parturition, lactation, and weaning in the F0 and F1 generations, and F1 generation offspring growth and development. Rats in the 150 and 500 ppm groups in both parental generations gained weight more slowly than the controls. No indication of adverse effects on reproductive performance was seen in the F0 or F1 rats. Male and female mating and fertility indices, pre-coital intervals, spermatogenic endpoints, reproductive organ weights, lengths of estrous cycle and gestation, live litter size, and postnatal survival were similar at all exposures. Ovarian follicle counts and corpora lutea counts for the F1 females at 500 ppm were similar to the controls. Degeneration of the olfactory epithelium lining the dorsal septum and the dorsal and medial aspects of the nasal turbinates occurred in the F0 and F1 500 ppm animals. At 500 ppm, F2 birth weights were reduced compared to the controls. F2 offspring from the 150 and 500 ppm groups gained weight more slowly than the controls. The authors concluded that 50 ppm was a NOAEL for F0 and F1 parental systemic toxicity; the NOAEL for F0 and F1 reproductive toxicity was 500 ppm, the highest level tested.

Male Reproductive Effects – Other Relevant Data

In vitro and *in vivo* mutagenicity studies (Bjorge *et al.*, 1996; Solveig-Walles and Orsen, 1983) have indicated mutagenic effects of styrene on testicular cells. No studies were identified which studied styrene in a dominant lethal protocol. A multigeneration reproductive toxicity study conducted in rats (Beliles *et al.*, 1985) did not demonstrate styrene-associated effects on fertility or testicular pathology. Nor were effects on testicular weight or pathology identified in nine-week-old rats following four weeks of styrene exposure in drinking water (Takao *et al.*, 2000). However, studies of the effects of styrene on adult and developing rats found effects on epididymal sperm counts, on the levels of certain testicular enzymes, and, in some cases, on testicular weight or histopathology (Srivastava *et al.*, 1989, 1992a, 1992b). Studies in human populations have indicated potential effects of styrene on sperm parameters (Jelnes, 1988; Kolstad *et al.*, 1999), but have not demonstrated an association between paternal occupational styrene exposure and spontaneous abortion.

In the lactation-exposure study (Srivastava *et al.*, 1992a), since the doses were given to the dams, and styrene passed to the pups through the dams' milk, the doses delivered to pups would most likely have been somewhat lower. However, in the absence of data on styrene secretion in milk, the dose to pups in this study is unknown.

Female Reproductive Effects - Human Studies

Harkonen and Holmberg (1982) compared the numbers of pregnancies and births among 67 female lamination workers (exposed to styrene) in Finland to the histories of 67 age-matched solvent-unexposed women who worked in textile and food production. The pregnancies and births were identified in interviews that also gathered information on styrene exposure and potential confounding variables. The number of pregnancies in the styrene-exposed women was lower than in the reference group (not statistically significant), but there was no consideration of whether the women in either group were attempting to become pregnant. The number of births was also lower, which was explained in part by a higher proportion of induced abortions among the exposed (statistical significance was not calculated). Other limitations of the study included small numbers and no description of the source of the "textile and food" reference group. Because the study did not consider whether the women were attempting to become pregnant, it does not provide useful evidence for effects of styrene on fertility.

Menstrual Function

Harkonen and Holmberg (1982) questioned subjects about menstrual function in their study of spontaneous abortion among female styrene-exposed workers (discussed above). Duration of menstrual cycle, regularity of cycle, and changes in menstruation during the exposure period were compared between 67 female lamination workers exposed to styrene and 67 age-matched solvent-unexposed women who worked in textile and food production. No significant differences in menstrual function were found. Limitations of the study included lack of details on the reference group of textile and food workers.

Menstrual function was studied in 623 women employed at 40 reinforced plastics facilities in the United States (Lemasters *et al.*, 1985a). Styrene exposed women (n = 174) were compared to unexposed women (n = 449) (office and other unexposed jobs) at the same facilities. No differences between exposed and unexposed women were found for five parameters of menstrual function: dysmenorrhea, intermenstrual bleeding, secondary amenorrhea, menstrual blood clots, and hypermenorrhea. Strengths of the study included a large sample size and exclusion of subjects who had conditions that might affect menses, including use of birth control pills and certain medical procedures and treatments. A limitation of the study was that only 70 percent of the women eligible women could be interviewed, creating some potential for selection bias.

In contrast to the negative findings in the studies discussed above, Cho *et al.* (2001) reported significantly increased menstrual cycle length among 276 styrene-exposed workers (14.5 percent greater than 35 days) compared to unexposed controls (8.5 percent). However, all but three of the 276 styrene exposed workers were also exposed to other aromatic solvents, including benzene, toluene, and xylene. Thus the study was not able to conclude that styrene per se increased cycle length.

Reproductive Hormone Function in Women

Mutti *et al.* (1984a) showed that the mean serum level of prolactin was twice as high in 30 women occupationally exposed to styrene (boat and silo manufacturing in Italy) compared to a non-exposed, age-matched reference group. The mean levels were 633 and 313 pmol/L, respectively ($p<0.001$). The purpose of the study was to evaluate

possible interference of styrene or its metabolites on five neuroendocrine parameters in serum: prolactin (PRL), human growth hormone (HGH), thyroid stimulating hormone (TSH), and the gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Only serum PRL appeared to be affected. However, a second study by the same research team on (apparently) a subset of the same population (16 volunteers out of 30 exposed women) on the effect of exogenous TSH challenge on PRL levels gave pre-challenge prolactin levels that conflicted with the first study. Pre-challenge serum PRL levels in the styrene-exposed were only slightly higher than in the non-exposed reference group, and the PRL levels in both groups prior to TSH challenge were approximately three times higher than in exposed workers in the first study (Arfini *et al.*, 1987). The inconsistencies in prolactin findings between the two papers were not acknowledged in the later paper. Luderer *et al*. (2004) studied 302 workers (259 men, 43 women) exposed to styrene in 17 workplaces. Acute blood styrene concentration was the strongest predictor of serum PRL level. Prolactin tended to increase with increasing styrene exposure in both men and women, but women generally had higher hormone levels. The authors concluded that styrene exposure enhances serum prolactin levels and that styrene likely has an acute effect on PRL secretion.

Female Reproductive Effects – Animal Studies

Limited information is available pertaining to the toxicity of styrene on the female reproductive system. Sixty young, sexually mature female white rats were subjected to daily inhalation of styrene at the "Lim_{ch} level" (Bakhtizina *et al.*, 1983). (The Lim_{ch} is a threshold level for chronic adverse effects used in the former Soviet Union. The maximum allowable workplace concentration (MAC) was set below the Lim_{ch}.) The styrene concentration and other exposure details, such as hours/day and days/week, were not stated. Sixty additional females served as controls. Animals were necropsied during the diestrus phase of the female reproductive cycle, after 15, 60, or 120 days of treatment.

The authors noted that "yellow bodies" (corpora lutea) of varying maturity could be identified in the gonads of styrene-exposed animals at all time points evaluated. This was taken to indicate that cyclicity was preserved in treated animals, although evidence of changes in the length of some phases of the cycle was mentioned. This conclusion was attributed to data obtained from vaginal smears, but data are not presented. Volumes of corpora lutea, interstitial glands, and follicles were presented as percentages of stroma, and differences from controls were observed at each of the time points. Changes in intracellular metabolic parameters were indicated by cytospectrophotometric study of enzymes in the ovary (NAD-diaphorase, glucose-6-phosphate dehydrogenase, 3β`-ol-steroid dehydrogenase, and alkaline phosphatase). However, the deficits in the design and reporting of this study severely limit its applicability to risk assessment.

The effects of inhaled styrene oxide on female rats and rabbits (Sikov *et al.*, 1986) indicated an adverse impact on reproduction. The study provides only supplemental information with respect to the reproductive effects of styrene.

A multigenerational reproductive toxicity study was included as part of a larger chronic drinking water study of styrene in rats (Beliles *et al.*, 1985). The styrene concentrations were 0, 125, and 250 ppm, yielding approximate daily doses of 7.7 or 14 mg/kg for males

and 12 or 21 mg/kg for females. No adverse effects were identified in animals exposed for two years, and in particular, no changes in the weights or histological appearance of ovaries or testes. No maternal toxicity was noted for the three generations of breeding. No significant changes in fertility were noted. There were no treatment related effects on absolute or relative pup organ weights, or on organ histology, for any of the three generations. There were significant effects on pup viability at birth for the F2 high-dose group. Viability was also affected at various postnatal time-points for high-dose pups of the F1 and F2 generations. Body weights of high-concentration group pups were decreased in the F3 generation on postnatal days 7 and 21. There is no direct evidence that the reduced pup weights and viability resulted from impaired milk production or negative effects on milk quality, but if lactational impairment was responsible, a LOAEL of 250 ppm (or 21 mg/kg-day) could be considered for the female reproductive toxicity of styrene, with a corresponding NOAEL of 125 ppm (or 12 mg/kg).

Endocrine Disruptor Effects

Because styrene administration has resulted in altered prolactin secretion and effects on the male and female reproductive systems, styrene might have additional effects as an endocrine disruptor. Thus Date *et al.* (2002) used *in vitro* and *in vivo* assays to study the possible endocrine-disrupting effects of styrene monomer (and of specific styrene dimers and trimers, which migrate from polystyrene containers into food). In the estrogen and androgen receptor binding assays, styrene monomer showed no binding activity at concentrations of 10^{-10} to 10^{-5} mol/L. The uterotrophic assay was used to evaluate *in vivo* estrogenic activity. When prepubertal and ovariectomized adult rats were dosed with 20 or 200 mg/kg styrene for 3 days by subcutaneous injection, uterine weight was not significantly increased.

For anti-androgenic activity the Hershberger *in vivo* assay was conducted in the presence of testosterone treatment. Castrated, testosterone-treated immature male rats were dosed with 20 or 200 mg/kg styrene for 7 days by oral gavage; styrene did not induce a decrease in the seminal vesicle, ventral prostate and levator ani plus bulbocavernosus muscle weights. As for other hormones, styrene at 10^{-5} mol/L showed no binding activity in the thyroid receptor assay. When ovariectomized rats were dosed with 20 mg/kg styrene for 3 days by subcutaneous injection, there was no change in rat serum prolactin. The authors concluded that styrene has no apparent estrogenic, androgenic, anti-androgenic and thyroid activity. The same conclusion was made for the styrene dimers and trimers tested. Ohno *et al*. (2001) reported that styrene monomer had no activity in the estrogen receptor binding assay, the luciferase reporter gene assay, and the human breast cancer cell MCF-7 proliferation assay.

Summary

Human reproductive toxicity was examined in studies of fertility, menstrual function, and reproductive hormone function. In the two studies that measured time-to-pregnancy, no effect by styrene was observed (Sallmen *et al.*, 1998; Kolstad *et al.*, 1999b). In a fertility-related study of semen quality, Jelnes (1988) found an association between styrene exposure and abnormal sperm morphology, but there were seven parameters of semen quality, thus the association could have been due to multiple comparisons

(chance). In another study of semen quality, Kolstad *et al.* (1999b) found decreased sperm density and total sperm count in styrene-exposed workers, but the investigators stated that "due to the small numbers these findings are only preliminary." No association between styrene exposure and menstrual function was detected in two studies (Harkonen and Holmberg, 1982; Lemasters *et al.*, 1985b), although a third study with small sample size showed increased variability in duration of the menstrual cycle and higher prevalence of irregular menses (Mutti, 1988). Mutti *et al.* (1984a) reported increased levels of serum prolactin in styrene exposed female workers compared to unexposed controls.

Other Toxicity in Animals

Acute Toxicity

Results from animal studies indicate that the acute toxicity of styrene via oral, dermal, and inhalation routes is relatively low; lethal concentrations range from 5,000 mg/kg (LD_{50}) to 8,000 mg/kg (100 percent mortality) for oral exposures, and 2,348 ppm (for 2 hours, 20 percent mortality) to 11,000 ppm (for 3 hours, 100 percent mortality) for inhalation exposures (U.S. EPA, 1991). Inhalation exposures led to eye and nasal irritation and a dose-related loss of equilibrium that progressed to unconsciousness at high exposures in both rats and guinea pigs. Skin and eye irritation resulted from external applications to these organs (U.S. EPA, 1991). Exposure-related histological damage in rats was concentrated in the lungs, with some liver and kidney changes reported. Levels as low as 1,400 ppm produced lesions in the rat lung (U.S. EPA, 1991). The acute toxicity of styrene oxide is greater than styrene, with an oral LD_{50} in rats reported as 2,550 mg/kg (U.S. EPA, 1991).

4-Vinylphenol is a minor metabolite of styrene. Carlson *et al*. (2002) administered 4-vinylphenol at 25, 50, or 100 mg/kg i.p. to mice. Acute liver toxicity was assessed by measuring serum sorbitol dehydrogenase (SDH) and by light microscopy. 4-Vinylphenol caused a dose-dependent increase in serum SDH (28-fold at the highest dose) and mild hepatocellular swelling. Bronchoalveolar lavage fluid showed a 25 percent increase in cell number and up to a 5-fold increase in lactate dehydrogenase activity, indicating acute lung toxicity. Microscopically, there was widespread, severe necrosis of the bronchioles by 12 hours, and evidence of re-epithelialization of the bronchioles by 48 hours.

Kaufmann *et al.* (2005) investigated whether styrene-7,8-oxide, ring-oxidized, and side-chain hydroxylated metabolites of styrene induced cell proliferation, apoptosis, pathological changes, and GSH depletion in the mouse lung. Phenylacetaldehyde and phenylacetic acid (i.p., 3 x 100 mg/kg-day) increased apoptosis and cell proliferation in the alveoli but not the terminal bronchioli. Only styrene-oxide (SO; 3 x 100 mg/kg-day) and 4-vinyl-phenol (3 x 35 and 3 x 20 mg/kg-day) increased cell proliferation in the large and medium bronchi and in terminal bronchioles (up to 19-fold). In alveolar cells marginal increases in alveolar cell proliferation were noted only with SO (1.6-fold). SO and 4-vinylphenol caused GSH depletion in the bronchiolar epithelium and histomorphological changes of the bronchiolar epithelium in large and medium bronchi and terminal bronchioles, i.e., flattened cells and loss of the typical bulging of Clara cells.

Subchronic Toxicity

Respiratory Effects

Several studies have shown respiratory irritation in test animals exposed by inhalation to styrene. In one high dose study (1,300 ppm, seven to eight hours/day, five days/week), rats, mice, and guinea pigs (but not rabbits and monkeys) exposed for six months exhibited nasal irritation (Spencer *et al*., 1942). Rats exposed to 30 or 800 ppm styrene, four hours/day for two months exhibited mild changes in the nasal mucosa (30 ppm) including increased mucus secretion and more severe changes in the nasal and tracheal epithelia three weeks post-exposure (Ohashi *et al.*, 1985).

Male mice exposed by inhalation to about 300 ppm styrene for five hours/day, five days per week for 1.5 months exhibited increases in the thickness of the bronchiolar walls and alveolar septa and in bronchiolar and alveolar hyperplasia (Morisset *et al.*, 1979). A small proportion of the styrene-exposed mice presented a different response, *i.e.*, thinned out bronchiolar and alveolar walls, an observation that suggested the presence of a subpopulation within this group. Mice exposed by gavage to styrene (1,350 mg/kg) prenatally on gestation day 17 and once/week for the first 16 weeks of life exhibited lung congestion (Ponomarkov and Tomatis, 1978).

Liver Toxicity

In rats exposed to 300 ppm styrene intermittently for 11 weeks, degenerative morphologic alterations in liver parenchymal cells were observed two weeks after exposure (Vainio *et al*., 1979, cited in ATSDR, 1992). In rats exposed to 400 mg/kg styrene for 100 days, small areas of focal necrosis were observed. Growth depression and increased liver weight occurred in rats orally administered 400 or 667 mg/kg-day for six months (Wolf *et al*., 1956); neither effect was seen at 133 mg/kg-day. Histopathologic changes in liver were reported in female mice exposed to styrene in air at 150 and 200 ppm and in male mice at 200 ppm for 13 weeks (Cruzan *et al.*, 1997).

Kidney Toxicity

Rats exposed to 300 ppm styrene for 11 weeks (Vainio *et al*., 1979) or 133 ppm styrene for 13 weeks (Viau *et al*., 1987) did not exhibit degenerative morphologic or functional alterations in the kidney (ATSDR, 1992). In a different study, female Sprague-Dawley rats, exposed by inhalation to 300 ppm styrene six hours/day, five days/week for 13 weeks, exhibited significant urinary excretion of albumin, marginal excretion of total proteins and retinol binding protein, increased kidney fibrosis, and cystic dilations (Mutti *et al.*, 1999). The authors observed that styrene also enhanced the kidney toxicity of the nephrotoxic drug adriamycin. Increased kidney weight was observed in female rats given styrene at 400 or 667 mg/kg-day for six months (Wolf *et al*., 1956, cited in ATSDR, 1992). Increased relative kidney weights were observed among male Wistar rats that received a urinary metabolite of styrene, phenylglyoxylic acid, in the drinking water at 0, 1,250, 3,750 or 5,000 mg/L (Ladefoged *et al.*, 1998). Rats receiving an i.v. injection (0.5 mmol/kg) of the styrene-glutathione conjugate exhibited vacuolization of the proximal tubule; reduced glomerular function was not observed (Chakrabarti and Malick, 1991).

Hematologic Effects

Female and male beagle dogs, exposed to 0, 200, 400, or 600 mg/kg-day styrene by gavage for up to 19 months (560 days), experienced changes in several hematologic endpoints, including increased hemosiderin deposits, intraerythrocytic Heinz bodies, and occasionally decreased cell counts and hemoglobin levels (Quast *et al*. 1979). From this subchronic study U.S. EPA determined a LOAEL of 400 mg/kg-day and a NOAEL of 200 mg/kg-day for styrene in dogs (U.S. EPA, 2004).

Ototoxicity

Studies in rats suggest that exposure to styrene causes hearing loss. Pryor *et al.* (1987) and Yano *et al.* (1992) reported hearing loss among weanling and adult F344 rats exposed to ≥800 ppm styrene by inhalation for three weeks. The effects were more pronounced at higher frequencies. Yano *et al.* (1992) suggested that the data were consistent with an enhanced response in the weanlings. Similarly, adult Wistar rats exposed to styrene in air for four weeks exhibited hearing loss at 600 ppm, but not at 100 or 300 ppm. Morphologic changes in the cochlea of styrene-exposed rats were reported in two studies. Yano *et al.* (1992) reported alterations in the inner and outer hair cells and Makitie *et al.* (2002) observed loss of outer hair cells in the cochlea of rats exposed to 600 ppm (but not to 100 or 300 ppm) styrene.

Chen *et al*. (2007) gave male Long-Evans rats styrene by gavage in olive oil at various doses for different time periods, five or six days/week, to study cochlear outer hair cell loss. Seven days of exposure to 800 mg/kg styrene produced detectable hair cell loss, while a dose as low as 200 mg/kg five days/week for three weeks produced just-detectable hair cell loss and a slight decrease in the cochlear hearing response threshold. Their microscopic analysis suggested that supporting cells in the cochlea known as Dieter cells might be the proximal target for styrene toxicity.

Chronic Toxicity

Respiratory Effects

Cruzan *et al.* (1998) exposed female and male rats for their lifetime to 0, 50, 200, 500, or 1,000 ppm styrene for six hours/day, five days/week. Changes in nasal tissue were observed at all doses. Among the male rats, exposure to 50 ppm styrene vapors for two years resulted in atrophy and/or degeneration of the olfactory epithelium and prominent Bowman's glands in the olfactory epithelium ($p = 7 \times 10^{-6}$ and 0.02, respectively, one-sided Fisher's Exact Test, compared to controls). Among the female rats, significant ($p = 0.04$) atrophy and/or degeneration of the olfactory epithelium was detected. The LOAEL was 50 ppm and the males appeared to be more sensitive. No non-neoplastic changes were observed in the lung.

Cruzan *et al.* (2001) exposed female and male CRL-CD-1 mice to 0, 20, 40, 80, or 160 ppm styrene for six hours/day, five days/week for their lifetime. Changes in nasal and lung tissue were observed at all doses. The authors list the lung pathologies as decreased eosinophilic staining of epithelial cells in terminal bronchioles (an indication of cytotoxicity), bronchiolar epithelial hyperplasia, bronchiolar epithelial hyperplasia extending into alveolar ducts, and bronchioloalveolar hyperplasia. Statistically

significant differences ($p \leq 0.002$, one-sided Fisher's Exact Test, compared to controls) were detected for both sexes except for male bronchioloalveolar hyperplasia ($p = 0.2$). The p-values were lower for females than for males, but the data were inadequate to determine whether the female is more sensitive than the male mouse under these conditions. Greater changes occurred in the olfactory epithelia than in the respiratory epithelia. A LOAEL of 20 ppm was identified for lung and nasal pathologies.

Liver Toxicity

Srivastava *et al.* (1982) gavaged male rats with 0, 200, or 400 mg/kg-day six days/week for 100 days. At the low dose, changes in enzyme activity were reported for liver microsomal benzo[a]pyrene hydroxylase, aminopyrine-N-demethylase, glutathione-S-transferase (CDNB), liver lysosomal β-glucuronidase, and isolated mitochondrial succinic dehydrogenase (Table 12). At 400 mg/kg-day, enzyme activity changes ($p<0.02$) were observed for serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase and for lysosomal acid phosphatase. Hepatic benzo[a]pyrene hydroxylase and aminopyrine-N-demethylase activities are associated with the centrilobular area of liver, whereas the two transaminases are associated with the periportal area of the liver. The authors reported histopathologic changes in the liver at the high styrene dose, consisting of "tiny areas of focal necrosis comprised of a few degenerated hepatocytes and inflammatory cells." A LOAEL of 200 mg/kg-day was identified based on changes in activities of five liver enzymes.

Table 12. Changes in Liver Enzymes due to Repeated Oral Styrene Administration

Parameter Measured	**Styrene daily dose (mg/kg)**		
	0	**200**	**400**
Serum enzyme			
alkaline phosphatase	318 ± 22.0	299 ± 31.8	308 ± 14.0
glutamic oxalacetic transaminase	41.7 ± 1.8	54.4 ± 1.9	67.3 ± 0.52[#]
glutamic pyruvic transaminase	86.13 ± 4.1	89.92 ± 3.7	112.00 ±11.8[#]
Enzyme in liver S9 fraction			
benzo[a]pyrene hydroxylase	100.7 ±13	193 ± 16[†]	244.0 ± 21[‡]
aminopyrine-N-demethylase	0.937 ± 0.22	1.803 ± 0.14[#]	2.242 ± 0.11[†]
glucose-6-phosphatase	48.82 ± 8.2	51.97 ± 1.37	40.125 ± 7.48
glutathione-S-transferase	1134 ± 83	830 ± 24*	791 ± 19*
Liver enzyme			
acid phosphatase (lysosomal)	31.22 ± 0.87	32.12 ± 0.93	25.55 ± 0.19[†]
β-glucuronidase	2.05 ± 0.05	1.63 ± 0.06[#]	1.23 ± 0.04[‡]
succinic dehydrogenase	37.37 ± 1.8	22.75 ± 3.8[#]	17.35 ± 4.1[†]

All values are mean ± SE for 5 animals. Activity units are per minute per mg protein.
* $p<0.05$ vs control; [#] $p<0.02$; [†] $p<0.01$; [‡] $p<0.001$.

In the Cruzan *et al.* (2001) study in mice exposed to styrene at up to 160 ppm for up to 24 months, liver toxicity was not observed. However, hepatocyte necrosis was considered responsible for the death of two females at the high dose within the first two weeks of exposure

Kidney Toxicity

Among some rats that received 500 mg/kg-day styrene by gavage on the 17th day of gestation and throughout life, hyperplasia of the kidney pelvis epithelium was reported. Two similarly-treated strains of mice did not exhibit kidney toxicity (Ponomarkov and Tomatis, 1978). The rat studies suggest that renal toxicity may occur at relatively high doses and may help to explain the marginal effect or lack of effect in humans whose recent or current exposures are at relatively low doses.

Immunotoxicity

Styrene suppresses antibody responses and enhances hypersensitivity responses in mice given 20 mg/kg orally for 5 days (Dogra *et al.*, 1989). Oral doses of 20, 30, or 50 mg/kg styrene daily for 5 days did not result in any overt toxicity in lymphoid organs or on hematologic parameters of male mice. Styrene produced a mild reduction in the adrenal and spleen weights and a slight reduction in the cellular viability of lymph nodes. In a dose-dependent manner, styrene suppressed the humoral immune response (IgM-producing plaque forming cells (PFCs) of spleen and serum anti-sheep red blood cell (SRBC) hemagglutination titer) to SRBC. The proliferative response to the B-cell mitogen lipopolysaccharide revealed a significant increase in incorporation of ^{3}H-thymidine with the middle and lowest doses of styrene. In cell-mediated immunity (CMI) studies, styrene exposure resulted in a dose-dependent enhancement in delayed type hypersensitivity (DTH) reaction to SRBC in skin and an increased blastogenic response of splenic lymphocytes to phytohaemagglutinin (PHA). Additionally, there was significant impairment in the NBT reduction, attachment, and phagocytic indices of nonadherent and adherent peritoneal exudate cells. This study yielded a 5 day LOAEL of 20 mg/kg-day for immunotoxic effects of styrene in mice.

Corsini *et al.* (1994) exposed female mice (6 or 7 per group) by inhalation to 0, 125, 250, or 500 ppm styrene 6 hours/day for 14 consecutive days. The primary effect on the immune system in the exposed animals was hypocellularity of the spleen.

Vaghef and Hellmann (1998) gave single i.p. injections of styrene (100, 250, 350, or 500 mg/kg) and styrene oxide (50, 100, 150, or 200 mg/kg) to female mice. Both substances induced significant DNA damage four hours after injection in lymphocytes (and in other cells - liver, bone marrow, and kidney) as measured by alkaline single cell gel electrophoresis (Comet Assay). Lymphocytes (along with liver cells) were the most sensitive. This is a secondary effect on the immune system due to breaking of the DNA in several types of cells, including lymphocytes.

Neurotoxicity

Acute Neurotoxic Effects

Inhalation of 1,300 ppm (6,000 mg/m³) styrene by rats and guinea pigs resulted in immediate irritation and lachrymation (Spencer *et al.*, 1942). No deaths occurred from exposure to 10,000 ppm styrene for 1 hour, while 3 hours of exposure resulted in 100 percent mortality in both species. At 5,000 ppm, 100 percent survival was observed following exposure of rats and guinea pigs for 2 and 3 hours, respectively, but 100 percent mortality was observed in both species following 8 hour exposure. Immediate deaths were due to central nervous system (CNS) depression. Delayed deaths occurred due to pulmonary edema and hemorrhage, which frequently developed as a result of styrene's acute lung irritant action.

Morgan *et al.* (1993) exposed $B6C3F_1$ mice (36 mice/sex/dose) to 125, 250, or 500 ppm of styrene by inhalation 6 hours/day for 3 days. Seven of 72 mice died or were terminated moribund following one 6-hour exposure to 500 ppm. While the liver was identified as the major target organ, the authors indicated that styrene's CNS depressant action also likely contributed to the overall toxicity.

Chakrabarti (1999) tested the hypothesis that a central component in styrene-induced neurotoxicity is malfunction of the vesicular transport of dopamine. In rats both styrene and styrene oxide inhibited the *in vitro* striatal binding of (tritiated) tyramine, a putative marker for dopamine vesicular transport. Both caused dose-related inhibition of dopamine uptake into purified striatal synaptic vesicles. Neither chemical significantly increased basal efflux of dopamine preloaded into striatal vesicles *in vitro*, nor inhibited significantly the uptake of norepinephrine or serotonin into striatal synaptic vesicles.

Gagnaire *et al*. (2006) exposed freely moving adult male rats to 750 or 1,000 ppm styrene, 6 hours/day, 5 days/week for 4 weeks and assayed extracellular levels of dopamine (DA), serotonin (5-HT), and their acid metabolites, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindolacetic acid (5-HIAA), in dialysates of striatal tissue. They also measured DA, 5-HT, and their acid metabolites in striatum, nucleus accumbens, and prefrontal cortex obtained postmortem from similarly exposed rats. Exposure to 1,000 ppm styrene (but not 750 ppm) caused a significant decrease in extracellular acid metabolite levels. Tissue levels were decreased to a lesser extent. The effects were observed 72 hours after discontinuing exposure and disappeared by 17 days later. There was no change in DA or 5-HT levels in the dialysates or tissues.

Chronic Neurotoxic Effects

Rosengren and Haglid (1989) investigated whether three months inhalation exposure to styrene at 90 or 320 ppm could induce long lasting astroglial alterations in Sprague Dawley rats, traceable four months after exposure ceased. Styrene exposure at 320 ppm raised concentrations of glial fibrillary acidic protein (GFA) in the sensory motor cortex and in the hippocampus. GFA is the structural protein of the astroglial filaments. These filaments form after damage to the central nervous system from any cause. The authors concluded that moderate exposures to styrene induce regional, long lasting astroglial reactions that are an indicator of brain damage.

Coccini *et al.* (1999) exposed male Sprague-Dawley rats six hours/day, five days/week to airborne styrene at 300 ppm for four weeks or 50 ppm for 13 weeks. Both exposures led to significant decreases of MAO (monoamine oxidase) B activity in several brain areas (cortex, striatum, hippocampus, brainstem, and cerebellum); MAO A was not affected. Decreases in MAO B activity were also found in the brainstem of rats given 400 mg/kg styrene or 100 mg/kg styrene oxide i.p. for two weeks.

Chakrabarti (2000) examined the central dopaminergic system in relation to neurobehavioral effects of subchronic styrene. Groups of adult male Sprague-Dawley rats were gavaged with 0, 0.25, or 0.5 g styrene/kg-day in corn oil for 13 consecutive weeks. Twenty-four hours after treatment with 0.5 g/kg ended, dopamine (DA) and its metabolites were significantly reduced in the brain corpus striatum, hypothalamus, and lateral olfactory tract regions. *In vitro* styrene caused a significant increase in dopamine release from rat striatal synaptosomes, similar to that of tyramine. Significant loss of motor function was observed on days 56, 70, and 84 of styrene treatment at 0.5 g/kg, and lasted over a month after the treatment. Treated rats recovered motor function within 45-60 days after treatment ended and had normal levels of dopamine and its metabolites. Specific spiroperidol binding was unaltered 7 or 15 days after subchronic treatment with styrene. These data imply that despite the dopaminergic neuron loss due to styrene, dopaminergic transmission was not reduced enough to cause dopamine receptor supersensitivity in the striatum. The subchronic neurotoxicity of styrene may be presynaptic and may involve impaired regulation of dopamine content and stimulation of dopamine release. In this study 0.5 g/kg styrene was a subchronic LOAEL and 0.25 g/kg was a NOAEL for neurotoxicity.

In a chronic toxicity/oncogenicity study Cruzan *et al.* (2001) exposed groups of 70 male and 70 female CD-1 mice to 0, 20, 40, 80, or 160 ppm airborne styrene for six hours/day, five days/week for two years. At 40 ppm and above, mice exhibited loss of olfactory nerve fibers. A NOAEL of 20 ppm was identified for this effect. Converting doses for continuous exposure, a NOAEL of 20 ppm equals 2.8 mg/kg-day (0.65 x 20 ppm x 6 hours/24 hours x 5 days/7days = 3.57 ppm = 15.2 mg/m^3 / 3.5 L/kg)**.**

At very high levels, styrene affects the auditory organs in animals. Makitie *et al.* (2002) exposed male Wistar rats to 600, 300, or 100 ppm styrene twelve hours/day, five days/week, for four weeks. They tested auditory sensitivity before and after exposure by auditory brain stem audiometry at 1.0, 2.0, 4.0, and 8.0 kHertz. Exposure to 600 ppm styrene caused a 3 decibel hearing loss only at 8 kHz. Quantitative morphological analysis of cochlear hair cells showed severe outer hair cell loss, particularly in the third outer hair cell row of the upper basal and lower middle coil, at 600 ppm styrene. The inner hair cells were usually intact. The authors believe that there is a concentration threshold for styrene ototoxicity in rats between 300 and 600 ppm.

Carcinogenicity in Animals

Cancer bioassay data are summarized in Table 13 and discussed in detail below. At the end of this section, cumulative doses received by rats in inhalation and ingestion studies are listed in comparable units, as estimated by OEHHA (see “Estimation of total doses in rat bioassays”).

Table 13. Summary of Rodent Carcinogenicity Bioassay Results

Route	Species / Strain	Sex	Tumor	Reference
Ingestion (gavage)	Mouse, O_{20}	Female progeny	Bronchiolar-alveolar adenomas and carcinomas	Ponomarkov and Tomatis, 1978
		Male progeny	Bronchiolar-alveolar adenomas and carcinomas	
	Mouse, $C_{57}Bl$	Female	N.S.	
		Male	N.S.	
	Mouse, $B6C3F_1$	Female	Hepatocellular adenoma[1]	NCI, 1979
		Male	Bronchiolar-alveolar adenomas and carcinomas	
	Rat, BDIV	Female progeny	N.S.	Ponomarkov and Tomatis, 1978
		Male progeny	N.S.	
	Rat, F344	Female	N.S.	NCI, 1979
		Male	N.S.	
	Rat, SD	Female	N.S.	Conti *et al.*, 1988; Maltoni *et al.*, 1982
		Male	N.S.	
Ingestion (drinking water)	Rat, SD	Female	Mammary gland tumors[2]	Beliles *et al.*, 1985
		Male	N.S.	
Inhalation	Mouse, CD1	Female	Bronchiolar-alveolar adenomas and carcinomas	Cruzan *et al.*, 2001; SIRC, 1998
		Male	Bronchiolar-alveolar adenomas and carcinomas	
	Rat, SD	Female	N.S.	Cruzan *et al.*, 1998; SIRC, 1996
		Male	Testes interstitial cell[1]	
	Rat, SD	Female	Malignant and benign mammary tumors; brain meningiomas[2]	Conti *et al.*, 1988; Maltoni *et al.*, 1982
		Male	N.S.	
	Rat, SD	Female	Leukemias/lymphosarcomas; mammary tumors[2]	Jersey *et al.*, 1978
		Male	N.S.	
IP Injection	Mouse, A/J	Female	N.S.	Brunnemann *et al.*, 1992
	Rat, SD	Female	N.S.	Conti *et al.*, 1988
		Male	N.S.	
SC Injection	Rat, SD	Female	N.S.	Conti *et al.*, 1988
		Male	N.S.	

Abbreviation: N.S. = no significant increases in treatment-related tumors.
[1] Trend test only, OEHHA analysis.
[2] Pairwise comparison, OEHHA analysis.

Administration by the Oral Route

Ingestion (Gavage): O_{20} Mice

Strain O_{20} mice were gavaged with styrene in olive oil during pregnancy and their progeny were similarly exposed from weaning onward (Ponomarkov and Tomatis, 1978). Twenty-nine pregnant mice received 1,350 mg/kg styrene on gestation day 17. The weaned offspring (45 males, 39 females) received the same dose once/week. Due to toxicity, treatment was suspended after 16 weeks; observation continued for 120 weeks or until death. Controls consisted of nine females that received olive oil on gestation day 17, their progeny that received olive oil for their lifetime after weaning (20 males, 22 females), and a set of mice that served as untreated controls (54 males, 47 females).

Preweaning mortality was higher among offspring of dams treated with styrene. The average age at death was earlier among styrene-exposed progeny (males: 32 weeks *vs*. 88 weeks vehicle and 94 weeks untreated controls; females: 49 weeks *vs*. 85 weeks vehicle and 99 weeks untreated controls). The average age of death for the pregnant female mice given styrene was 74 weeks (*vs*. 85 weeks for vehicle controls). No body weight differences were reported. Multiple centrilobular necrosis of the liver, spleen hypoplasia, and lung congestion were observed in the styrene-treated mice that died within the first 20 weeks of the study. In mice that died up to the 45th study week, there was evidence of liver inflammatory reactions and bronchitis/peribronchitis. Mice that died late in the study exhibited multiple abscess-like, round-shaped cavities in the liver that were filled with polymorphonuclear leukocytes.

Lung tumor incidences among progeny of mice in this experiment are presented in Table 14. Statistically significant increased incidences of total lung tumors were observed for styrene-treated male and female mice relative to both the vehicle and untreated control groups ($p < 0.05$, by Fisher's exact test). In female mice, lung adenomas and adenocarcinomas were each significantly increased above untreated control, while only the adenocarcinoma incidence was significantly increased above vehicle controls. In male mice, only lung adenomas were significantly increased above the vehicle controls. The average age of death among styrene-treated offspring with lung tumors was 49 and 58 weeks for male and female mice, respectively, compared to 84 and 85 weeks for vehicle controls, respectively, and 87 and 91 weeks for untreated controls, respectively. The proportion of benign and malignant tumors was not different among the groups.

Table 14. Lung Tumors among Progeny of O20 Mice Treated with Styrene Once During Gestation and Weekly Post-Weaning for 16 Weeks[1]

Lung Tumors	Styrene Treatment Group and Incidence [a]		
	0 (vehicle)	0 (untreated)	1,350 mg/kg
Female Progeny			
Adenomas	10/21	11/47	14/32 [b]
Adenocarcinomas	4/21	14/47	18/32 [b,c]
Total lung tumors	14/21	25/47	32/32 [b,c]
Male Progeny			
Adenomas	4/19	22/53	12/23 [c]
Adenocarcinomas	4/19	12/53	8/23
Total lung tumors	8/19	34/53	20/23 [b,c]

[1] Data from Ponomarkov and Tomatis, 1978.
[a] Denominator reflects number of survivors to the time of the first tumor (authors did not state which site or tumor type, thus presumably any).
[b] Increase vs. untreated controls by Fisher's Exact Test pairwise comparison (1-tailed), $p < 0.05$.
[c] Increase vs. vehicle controls by Fisher's Exact Test pairwise comparison (1-tailed), $p < 0.05$.

Ingestion (Gavage): C_{57}Bl Mouse

Strain C_{57}Bl mice were exposed during pregnancy to styrene (in olive oil) by gavage, and their progeny were subsequently exposed by gavage from weaning throughout their lifetime (Ponomarkov and Tomatis, 1978). Fifteen pregnant mice received 300 mg/kg styrene in olive oil on day 17 of gestation and their progeny (27 males, 27 females) were treated weekly by gavage with the same dose from weaning throughout their lifetime. Controls consisted of five mice that received olive oil on gestation day 17, their progeny that received olive oil weekly for their lifetime after weaning (12 males, 13 females), and a set of mice that served as untreated controls (51 males, 49 females). Survivors were autopsied at 120 weeks, whereas other mice were killed when moribund.

The average age of death was similar among styrene-treated and control animals (91 to 114 weeks). Survival among the groups was also similar, although by 110 to 120 weeks the mortality was about 50 percent. No significant treatment-related increases in tumor incidence, organic toxicity or treatment-related effects on body weights were reported.

Ingestion (Gavage): B6C3F_1 Mouse

Female and male B6C3F_1 mice (50/sex/dose treated, 20/sex vehicle controls) were gavaged with styrene in corn oil at 0, 150, or 300 mg/kg five days/week for 78 weeks and were observed for an additional 13 weeks (NCI, 1979). Necropsy was performed on all animals. High dose male mice exhibited a dose-dependent decrease in survival rates compared to control mice. However, survival was adequate for development of late-forming tumors among all dose groups. Compared to controls, a slight dose-related body

weight reduction was observed in treated females. Inflammatory and proliferative lesions occurred with equal frequency among styrene-treated and control mice.

Lung tumor incidences are shown in Table 15. The incidence of combined alveolar/bronchiolar carcinoma and adenoma significantly increased in the high dose males relative to controls ($p < 0.05$) and marginally increased in the mid-dose group ($0.05 < p < 0.1$), with a significant positive trend. No significant increases in tumor incidence were observed among females, although a significant positive trend for hepatocellular adenoma was observed. NCI concluded: "The findings of an increased incidence of a combination of adenomas and carcinomas of the lung provided suggestive evidence for the carcinogenicity of styrene in male $B6C3F_1$ mice. However, it is concluded that, under the conditions of this bioassay, no convincing evidence for the carcinogenicity of the compound was obtained in … $B6C3F_1$ mice of either sex."

Table 15. Tumors among Mice Treated with Styrene by Gavage for Two Years[1]

Sex and Tumor Type [2]	Dose (mg/kg)			Trend [3]
	0	150	300	
Females				
Alveolar/bronchiolar adenomas	0/20	1/43	3/43	N.S.
Hepatocellular adenomas	0/20	1/44	5/43	p = 0.018
Males				
Alveolar/bronchiolar adenomas	0/20	3/43	4/43	N.S.
Alveolar/bronchiolar carcinomas	0/20	3/44	5/43	N.S.
Alveolar/bronchiolar combined adenomas and carcinomas	0/20	6/44 [a]	9/43 [b]	p = 0.015

[1] Data from NCI, 1979.
[2] Denominator is animals surviving to time of first tumor or 52 weeks, whichever is earlier.
[3] p-value of Cochran-Armitage trend test. N.S. = not significant ($p > 0.05$). Significance of increase in tumor incidence by pairwise comparison with controls (Fisher's exact test):
[a] $0.05 < p < 0.1$.
[b] $p < 0.05$.

Ingestion (Gavage): Sprague-Dawley Rat

As reported by Conti *et al.* (1988), Maltoni (1978), and Maltoni *et al.* (1982), female and male Sprague-Dawley rats (40/sex/dose group) received styrene in olive oil at doses of 0, 50, or 250 mg/kg four to five days/week for 52 weeks and were observed until spontaneous death; here, evaluation commenced at 140 weeks.[1]

[1] The experiment duration and other relevant details were not specified in Conti *et al.* (1988). A letter from Maltoni to The International Cooperative Study Group stated that tumor evaluation began at 140 weeks, presumably the time of the last spontaneous death (Maltoni, 1978), and other details. Maltoni *et al.* (1982) reported only on brain tumors.

Female rats in the high-dose group experienced a higher mortality than rats in other groups (data not presented). At the time the first tumor appeared (19 weeks), 36/40 high-dose females and 38/40 high-dose males were alive (Maltoni, 1978). No treatment related effects on body weight were observed.

No significant increases in tumor incidence were observed among styrene-treated rats compared to control rats. The authors attributed the decreased incidences of total benign and malignant tumors and of benign and malignant mammary tumors among the high-dose female rats to the higher mortality rate (Conti *et al.*, 1988). IARC (1994a) noted the incomplete data reporting and considered 52 weeks to be a short exposure.

Ingestion (Gavage): Fischer 344 Rat

Male and female Fischer 344 rats (50/sex/dose, treated; 20/sex control) received styrene in corn oil by gavage, five days/week at doses of 0, 500, 1,000 or 2,000 mg/kg (NCI, 1979). The study was initiated with the medium- and high-dose groups, treated for 78 weeks plus a 27-week observation period. Due to toxicity at the high dose, another experiment was initiated with the low-dose rats treated by daily gavage for 103 weeks with a one-week observation period. The vehicle controls received corn oil by gavage daily for 104 to 105 weeks.

A dose-dependent body weight decrease occurred in treated males, with no other clinical symptoms. Mortality was significantly higher at the high dose ($p < 0.001$) for each sex, compared to the vehicle control. Among high dose male and female rats, detection of late developing tumors was difficult because of inadequate numbers (males: 6 surviving of 50 treated, versus 18/20 for vehicle controls; females: 7/50 versus 18/20 for vehicle controls). No increased incidences of neoplastic lesions were observed in the styrene-treated male or female rats, compared to vehicle controls.

Ingestion (Gavage): BD IV rat

Ponomarkov and Tomatis (1978) gave 21 pregnant female BD IV rats 1,350 mg/kg styrene by gavage in olive oil on gestation day 17. Their progeny received 500 mg/kg styrene once/week for life after weaning. Controls consisted of ten pregnant rats given olive oil alone on gestation day 17 and progeny that received olive oil once/week for life after weaning. The experiment was conducted for 120 weeks. Several rats that died by 50 to 60 weeks of the study exhibited moderate lung and kidney congestion and small necrotic foci in the liver parenchyma. Rats that died later in the study did not exhibit liver damage, but lesions of the forestomach (atrophy, desquamation, necrosis with inflammation) and kidney (pelvis epithelial hyperplasia) were frequently observed. A small number of rare tumors was found in the styrene-treated rats, including three stomach tumors (an adenoma, a fibrosarcoma, and a carcinosarcoma), a hepatocellular adenoma, and three neurinomas (one each of heart, intestine, and trigeminus) compared to one fibrosarcoma, no hepatocellular adenomas, and one meningioma in control rats.

Ingestion (Drinking Water): Sprague-Dawley Rat

Beliles *et al.* (1985) exposed female and male Sprague-Dawley rats [COBS (SD) BR] to styrene at target levels of 125 or 250 ppm (50 males and 70 females/dose) in drinking water. Controls (76 male, 106 female) received drinking water without styrene. An F_1

generation was produced for a reproductive toxicity study from parental (F_0) rats on the drinking water study for about 90 days (10 males and 20 females, treated; 15 males and 30 females, control). The F_1 mice were also evaluated for tumorigenicity. Some F_1 and F_2 pups were selected to produce F_2 and F_3 generations, respectively. After weaning of their pups, F_0 parental rats were returned to the study, while F_1 and F_2 parental rats were killed after weaning of their pups. Macroscopic and histological examination was performed on 10 rats/sex/dose group at 52 weeks exposure and on surviving rats at the end of exposure (104 weeks). Two-year mortality was similar among the control and treatment groups. Mean body weight for high dose female rats was decreased by 11 percent ($p < 0.05$) compared to controls. Food consumption was similar among dose groups, although daily water consumption was decreased among the styrene-exposed male and female rats in each dose group compared to control.

Beliles *et al.* (1985) reported no evidence of styrene-related tumorigenicity. However, an OEHHA analysis of the histopathologic results in Table 6, p. 863 of Beliles *et al.* (1985) showed a significant increase in incidence of mammary tumors in high dose female rats (Table 16). No histopathological information (*e.g.*, benign compared to malignant) was provided. A nearly significant increase in lung tumors (type unspecified; $p = 0.078$ by Fisher's exact test) was observed among high dose male rats and in hematopoietic tumors (type unspecified; $p = 0.057$, by Fisher's exact test) among the low dose female rats. No evidence was presented for tumorigenicity in the F_1 or F_2 generations. IARC (1994a) noted the low styrene doses in this study.

Table 16. Tumors in Rats Exposed to Styrene in Drinking Water for Two Years[1]

Sex and Tumor Type	Styrene Concentration (ppm)			Trend [2]
	0	125	250	
Females				
Mammary gland tumors	54/96 (56%)	20/30 (67%)	45/60[a] (75%)	p = 0.010
Hematopoietic tumors	5/96 (5%)	5/30 [b] (17%)	1/60 (1.7%)	N.S.
Males				
Lung tumors	2/63 (3%)	0/22 (0%)	5/40[b] (12%)	p = 0.053

[1] Data from Beliles *et al.*, 1985; denominator represents the number of animals examined.
[2] p-value of exact test for linear trend.
Significance of increase in tumors by pairwise comparison with controls (Fisher's exact test):
[a] $p < 0.05$.
[b] $0.05 < p < 0.1$.

Studies using Inhalation Exposure

Inhalation: CD-1 Mouse

Female and male CD-1 mice (50/sex/dose) were exposed to styrene vapors at target levels of 0, 20, 40, 80, or 160 ppm for six hours/day, five days/week for 97 weeks (females) or 104 weeks (males) (Cruzan *et al.*, 2001; SIRC, 1998). The average analyzed air levels met the targets and the nominal air styrene levels (amount of styrene used

divided by the chamber airflow) were 21, 42, 83, and 161 ppm. Interim necropsies (n = 6 to 10/dose/sex) were conducted at 52 and 78 weeks; these mice were not included in tumor incidence analyses reported by Cruzan *et al.* (2001). The commercial styrene test material consisted of 98.9 to >99.5 percent styrene with small amounts of benzene (<1 to 8 ppm), ethylbenzene (149 to 291 ppm), styrene-7,8-oxide (<1 to 7 ppm), styrene dimer (<1 to 7 ppm) and tertiary butyl catechol (4 to 34 ppm).

Mortality was high among control and treated mice in all dose groups. Because survival among control females fell below 50 percent, all female groups were terminated after 97 weeks of exposure. Among control males, mortality was 28 percent prior to terminal necropsy at 104 weeks. Lang and White (1996) observed that among CD-1 mice, group mortality at 104 weeks often exceeds 50 percent and cannot always be predicted by mean body weights. The Cruzan *et al.* (2001) data show reduced body weight gain (>10 percent compared to controls) for male mice exposed to 80 or 160 ppm styrene and for female mice exposed to 160 ppm styrene, as well as reduced food consumption in these groups. Water consumption increased in the treated groups of both sexes, particularly at 160 ppm during the first year of exposure. Changes in hematology and biochemistry endpoints occurred early in the study and returned to control values by the end of the study. Urinary pH was increased among dosed females relative to controls. The authors considered this of no toxicologic significance, because the male mice did not exhibit a similar increase and all values were within normal ranges for the age and strain of mice.

Levels of styrene and styrene oxide in blood showed a dose-dependent increase at week 74. For concentrations of 20, 40, 80, and 160 ppm air styrene, respectively, blood styrene levels in males were 69, 177, 654, and 1461 nanograms per gram (ng/g) and in females were 30, 106, 527, and 1743 ng/g, while blood styrene oxide levels in males were <1, 2.5, 12, and 34 ng/g and among female mice were <1, 1.4, 6.2, and 20 ng/g. Hepatocyte necrosis was observed in two female mice within the two weeks of initial exposure to 160 ppm styrene. Liver toxicity was considered contributory to their death but was not observed in other mice after 52, 78, or 104 weeks of exposure.

At the time of the first interim necropsy (following 52 weeks exposure), non-neoplastic lesions were observed in the nasal passages and lungs of styrene-treated animals of both sexes. In animals necropsied after 78 weeks exposure, there was an increased incidence compared to controls of nasal passage lesions in animals of both sexes; non-neoplastic lesions were also observed in lungs in animals from all styrene-exposed groups. At the end of the study, treatment-related non-neoplastic lesions were observed in nasal passages and lungs of both sexes at all styrene exposure levels. These lesions of the bronchiolar epithelium included decreased eosinophilic staining in the terminal bronchioles, bronchiolar epithelial hyperplasia, and focal bronchioloalveolar hyperplasia extending into the alveolar ducts. Foci of bronchiolar-alveolar hyperplasia that were not contiguous with the changes in the terminal bronchioles were also reported.

Tumor incidence is summarized in Tables 17 and 18. At the end of exposure, styrene-exposed male mice showed a statistically significant increased incidence of bronchiolar-alveolar adenomas and combined bronchiolar-alveolar adenomas and carcinoma at 40, 80, and 160 ppm styrene. Female mice had statistically significant increased incidences of bronchiolar-alveolar adenomas and combined bronchiolar-alveolar adenomas and carcinomas at 20, 40, and 160 ppm. An increased incidence of bronchiolar-alveolar

carcinomas was observed in female mice at 160 ppm styrene. The results of the tumor incidence comparisons were supported by a time-to-tumor analysis (SIRC, 1998).

Table 17. Incidence of Bronchiolar/Alveolar Lung Tumors in Mice Exposed to Styrene by Inhalation and Necropsied at 97 Weeks (Females) or 104 Weeks (Males)[1]

Tumor Site and Type		Concentration (ppm in Air)					Trend [2]
		0	20	40	80	160	
Females							
Lung	Adenomas	6/50 (12%)	16/50 [a] (32%)	16/50 [a] (32%)	11/50 (22%)	24/50 [b] (48%)	$p < 0.001$
	Carcinomas	0/50 (0)	0/50 (0)	2/50 (4%)	0/50 (0)	7/50 [b] (14%)	$p < 0.001$
	Combined adenomas and carcinomas	6/50 (12%)	16/50 [a] (32%)	17/50 [a] (34%)	11/50 (22%)	27/50 [b] (34%)	$p < 0.001$
Males							
Lung	Adenomas	15/50 (30%)	21/50 (42%)	35/50 [b] (70%)	30/50 [b] (60%)	33/50 [b] (66%)	$p < 0.001$
	Carcinomas	4/50 (8%)	5/50 (10%)	3/50 (6%)	6/50 (12%)	7/50 (14%)	N.S.
	Combined adenomas and carcinomas	17/50 (34%)	24/50 (48%)	36/50 [b] (72%)	30/50 [b] (60%)	36/50 [b] (72%)	$p < 0.001$

[1] Data from Cruzan *et al.*, 2001; SIRC, 1998.
[2] p-Value of Cochran-Armitage trend test. N.S. = not significant ($p > 0.05$). Significant increase above controls by pair wise comparison (Fisher's exact test):
[a] $p < 0.05$.
[b] $p < 0.01$.

The incidence of bronchiolar-alveolar carcinoma in the high-dose female mice (14 percent) is outside the historical control incidences of 0 to 4 percent for female mice reported by Cruzan *et al.* (2001) (non-inhalation historical controls of the same strain and age of mice from the laboratory). Lang (1995) reported an overall historical control incidence of 6 percent bronchiolar-alveolar carcinoma among female CD-1 mice from 13 different laboratories. Among male mice, the high-dose bronchiolar-alveolar carcinoma incidence (14 percent) was within the historical control range of 4 to 26 percent reported by Cruzan *et al.* (2001). This was also within the range noted by Sher *et al.* (1982) for male CD-1 mice aged 81 to 105 weeks in a different laboratory (0 to 17 percent), but was slightly higher than the control incidence reported by Lang (1995) (12 percent).

Cruzan *et al.* (2001) reported no increased incidences of pulmonary neoplastic lesions in any dose group of either sex at 52 or 78 weeks of exposure, based on the incidence data presented in Table 18. The number of mice evaluated at the interim necropsies, however, was small (six to ten/sex/dose) and the bioassay protocol may not have been sufficiently sensitive to detect an increased incidence in lung tumors, given the low number of mice at the interim necropsies and the incidence of lung tumors in the mice examined

(50/sex/dose) at the terminal necropsy. Cruzan *et al.* (2001) (SIRC, 1998) did not combine the interim necropsy data in their evaluation of increases of tumor incidence, but suggested that the lack of tumorigenic response at the early times was indicative of a pattern of late-forming lung tumors.

Table 18. Incidence of Bronchiolar/Alveolar Tumors, Including Observations from Interim Necropsies (12 and 18 Months) in Mice Exposed to Styrene by Inhalation[1]

Lung Tumor Type and Necropsy Group [2]		Concentration (ppm styrene in air)					Trend [3]
		0	20	40	80	160	
Females							
Adenomas	Interim I	1/10	2/10	0/9	2/10	0/10	N.S.
	Interim II	1/8	4/10	5/10	1/10	3/10	N.S.
	Total	8/66	22/67	21/69	14/70	27/67	p = 0.005
Carcinomas	Interim I	0/10	0/10	0/9	0/10	0/10	N.S.
	Interim II	0/8	0/9	1/8	0/9	0/10	N.S.
	Total	0/51	0/49	3/51	0/54	7/54	p < 0.001
Combined adenomas and carcinomas	Interim I	1/10	2/10	0/9	2/10	0/10	N.S.
	Interim II	1/8	4/10	5/10	1/10	3/10	N.S.
	Total	8/66	22/67	22/69	14/70	30/67	p = 0.0009
Males							
Adenomas	Interim I	1/10	2/10	0/10	2/10	0/10	N.S.
	Interim II	3/10	5/10	5/10	7/10	4/9	N.S.
	Total	19/70	28/68	40/70	39/70	37/69	p = 0.003
Carcinomas	Interim I	0/10	0/10	0/9	0/10	0/10	N.S.
	Interim II	1/9	0/10	0/10	1/9	0/6	N.S.
	Total	5/54	5/55	3/57	7/57	7/51	N.S.
Combined adenomas and carcinomas	Interim I	1/10	2/10	0/10	2/10	0/10	N.S.
	Interim II	4/10	5/10	5/10	7/10	4/9	N.S.
	Total	22/70	31/68	41/70	39/70	40/69	p = 0.004

[1] Data from SIRC, 1998.
[2] Effective number equals number of animals alive at week 38 for female adenoma, week 79 for female carcinoma, week 38 for female adenoma/carcinoma combined, week 35 for male adenoma, week 79 for male carcinoma, and week 35 for male adenoma/carcinoma combined.
[3] p-Value of Cochran-Armitage trend test. N.S. = not significant (p < 0.05).

OEHHA analyzed the tumor incidence data for all of the animals in the study, including those necropsied before the end of the study (i.e., 70 animals/sex/dose group) presented

by SIRC (1998). In Poly-3 pair-wise comparisons of dosed and control animals (a modification of the Cochran-Armitage test which adjusts the value of the incidence denominator for early mortality assuming that cancer risk is a function of the third power of age), the incidences of combined lung adenomas and adenocarcinomas among the female mice exposed to styrene were significantly increased for three dose groups (20 ppm, $p = 0.003$; 40 ppm, $p = 0.002$; 160 ppm, $p = 0.00001$). Significant positive trends for lung adenomas and lung carcinomas (independently) were also observed ($p = 0.005$ and 0.0002, respectively, by the Poly-3 trend test; $p = 0.005$ and 0.0001, respectively, by the Cochran-Armitage trend test).

SIRC (1998) analyzed dose-related effects on tumor diameter by grouping total tumors into four categories (≤ 1 mm, 2 to 4 mm, 5 to 8 mm, and 9 to16 mm), finding "an increase in the percentage of small tumors (≤ 1 mm diameter) in mice receiving styrene, particularly in males" (41.2 to 56.5 percent treated *vs.* 12.5 percent controls), but found "no obvious dose-relationship." "In females, the largest tumors (9 to 16 mm) were found in mice receiving styrene at 40 or 160 ppm; these represented 9.1 percent and 8.5 percent of the total number of tumors in these dose groups. The significance of these findings is unclear." The authors suggested that tumor diameters among styrene-exposed male mice were smaller than the tumor diameters among control mice and that this apparent size difference was related to the significance of the increased tumor incidence.

To investigate further the observation regarding tumor diameter, OEHHA calculated the average diameter for each tumor group (total, benign, and malignant) in each sex and dose group from the data in Table 66 of SIRC (1998), comparing pairwise the diameters in each dose group versus the control group (two-tailed t-test, significance defined as $p < 0.05$). Statistically significant decreased tumor diameters were observed for total tumors among male mice exposed to 40 and 80 ppm styrene. For benign tumors, significantly decreased diameters were observed only for male mice exposed to 80 ppm styrene. For malignant tumors, the diameters were decreased in the 40 and 160 ppm groups and increased in the 20 and 80 ppm groups; however, the changes were not statistically significant. A similar analysis of tumor sizes among the female styrene-exposed mice did not reveal significant differences compared to controls.

Cruzan *et al.* (2001) suggested that styrene's respiratory irritation may play a role in changes to the lung, and presented results on cell-type specific toxicity of styrene (Cruzan *et al.*, 2001; SIRC, 1998). Toxicity was observed in mouse epithelial Clara cells but not in the alveolar type II cells. The cellular composition of lung tumor tissues from male and female control and high dose (160 ppm) styrene-exposed mice was evaluated using immunochemical staining agents specific to Clara cells and alveolar type II cells (*i.e.*, Clara cell 10 kDa protein for epithelial Clara cells and type II surfactant protein for alveolar type II cells) (Cruzan *et al.*, 2001; Brown, 1999). The bronchiolar-alveolar adenomas and carcinomas in control and treated mice stained with moderate intensity for alveolar type II cells and comprised over 50 percent of the total cells in the tumor. On the other hand, the majority of tumors were negative for Clara cells; less than 5 percent were positive, with barely perceptible staining. Clara cell positive staining was observed in the bronchioles next to the tumors. Tumors were observed in the peribronchiolar region, as well as in regions that were devoid of bronchioles, and in some cases, a bronchiole could be seen within the tumor; however, there was no preponderance of a

specific localization of tumors relative to bronchioles. Clara cell positive staining was observed in the hyperplastic bronchiolar epithelium and in the bronchiolar hyperplasia extending to the alveolar ducts; type II cell staining was negative in these regions. The separation of the cellular origin of the lung tumors (alveolar type II cells) from the cellular origin of the bronchiolar hyperplastic tissue (bronchiolar Clara cells) suggests that the styrene-related toxic responses described by Cruzan *et al.* (2001), SIRC (1998), and Brown (1999) can be separated from the tumorigenic response, and that the mechanism of the tumorigenic action should include a discussion about alveolar responses, in particular, the type II cell. The increased incidence of alveolar macrophages associated with neoplasia in the high-dose males and females ($p < 0.05$, compared to controls) (SIRC, 1998) also suggests a role for the alveolar region in the tumorigenic response to styrene.

Alternatively, studies by Szabo *et al.* (1998) and Linnoila *et al.* (2000) indicate that the marker protein CC10 antagonizes lung cell carcinogenesis by reducing invasiveness, decreasing adhesiveness to fibronectin and reducing anchorage-independent growth. Furthermore, CC10 may be reduced or lost during tumor development (Hicks *et al.*, 2003; Yao *et al.*, 2003) and thus may not be a suitable marker of regional lung tumor cell origin.

To further elucidate the lung cells targeted by styrene in mice, Gamer *et al*. (2004) exposed female CD-1 mice to 0, 40, or 160 ppm styrene for 6 h/day (1) on 1 day, (2) on 5 consecutive days, or (3) for 20 days during a 4 week period. In addition female Crl:CD rats were exposed at 0, 160, or 500 ppm for 6 h on one day or for 6 h/day on 5 consecutive days. No signs of lung toxicity were observed in rats. The cytology of cells in lung lavage fluid provided no signs of an inflammatory response. In mice, both 40 and 160 ppm styrene led to lower levels of Clara cell secretory protein CC16 in lung lavage fluid, after 1 and 5 exposures, and in mouse blood serum throughout the study. Electron microscopy showed vacuolar degeneration and necrosis of Clara cells. Thus styrene can cause destruction of Clara cells in mice.

Inhalation: Sprague-Dawley Rat – Cruzan and Coworkers

Female and male Sprague-Dawley (CRL-CD, SD) rats (70/sex/dose) were exposed to styrene vapors at target concentrations of 0, 50, 200, 500, or 1,000 ppm six hours/day, five days/week for up to 24 months (Cruzan *et al.*, 1998; SIRC, 1996). The average air styrene levels in the chambers were 0, 50 ± 2.6 (SD), 202 ± 6.4, 501 ± 15.8, and 997 ± 36 ppm, respectively. The test material was >99.5 to 99.7 percent styrene, with relatively low contamination by benzene (<1 to 24 ppm), ethylbenzene (143 to 731 ppm), styrene dimer (<1 to 6 ppm), and styrene oxide (<5 to 6 ppm). The rats were virus antibody-free at the start and exhibited no evidence of infectious disease during the experiment. Necropsies were performed at 52 weeks on 10 males and nine females per dose group and at 105 to 107 weeks on all surviving rats. Lifetime tumor incidences did not include data from the animals necropsied at 52 weeks. [Because of a technical problem causing liquid styrene to drip into chambers during week 61, and subsequent dermal exposures, six males in the 500 ppm and eight males in the 1,000 ppm groups died or were killed within the next two weeks and were not counted in the mortality or tumor analyses.]

At 95 weeks blood styrene levels ranged from 0.29 to 30 μg/mL in female and 0.43 to 33 μg/mL in male rats across styrene-treated groups. Blood levels of styrene-7,8-oxide were below the quantification level for both sexes at 50 ppm styrene, while at 200, 500, and 1,000 ppm, they were 0.028, 0.092 and 0.15 μg/mL (females) and 0.066, 0.12, and 0.18 μg/mL (males), respectively (Cruzan *et al.*, 1998).

No differences in survival at 24 months between the styrene-exposed male rats in all dose groups (56 to 60 percent) and male controls (58 percent) or between female rats in the two lowest dose groups (47 to 48 percent) and female controls (48 percent) were reported. Females in the 500 and 1,000 ppm treatment groups exhibited increased survival (67 to 82 percent) compared to controls. Survival among control females was significantly less than among control males. The styrene-exposed rats had decreased weight gain and decreased food consumption compared to controls, especially at higher concentrations. Males exposed to 500 and 1,000 ppm styrene consumed less food during the first 26 weeks, and females exposed to 500 and 1,000 ppm styrene consumed less food throughout the study, although the decrease was more evident during the first year of exposure. No hematologic or clinical chemistry differences of toxicologic significance or changes in organ weights relative to body weights were observed among the styrene-treated rats compared to control rats.

The authors noted increased water consumption ($p < 0.05$, compared to controls) for males exposed to 500 or 1,000 ppm throughout the study and for females exposed to 500 or 1,000 ppm during the first six months. Among the styrene-exposed males, significant decreases in urinary pH ($p < 0.05$) occurred during the first six months in the 200 and 1,000 ppm groups (pH = 6.2 and 6.0, respectively, compared to pH = 7.0 among controls). At week 104, the urinary pH of 6.3 in the 1,000 ppm group was significantly lower than the pH 6.9 of controls. Among the females, significant decreases in urinary pH occurred at six months in the 500 and 1,000 ppm groups (pH 5.8 and 5.6, respectively, compared to pH 6.1 for controls, $p < 0.05$). The decreased urinary pHs reflect increased levels of MA + PGA; however, no data were presented. Significantly increased osmolality was observed among male rats exposed to 200, 500, or 1,000 ppm styrene for 26 weeks and to 1,000 ppm styrene for 52 weeks. Significantly increased osmolality (compared to controls) was also reported for female rats exposed to 500 or 1,000 ppm styrene for 26 or 52 weeks, or to 1,000 ppm for 78 or 104 weeks.

Post-mortem examination found pale subpleural foci in female rats exposed to 1,000 ppm styrene. Histopathologic examination revealed a statistically significant increase in subpleural lesions containing foamy alveolar macrophages (8/60 in 1,000 ppm females, compared to 1/60 in controls). These lesions were considered by the authors to be spontaneous findings and incidental to the study. Foamy alveolar macrophages occur in response to an impairment of the normal breakdown of lipid and are often associated with drug-induced pulmonary lesions (Hruban, 1976).

Non-neoplastic atrophy and degenerative changes in the olfactory epithelium were observed in the nasal passages of styrene-exposed male and female rats at 104 weeks. The lesions in the lower dose groups tended to be located in the more anterior areas, whereas those in the 500 and 1,000 ppm styrene groups were more extensive and also found in the turbinates. Changes in the Bowman glands, which occur beneath the olfactory epithelium, were also observed.

Macroscopic analysis revealed no treatment-related changes at 52 weeks exposure. At terminal necropsy (104 weeks), changes in three tumor types were seen: testicular interstitial cell tumors in males, and pituitary and mammary tumors in females (Table 19).

Table 19. Incidence of Tumors in Rats Exposed to Styrene by Inhalation and Necropsied at 104 Weeks[1]

Tumor Site and Type	Dose (ppm in Air)					Trend [2]
	0	50	200	500	1000	
Females						
Pituitary adenoma	45/60	42/49	35/42	29/37	31/59	--
Mammary adenocarcinomas	20/60 (33%)	13/44 (30%)	9/43 (21%)	5/38 (13%)	2/59 (3.3%)	[$p < 10^{-6}$]
Mammary fibroadenoma	21/60 (35%)	16/44 (36%)	13/43 (30%)	18/38 (47%)	17/59 (29%)	N.S.
Males						
Testicular interstitial cell tumors	2/60	2/60	2/60	4/54	6/52 [3]	p = 0.015

[1] Data from Cruzan *et al.*, 1998
[2] p-Value of exact test for linear trend. N.S. = not significant ($p > 0.05$). p-Values for negative trends are indicated in brackets. Significant decrease in incidence relative to controls.
[3] p-Value of pairwise comparison by Fisher's exact test: $0.05 < p < 0.1$.

An increased incidence of testicular interstitial cell tumors was observed among male rats exposed to 500 and 1,000 ppm styrene (4/54 and 6/52, respectively, compared to 2/60 in each of the other dose groups; p = 0.3 and 0.09, respectively, Fisher's exact test). Although the pairwise comparisons were not statistically significant, the authors reported a positive trend test (p = 0.015). However, the incidence in all groups was within the historical control range, and there was no exposure-related increase in hyperplasia or seminiferous tubular atrophy, which are commonly associated with chemically-induced interstitial cell tumors.

Among female rats, a decreased incidence of pituitary adenoma was reported. The decreased incidence was statistically significant in the 1,000 ppm group (31/59 compared to 45/60 for controls, p = 0.007; OEHHA analysis). The incidence of pituitary adenocarcinoma was similar across all dose groups, including controls. Mammary adenocarcinoma incidence was significantly decreased in female rats exposed to 500 and 1,000 ppm (p = 0.02 and p < 0.001, respectively; OEHHA analyses). No significant decreases were detected in fibroadenoma incidence in any treated group relative to controls, although a non-significant increase in fibroadenoma incidence was observed in the 500 ppm group. The authors report decreased incidences of mammary adenocarcinoma and fibroadenoma among styrene-treated females, although a significant decrease in fibroadenoma does not appear to be supported by the data.

The control incidence of mammary adenocarcinoma (33 percent) reported by Cruzan *et al.* (1998) is considerably higher than that reported in other studies. Control incidences of mammary adenocarcinomas in Sprague-Dawley rats have been reported as 8 percent (Prejean *et al.*, 1973), 5.8 percent (Jersey *et al.*, 1978), 9 to 10 percent (Keenan *et al.*, 1995), 8.8 percent (Chandra *et al.*, 1992), and 17 percent (McMartin *et al.*, 1992). Conti *et al.* (1988) reported a control incidence of 10 percent for female rat malignant mammary tumors (histologically undefined). Overall, the data provided by Cruzan *et al.* (1998) do not appear to support an association between increased air styrene levels and decreased mammary fibroadenomas in these rats. There was a significant decrease in mammary adenocarcinomas relative to concurrent controls, although historical control data suggest that the control incidence may be unusually high.

Inhalation: Sprague-Dawley Rat – Maltoni and Coworkers

Conti *et al.* (1988) and Maltoni *et al.* (1982) exposed male and female Sprague-Dawley rats (30/sex/dose and 60/sex controls) by inhalation to 0, 25, 50, 100, 200, or 300 ppm styrene for four hours/day, five days/week for 52 weeks and observed them until death (total time not stated)[2]. The last animals died at 138 weeks (Maltoni, 1978).

Quantitative data on survival and toxicity among styrene-exposed rats were not presented. Conti *et al.* (1988) stated that "No differences related to styrene treatment were observed, apart from a higher mortality rate in females treated by ingestion with the highest dose. … No relevant body weight differences were observed between treated groups and controls." According to Maltoni (1978), when the first mammary carcinoma was observed (by 12 weeks), all rats were alive. Adequate survival among the rats in the inhalation bioassay is tacitly suggested by the same authors' acknowledgement of a problem with toxicity in a related bioassay (Conti *et al.*, 1988).

Conti *et al.* (1988) reported an increased incidence of mammary tumors in the female rats exposed to styrene by inhalation (Table 20). Statistically significant increases in malignant mammary tumors were found in the three highest dose groups by pairwise comparison with the control group. Significant increases in combined benign and malignant tumors were found in all styrene-treated groups by pairwise comparison with the control group, except at 50 ppm. Significant positive trends were also observed for both malignant mammary tumors and combined benign and malignant tumors. IARC (1994a) evaluated the malignant mammary tumor data and also reported a positive trend. The mammary tumors were not histologically identified by Conti *et al.* (1988), but were only identified as benign and/or malignant. In preliminary results of a histologic evaluation of mammary tumors, Maltoni (1978) listed fibroadenomas and fibromas as benign tumors, and carcinomas, sarcomas, and carcinosarcomas as malignant. For combined mammary carcinoma and carcinosarcoma among the female rats, Maltoni (1978) reported tumor multiplicities of 1.3, 2.2, 2.3, 2.7, and 1.7 at 0, 25, 50, 100, 200, and 300 ppm styrene, respectively.

[2] This bioassay was part of a set of studies of styrene given by different exposure routes. Non-inhalation exposures are discussed separately above. Because the inhalation exposure regimen described in Maltoni *et al.* (1982) is the same as that described in Conti *et al.* (1988), we assume that the two reports describe the same study. Maltoni *et al.* (1982) reported only on the incidence of styrene-related brain tumors.

Table 20. Incidence of Tumors among Female Rats Exposed to Styrene by Inhalation and Observed until Death[1]

Tumor Site and Type	Concentration (ppm in Air)						Trend [2]
	0	25	50	100	200	300	
Malignant mammary tumors	6/60 (10%)	6/30 (20%)	4/30 (13%)	9/30 [a] (30%)	12/30 [a] (40%)	9/30 [a] (30%)	$p < 0.01$
Combined benign and malignant mammary tumors	34/60 (57%)	24/30 [a] (80%)	21/30 (70%)	23/30 [a] (77%)	24/30 [a] (80%)	25/30 [a] (83%)	$p < 0.001$
Meningiomas	0/60	0/30	0/30	3/30 [b]	0/30	0/30	N.S.

[1] Data from Conti *et al.*, 1988; Maltoni *et al.*, 1982.
[2] p-Value of Cochran-Armitage trend test. N.S. = not significant ($p > 0.05$).
[a] Significant increase by pairwise comparison ($p \leq 0.05$, Fisher's Exact Test), OEHHA analyses.
[b] Significant increase vs control ($p \leq 0.05$, Fisher's Exact Test)

Although the histological type(s) of malignant mammary tumors in this study was not reported, the control group incidence (10 percent) is consistent with the incidence of mammary adenocarcinoma in a number of other studies of female Sprague-Dawley rats (5.8 to 17 percent; see discussion above of Cruzan *et al.*, 1998), with the exception of the Cruzan *et al.* (1998) study itself (33 percent).

Maltoni *et al.* (1982) found no evidence of styrene-related brain tumors, identified as gliomas or meningiomas. An OEHHA analysis of the data in Table 20 of Maltoni *et al.* 1982), however, shows that female rats exposed to 100 ppm styrene exhibited a significantly higher incidence of meningiomas compared to control female rats (3/30 treated compared 0/60 controls, $p = 0.034$, by Fisher's exact test). No other groups of female rats exhibited brain tumors. Conti *et al.* (1988) found no significant differences in leukemias (including "a variety of hemolymphoreticular neoplastic diseases at different sites"), pheochromocytomas, or pheochromoblastomas in styrene-exposed rats compared to the controls.

Inhalation: Sprague-Dawley Rat – Jersey and Coworkers

Male and female Sprague-Dawley rats (96/sex/dose) were exposed by inhalation to 0, 600, or 1,200 ppm styrene for six hours/day, five days/week for 18 months (males) and 21 months (females) (Jersey *et al.*, 1978). The high dose was lowered to 1,000 ppm after two months. Observation continued until 24 months. For the first ten months, the same chamber was used for each styrene level; the 1,000 ppm group was exposed in the morning and the 600 ppm group in the afternoon. Control rats were left in the normal facility holding room during exposure. Rats that died prior to the 24 month scheduled necropsy were examined at time of death. For the 24-month toxicologic evaluation, 84 to 86 rats/sex/dose group were observed.

At the end of 24 months, survival among all groups was poor with no apparent dose-response (6 to 21 percent among males and 26 to 45 percent among females). Infection with *Mycoplasma pulmonis* was observed in 14 to 83 percent of male rats and in 18 to 28 percent of female rats. The infection caused or contributed to the deaths of 8 to 71 percent of the male rats and 11 to 20 percent of the female rats; the lowest infection-related death rates occurred among the rats exposed to 600 ppm styrene.

Postmortem autolysis was observed among rats that died spontaneously. Using a scale of "slight" to "severe," "slight" autolysis was most prevalent (14 to 20 percent among females and 26 to 38 percent among males). All tissues, regardless of degree of autolysis, were examined microscopically and all rats were included in the incidence calculation.

Female rats exposed to 600 ppm styrene had a significantly increased incidence of mammary adenocarcinomas relative to controls (Table 21). No mammary tumors were observed in the high-dose female group. Among the seven adenocarcinomas, one metastasized to the lungs and ovaries. The concurrent mammary adenocarcinoma control incidence of 1.2 percent among the female rats was unusually low compared to the mean historical control rate (5.8 percent over 8 studies; range 0 to 9.3 percent) in the same laboratory over the previous five years.

Table 21. Incidence of Tumors among Rats Exposed to Styrene by Inhalation and Necropsied at 21 Months (Females) or 18 Months (Males)[1]

Sex and Tumor Type	Styrene Concentration (ppm)			Trend [2]
	0	600	"1,000"	
Females				
Mammary adenocarcinomas	1/85	7/85 [a]	0/85	N.S.
Leukemias and lymphosarcomas	1/85	6/85 [b]	6/85 [b]	p = 0.035
Males				
Leukemias and lymphosarcomas	1/85	5/86	1/85	N.S.

[1] Data from Jersey *et al.*, 1978.
[2] p-Value of exact test for linear trend. N.S. = not significant ($p > 0.05$). p-Value of increase above controls by pairwise comparison (Fisher's exact test):
[a] $p < 0.05$,
[b] $0.05 < p < 0.1$.

Among female rats, the incidence of leukemia/lymphosarcoma was marginally increased in both dosed groups compared to controls (Table 21). A non-significant increased incidence of leukemia/lymphosarcoma was observed at the lower dose in male rats. Among female rats, the concurrent and historical control rates were similar (1.2 and 1.4 percent, respectively).

Several aspects of the Jersey *et al.* (1978) bioassay make conclusions about the tumorigenicity of styrene difficult, including the exposure conditions, autolysis of tissues, and dose levels. More specifically:

1) Despite monitoring chamber air styrene levels, the use of the same chamber for both styrene exposure groups for about half of the exposure duration increased the chance of incorrect animal exposures.
2) The failure to use exposure chambers for control rats prevents classification of this group as a true "vehicle control."
3) Postmortem tissue autolysis was evident in all groups, and information was not provided on how many tissues were of sufficient quality to be included in the analysis for each tumor site. Nevertheless, the total number of rats scheduled for the 24-month evaluation (84 to 86) was used to calculate tumor incidence, and thus the incidence values probably underestimate fractional tumor responses due to "dilution" by inclusion of animals whose tissues had deteriorated enough to prevent accurate determination of the presence of tumors.

The study authors recognized the study's limitations in evaluating the chronic toxicity and carcinogenicity of styrene, stating "In light of recent pharmacokinetic evidence indicating that the levels of exposure used in this study overwhelm detoxification of styrene by rats, together with the high incidence of spontaneous disease and the uncertainty of the results obtained in this study, it is recommended that another study be initiated."

Studies by Injection

Intraperitoneal Injection: A/J Mouse

Brunnemann *et al.* (1992) administered 20 intraperitoneal (i.p.) injections of styrene in olive oil three times/week (6.7 weeks total) to female A/J mice (25/dose), a strain sensitive to the development of lung tumors. The total dose was 200 μmol/mouse. Twenty weeks after the final injection, the mice were killed and lung adenomas were counted (27 week study). The increased incidence of adenomas in treated mice was not significant (3/25) compared to controls (1/25), and adenocarcinomas did not occur. The number of lung tumors per mouse was not significantly higher among the styrene-treated mice than among olive oil treated control mice. Limitations of the A/J mouse test system include the short-term exposure and study duration.

Intraperitoneal Injection: Sprague-Dawley Rat

Female and male Sprague-Dawley rats (40/sex/dose) received four i.p. injections of 50 mg styrene (total dose 200 mg/rat) in olive oil or only olive oil (controls) at two month intervals (Conti *et al.*, 1988). The animals were then maintained until natural death. Survival was considered adequate, but details were not provided. The authors reported no significantly increased incidences of total benign and malignant tumors, malignant tumors, leukemias, pheochromocytomas, or pheochromoblastomas.

Subcutaneous Injection: Sprague-Dawley Rat

Conti *et al.* (1988) also administered by single subcutaneous injections, 50 mg styrene (in olive oil) or olive oil only to male and female Sprague-Dawley rats (40/sex/dose). The animals were then maintained until natural death. Survival was considered adequate, but details were not provided. The authors reported no significantly increased incidences of

total benign and malignant tumors, malignant tumors, leukemias, pheochromocytomas, or pheochromoblastomas.

Discussion of Bioassay Results

Bioassays relevant to styrene carcinogenesis have been conducted in both mice and rats, with exposures *via* gavage, drinking water ingestion, inhalation, and injection. Gavage studies in rats, as well as injection studies in both rats and mice, lacked positive findings. In mice, lung tumors (malignant, and benign plus malignant) were reported in several bioassays: 1) among male and female mice in a multidose inhalation exposure (Cruzan *et al.*, 2001; SIRC, 1998); 2) in male mice exposed to a single dose by gavage (NCI, 1979); and 3) in males and females exposed to a single dose *in utero* followed by postnatal gavage exposure (Ponomarkov and Tomatis, 1978). In female rats, mammary tumors, including adenocarcinomas, were reported in both a multidose inhalation study (Conti *et al.*, 1988) and in a two-dose drinking water ingestion study (Beliles *et al.*, 1985). There was a suggestion of mammary tumors and leukemia in female rats in another inhalation study (Jersey *et al.*, 1978), although this study had methodologic issues. A fourth rat inhalation study (Cruzan *et al.*, 1998) failed to find an increased incidence of mammary tumors.

If the mammary tumors, meningiomas, and/or leukemias/lymphosarcomas observed in inhalation and drinking water studies are related to styrene treatment, then the absence of tumors among rats treated with styrene by gavage could represent an effect of exposure route or an effect of dose. Styrene administered by gavage may be detoxified by epoxide hydrolases in the liver (Oesch, 1972), whereas inhaled styrene may undergo biotransformation to styrene-7,8-oxide in the lung and/or blood before entering the liver (Belvedere and Tursi, 1981; Belvedere *et al.*, 1983; Guengerich, 1990; Nakajima *et al.*, 1994; Carlson, 1998; Tuynman *et al.*, 2000). The lack of tumor formation in rats treated by gavage may also be due to a difference in the absorbed dose of styrene relative to that following inhalation.

To compare the absorbed styrene doses in the rat studies, OEHHA calculated total doses as mg/kg styrene. Although an accurate analysis requires knowledge of intake values over time and data on fractional absorption at a given external dose, data-derived default assumptions provided a basis for total dose estimates for the purpose of this evaluation. Higher levels of absorbed styrene may lead to a change in a styrene metabolism, as suggested by the decreased *in vitro* covalent binding of styrene-7,8-oxide to rat liver homogenate or microsomal fraction in the presence of increasing concentrations of GSH (Marniemi *et al.*, 1977). Competing toxicity at higher styrene levels may also mask a tumorigenic response due to non-neoplastic effects, *e.g.*, nephrotoxicity, respiratory toxicity, neurotoxicity, or hepatic toxicity (Chakrabarti and Tuchweber, 1987; Mutti *et al.*, 1999; Coccini *et al.*, 1997; Arlien-Soborg, 1992; Chakrabarti and Brodeur, 1981).

Lung tumors were increased in both sexes of CD-1 mice exposed to styrene by inhalation (Cruzan *et al.*, 2001/SIRC, 1998), in male and female O_{20} progeny mice exposed on gestation day-17 and then by gavage for 16 weeks after birth (Ponomarkov and Tomatis, 1978), and in male $B6C3F_1$ mice exposed by gavage (NCI, 1979). No increase in lung tumors was reported in female A/J mice that were exposed by intraperitoneal injection

(Brunnemann *et al.*, 1992); the duration (27 weeks) may have been inadequate. Stoner (1991) has suggested that for weak carcinogens, a minimum of 36 weeks may be necessary. Styrene consistently induces lung tumors in inhalation-exposed and gavage-exposed mice.

The inhalation study was multi-concentration, and lung adenomas and carcinomas combined were significantly increased at three styrene concentrations in both males and females (Cruzan *et al.*, 2001; SIRC, 1998). The increased incidence of bronchiolar-alveolar carcinoma in female mice was statistically significant at the highest level (160 ppm) and was outside the reported historical control incidences. In females, the trends for increased adenomas ($p = 0.005$) and carcinomas ($p = 0.0002$) were also statistically significant, using the Poly-3 trend test and tumor results for both interim and terminal necropsies.

Cruzan *et al.* (2001) suggested that a high incidence of spontaneous tumors in these animals was a factor in their suggestion of a non-genotoxic mode-of-action for styrene's carcinogenicity. However, the lung bronchiolar-alveolar carcinoma concurrent control data in this study (0 percent females, 8 percent males) are within the range of the reported facility control data (0-4 percent females, 4-26 percent males). The lack of an increased incidence of lung tumors among the mice at 52 or 78 weeks was also considered by Cruzan *et al.* (2001) to reflect the presence of late-forming tumors, which had implications for the carcinogenic mode-of-action of styrene. The authors did not discuss the statistical power of the interim necropsy sampling protocol to detect differences in tumor incidence at those time points relative to the tumor rates in the terminal necropsy.

OEHHA analyzed a further suggestion by the authors that the observed tumorigenicity was qualified by a preponderance of small tumors (SIRC, 1998). A difference in tumor size was seen only for total tumors. When benign and malignant tumors were separated, only benign tumors in mice exposed to 80 ppm styrene were smaller than benign tumors in control mice ($p < 0.05$). Differences in size of malignant tumors were not statistically significant. The results suggest that decreasing tumor diameter in the styrene-exposed mice is not a general pattern. Even if there were decreased tumor diameters, the significance of such a finding is questionable. Rehm *et al.* (1994) commented that Type II tumors of the pulmonary acinus vary in size from tiny foci to lesions 1.5 cm in diameter. They also suggested that small lesions are important in the tumor count and that an optimal histopathologic protocol would examine every tenth section to identify small tumors. A review of the microscopy protocol reported by SIRC (1998) suggested that for the lobes and mainstem bronchi of the lungs, four sections per organ were examined.

The mouse lung tumors described by Cruzan *et al.* (2001) and SIRC (1998) were characterized morphologically as solid, papillary, or mixed solid and papillary, and no difference in the distribution of these tumor types was observed among different doses (64 to 81 percent were papillary). The authors suggested that the uniform distribution of the tumor types among dose groups, including controls, reflected an increase in a pre-existing tumor type. Pulmonary solid tumors appear to be associated with the alveolar region of the lung. Since papillary structures develop in this region, the lesion can be diagnosed as carcinoma (Kauffman, 1981; Gunning *et al.*, 1991; Rehm *et al.*, 1994). Lung papillary tumors also have been observed in the bronchiolar region (Kauffman,

1981; Thaete and Malkinson, 1990; Gunning *et al.*, 1991). The distribution of solid, papillary, and solid/papillary tumors in the lung may be carcinogen-specific (Gunning *et al.*, 1991; Rehm *et al.*, 1994; Mason *et al.*, 2000) and may represent different stages in the progression of tumors towards malignancy (Malkinson, 1992; Rehm *et al.*, 1994).

Mammary tumors in female rats, including adenocarcinomas, were reported in a multi-dose inhalation study (Conti *et al.*, 1988) and in a two-dose drinking water ingestion study (Beliles *et al.*, 1985). In addition, there was a suggestion of mammary tumors in female rats in another inhalation study (Jersey *et al.*, 1978). A second multi-concentration inhalation study (Cruzan *et al.*, 1998) found no increased incidence of mammary tumors in female rats, and in two high dose groups, a decreased incidence of adenocarcinoma. None of the rat gavage or injection studies found an increased incidence of tumors.

In examining the differences between the multi-concentration Sprague-Dawley rat inhalation studies of Conti *et al.* (1988) and Cruzan *et al.* (1998) that may have influenced the differing mammary tumor response, one consideration is the styrene levels to which the animals were exposed. Conti *et al.* (1988) exposed rats to 25, 50, 100, 200, or 300 ppm styrene four hours/day for one year and observed them until natural death; Cruzan *et al.* (1998) exposed rats to 50, 200, 500, or 1,000 ppm styrene for six hours/day for two years. OEHHA estimated the total absorbed dose of the rats exposed to 200 ppm styrene (14,944 mg/kg) in the Conti *et al.* (1988) study is about one-third that of the rats exposed to the same air concentration (44,706 mg/kg) in the Cruzan *et al.* (1998) study. In total dose, all except the lowest concentration of the Cruzan *et al.* (1998) study were far above those in the Conti *et al.* (1988) study (Table 20).

Given the distribution of concentrations and mammary tumor incidence seen in exposed female rats in these two studies, it is possible that mammary tumors may be expressed at lower styrene air levels; other styrene-related toxicities may become more important at higher exposure levels. Observations of nephrotoxicity among styrene-exposed rats (Chakrabarti and Tuchweber, 1987; Mutti *et al.*, 1999) and decreased urinary pH and increased osmolality among female rats exposed to 500 or 1,000 ppm styrene for two years support this interpretation (Cruzan *et al.*, 1998; SIRC, 1996), as do the results of toxicokinetic modeling (Ramsey and Andersen, 1984; Filser *et al.*, 1993) that revealed the saturation of styrene metabolism above 200 ppm.

Rats exposed to styrene by i.p. injection or inhalation develop kidney dysfunction. Chakrabarti and Tuchweber (1987) reported that i.p. exposure of rats to styrene resulted in dose-dependent histopathologic changes of the proximal tubule, decreased anion transport across isolated renal cortical slices, and urinary excretion of γ-glutamyltransferase and other enzymes. The metabolites proposed as the active kidney toxicants are the styrene-glutathione conjugates and subsequent metabolites, formed through the action of glutathione S-transferase and other enzymes on the reduced glutathione pathway (Chakrabarti and Malick, 1991). The Csanady *et al.* (1994) analysis suggested that in the rat, the glutathione pathway becomes operative at relatively higher styrene levels. Experimental evidence for renal toxicity of styrene was presented by Mutti *et al.* (1999), who described a rat model for renal disease; rats exposed by inhalation to 300 ppm styrene for three months developed signs of mild kidney toxicity. At the higher styrene levels, styrene oxide may have been diverted from a neoplastic

pathway into a non-neoplastic glutathione pathway in the kidney. A potential for styrene-related kidney toxicity in humans was also suggested by studies of Welp *et al.* (1996) and Verplanke *et al.* (1998).

The data presented by SIRC (1996) show increased pelvic/papillary urothelial hyperplasia in the decedent animals exposed to 200 ppm styrene, and increased osmolality and urinary glucose levels among the high-dose female rats. Significantly increased osmolality was observed among male and female rats, particularly those exposed to higher styrene doses in the Cruzan study. Increased urinary glucose levels (compared to controls) were noted at various times in various dose groups. Chakrabarti and Malick (1991) commented that increased urinary glucose in the absence of increased blood glucose might reflect damage to the renal proximal tubule, a major site of glucose resorption. High levels of the styrene metabolites, which Cruzan *et al.* (1998) suggested were responsible for the decreased urinary pH, may have resulted in increased osmolality; however, levels were not presented.

Another consideration in examining the differences between the Conti *et al.* (1988) and the Cruzan *et al.* (1998) rat inhalation studies is the relationship between nutritional status and tumorigenicity. In the Conti *et al.* (1988) study, qualitative descriptions suggested that survival was adequate and that toxicity was not apparent. Conti *et al.* (1988) reported an absence of body weight differences between control and treated rats. The female rats described by Cruzan *et al.* (1998) and SIRC (1996) developed treatment-related changes in nutritional status at the higher doses (doses at which decreased mammary tumors occurred), including decreased food consumption, decreased weight gain, increased survival, and increased water consumption. Cruzan *et al.* (1998) suggested that factors in addition to decreased body weight might be responsible for the observed decrease in mammary tumor incidence. The 1,000 ppm female rats had increased survival compared to the 500 ppm females despite the equally reduced body weight gains in these two groups (compared to controls), and that there was a decreased incidence of pituitary adenomas in only the 1,000 ppm group.

Several publications have described studies on the relationship between body weight and mammary tumor incidence in rodents. Keenan *et al.* (1995) reported a decrease in the incidence of mammary fibroadenomas among Sprague-Dawley rats fed a moderately restricted diet compared to those on an *ad libitum* feeding protocol. The authors noted that the time of tumor onset, not tumor progression, was affected. Fischer 344 (F344) rats used in the NTP bioassays exhibited decreases in chemical-induced (styrene not included) mammary gland tumor rates that correlated with decreases in body weights (Haseman and Johnson, 1996). Among untreated control female F344 rats, mammary tumor incidences and body weights increased over a 10-12 year interval (Rao *et al.*, 1990; Haseman and Rao, 1992).

Nohynek *et al.* (1993) and Keenan *et al.* (1999) noted that increased survival could result if decreased weight gain is not pathologic. Seilkop (1995) carried out a statistical analysis of individual animal data on mammary tumors and body weights among the female F344 rats in the NTP database. In addition to finding a relationship between decreased mammary tumor incidence and decreased body weight among female rats, the author reported reduced statistical power to detect an increase relative to control rates for rats with decreased body weights. Such results suggest that the relationship between

reduced body weight and decreased mammary tumor incidence is complex. This phenomenon may have played a role in the decreased incidence of rat mammary tumors reported by Cruzan *et al.* (1998).

A final consideration in examining the rat mammary tumor results is the role of control tumor rates in establishing a meaningful baseline to which tumor incidence in treated animals can be compared. Mammary fibroadenoma occurs at high rates among untreated female Sprague Dawley rats; spontaneous incidences of fibroadenoma have been reported as 19 percent (Chandra *et al.*, 1992), 25-32 percent (Keenan *et al.*, 1995), and 32 percent (Prejean *et al.*, 1973). As discussed by Huff *et al.* (1991), the presence of a high background rate may render detection of chemically induced tumors difficult. Mammary adenocarcinoma rates range widely in these studies of styrene-exposed rats, which also complicates interpretation.

Data from the drinking water study of Beliles *et al.* (1985) suggested an increased incidence of mammary tumors in female rats despite a high background rate in controls (54/96), with significantly increased incidence for female rats in the high dose group (45/60, $p = 0.01$, by Fisher's exact test). Despite relatively high background mammary tumor rates in the rat colony used in Conti *et al.* (1988), statistically significant increases in combined benign and malignant tumors were observed. An OEHHA analysis of Conti *et al.* (1988) data showed a positive trend for malignant mammary tumors, as well as a positive trend for combined benign and malignant mammary tumors. Jersey *et al.* (1978) suggested that the increased incidence of mammary adenocarcinoma in female rats exposed by inhalation (600 ppm styrene, 8.2 percent) could be an artifact of an unusually low concurrent control rate (1.2 percent); however, the control rate was within the historical range of rates from the same laboratory (0 to 9 percent). The incidence of mammary adenocarcinoma in control animals (33 percent) reported by Cruzan *et al.* (1998) is considerably higher than that reported by other authors. Among Sprague-Dawley rats, average control incidences of mammary adenocarcinomas are 8 percent (Prejean *et al.*, 1973), 5.8 percent (Jersey *et al.*, 1978), 9-10 percent (Keenan *et al.*, 1995), 8.8 percent (Chandra *et al.*, 1992), and 17 percent (McMartin *et al.*, 1992).

To investigate the possibility that control rates reported by Conti *et al.* (1988) (57 percent, benign plus malignant; 10 percent, malignant) were unusually low among female rats in that particular Sprague-Dawley colony, OEHHA identified three additional bioassays in two reports (Maltoni *et al.*, 1988b; Maltoni *et al.*, 1988a) wherein control rates for mammary tumors among this colony were presented. We compared control incidences of benign plus malignant tumors or malignant tumors only in these bioassays (controls for chemicals other than styrene) with female control data reported by Conti *et al.* (1988). For these sets of controls, the incidence of combined benign and malignant tumors in controls differed from that in the Conti *et al.* (1988) study in both directions (108/150, higher, $p = 0.03$; 42/60, higher, $p = 0.13$; 24/60, lower, $p = 0.07$; compared to 34/60 for styrene controls, 2-tailed test of proportions), but only one difference was statistically significant ($p \leq 0.05$). The incidence of malignant tumors did not differ significantly, although the values fluctuated in both directions (20/150, higher, $p = 0.51$; 5/60, lower, $p = 0.75$; 2/60, lower, $p = 0.14$; compared to 6/60 for the styrene controls). Within the colony used for carcinogenicity studies by the Maltoni laboratory, control

rates of mammary tumors in the Conti *et al.* (1988) study appear to be quite similar and not unusually low.

Estimation of Total Doses in Rat Bioassays

To facilitate the comparison of the rat bioassays, OEHHA calculated "total doses" in mg/kg received by the animals during the course of each study (Table 22). These estimates necessitated several assumptions that are described below.

Table 22. Estimated Total Doses in Rat Ingestion and Inhalation Bioassays

Study Reference	Route	Dose Group	Total Dose (mg/kg)
Conti *et al.*, 1988; Maltoni *et al.*, 1982	Inhalation	25 ppm	1,868
		50 ppm	3,736
Beliles *et al.*, 1985	Drinking water	125 ppm	5,650
Conti *et al.*, 1988; Maltoni *et al.*, 1982	Inhalation	100 ppm	7,472
	Gavage	50 mg/kg	10,374
Cruzan *et al.*, 1998; SIRC, 1996	Inhalation	50 ppm	11,177
Beliles *et al.*, 1985	Drinking water	250 ppm	11,299
Conti *et al.*, 1988; Maltoni *et al.*, 1982	Inhalation	200 ppm	14,944
		300 ppm	22,416
Cruzan *et al.*, 1998; SIRC, 1996	Inhalation	200 ppm	44,706
Conti *et al.*, 1988; Maltoni *et al.*, 1982	Gavage	250 mg/kg	51,870
Ponomarkov and Tomatis, 1978	Gavage	500 mg/kg	60,060
Cruzan *et al.*, 1998; SIRC, 1996	Inhalation	500 ppm	111,766
Jersey *et al.*, 1978	Inhalation	600 ppm	134,488
Cruzan *et al.*, 1998; SIRC, 1996	Inhalation	1,000 ppm	223,532
Jersey *et al.*, 1978	Inhalation	1,000 / 1,200 ppm	224,147
NCI, 1979	Gavage	500 mg/kg	257,397
		1,000 mg/kg	389,844
		2,000 mg/kg	779,688

Studies in which animals were exposed to styrene in drinking water reported in units of mg/mL were converted to units of mg/kg-day based upon the following assumptions: a drinking water ingestion rate in rats of 63.3 mL/kg-day (average for male and female rats; Beliles *et al.*, 1985), a gastrointestinal absorption fraction of 1.0 (Csanády *et al.*, 1994), and a rat body weight of 0.35 kg (Anderson *et al.*, 1983).

Studies in which animals were exposed to styrene by inhalation reported in ppm were converted to mg/kg-day based upon a styrene conversion factor of 4.26 mg/m^3 per ppm (at 25°C), a rat breathing rate of 0.218 m^3/day (Anderson *et al.*, 1983), a pulmonary absorption fraction of 0.65 (see Metabolism and Pharmacokinetics section), and a rat body weight of 0.35 kg (Anderson *et al.*, 1983). We assumed that rats and mice have the same uptake factor as humans (e.g., Csanady *et al*., 2003). Total doses were calculated as a product of the dose rate (mg/kg-day) and the number of days exposed.

Summary

Rodent bioassay results indicate that styrene is carcinogenic in male and female mice and female rats. At higher styrene exposure levels the sensitivity of cancer bioassays in rodents may be decreased by competing non-neoplastic pathways. Lung tumors were observed in female and male mice exposed by inhalation for their lifetime (Cruzan *et al.*, 2001; SIRC, 1998) and in male mice exposed by gavage (NCI, 1979). Lung tumors developed in the progeny of mice exposed *in utero* and then by gavage (Ponomarkov and Tomatis, 1978). Increased mammary gland tumors were observed in female rats exposed by inhalation or drinking water ingestion (Beliles *et al.*, 1985; Conti *et al.*, 1988), although finding such effects only at the lower end of the dose-response continuum makes their toxicological significance questionable. On the other hand, the reports of lung tumors in styrene-treated mice and mammary tumors in styrene-treated rats are considered to be consistent with the tumor profiles of several alkenes and alkene epoxides (Melnick, 2002; see “Mechanism of Carcinogenicity”).

Other Toxicity in Humans

Acute Toxicity

Human volunteers who were acutely exposed to styrene developed ocular and respiratory tract irritations (reviewed in OEHHA, 1999). People with asthma may be more sensitive to the pulmonary effects of styrene (OEHHA, 1999). Central nervous system disturbances were observed in acutely exposed human volunteers (ACGIH, 1997; OEHHA, 1999). The CNS disturbances included impairment of balance, drowsiness, muscle weakness, decreased reaction times, reduced ocular tracking, and depression. Humans who inhaled high levels of styrene (500 to 800 ppm) for the first time found the exposure objectionable after one to two minutes, whereas workers exposed to the same levels for longer periods had only mild to moderate complaints of irritation (Gotell *et al.*, 1972). These observations suggest that a tolerance may have been induced.

Arnedo-Pena *et al.* (2003) reported on an accidental exposure to styrene in tap water in Spain, which occurred subsequent to repairs on a water tank serving two apartment buildings. Effects were investigated using a questionnaire on subjective symptoms; reports were obtained from 84 out of 93 persons living in the buildings. Symptoms were reported from 46 people (55 percent of the sample). Styrene concentrations up to 800 ppb were found in the water two days after the incident. Reported symptoms included irritation to the throat, nose, and eyes, as well as general gastrointestinal symptoms.

Symptoms were associated with drinking the water, being exposed to the vapors, and eating foods prepared from the tap water.

Seeber *et al*. (2004) investigated the risk for neurobehavioral impairments in simulated work exposure to styrene. In one experiment 16 volunteers (eight in the morning, eight in the afternoon) were exposed to 0.5 and 20 ppm styrene for 3 hours. In the second, 24 volunteers (12 morning, 12 afternoon) were exposed for 4 hours to 0.5 and 20 ppm styrene and also to an exposure changing between 0.5 and 40 ppm styrene (Time-Weighted-Average (TWA) = 14 ppm). The authors measured simple reaction, choice reaction, attention, acute symptoms, and well-being, and did not find exposure-related performance effects. However, a six hour time change resulted in delayed choice reactions in the morning hours. The authors concluded that acute styrene did not adversely affect well-being.

Ska *et al*. (2003) evaluated neurotoxicity of acute peak exposures in adults not previously exposed to styrene and with no known exposure to any other neurotoxicants. Volunteers (n = 24) were exposed to one of five exposure scenarios over 6 hours: (1) constant exposure to 106 mg/m^3 (25 ppm); (2) variable exposure with a mean of 106 mg/m^3 and four 15 min peaks of 213 mg/m^3 (50 ppm); (3) constant exposure to 213 mg/m^3; (4) variable exposure with a mean of 213 mg/m^3 and four peaks of 426 mg/m^3 (100 ppm); and (5) two exposures to 5 mg/m^3 (1.2 ppm; control). Before and after styrene exposure, sensory tests (visual and olfactory), neuropsychological tests (reaction time, attention, memory, psychomotor function), and self-evaluation questionnaires of mood and symptoms were given in a test-retest design. The different peak exposure scenarios did not influence the performance on any test or induce subjective signs and symptoms.

Several studies have investigated the effects of styrene as well as other solvents on human respiratory tissues. Röder-Stolinski *et al*. (2008) showed in a human epithelial cell culture that styrene stimulates expression of inflammatory mediators, including the chemotactic cytokine monocyte chemoattractant protein-1 (MCP-1). They concluded that the inflammatory response to styrene is mediated through oxidative stress related to depletion of intracellular GSH levels, followed by activation of the nuclear factor-kappa B (NF-κB) signaling pathway. Activation of other inflammatory mediators follows, and their corresponding genes are upregulated. These inflammatory processes could lead to respiratory diseases such as bronchitis.

Another concern is sensitization to other respiratory diseases by exposure to irritating solvents, particularly in infants and children. Rolle-Kampczyk *et al.* (2002) report that obstructive bronchitis was observed more frequently in children exposed to increased concentrations of styrene, as well as benzene, toluene, and m,p-xylene in residential air over a 5-year period in the Leipzig Allergy Risk Study in Germany. The solvent exposures were at least partly associated with passive smoking, which is obviously a confounding risk factor. Apartment redecoration, with exposure to a variety of chemicals off-gassing from fresh paints and other new materials, was also a risk factor (Diez *et al*., 2003).

Subchronic Toxicity

Liver Toxicity in Workers

Styrene-exposed workers (50 to 120 ppm for a mean of 5.1 years) did not exhibit higher levels of liver enzymes in the serum (aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase) or of bile (cholic, deoxycholic) acids compared to controls (Harkonen *et al*., 1984b).

Asthma in Workers

A few case reports indicate that asthma is associated with styrene exposure (e.g., Moscato *et al*., 1987; Hayes *et al*., 1991).

Ototoxicity in Workers

Morata *et al.* (2002) studied hearing loss in workers exposed to styrene. The styrene-exposed subjects [with or without exposure to noise at a time-weighted average (TWA) of 85 dB] worked in 11 plants of the fiberglass products industry, while workers exposed to noise only came from three companies that manufactured metal products. Controls unexposed to either styrene or noise worked in a mail distribution terminal. Styrene exposure was measured by personal air samples and urinary mandelic acid (MA). Air levels did not exceed 20 ppm. Noise exposure was estimated by personal noise dosimeters and measurement of background levels. Hearing ability was measured as "pure-tone thresholds" in each ear at frequencies of 1, 2, 3, 4, 6, and 8 kHz. The authors report hearing loss at 2, 3, 4, and 6 kHz among styrene-exposed groups compared to both non-exposed workers and noise only-exposed workers. Age, tenure, styrene level, noise level and MA level all contributed to risk of hearing loss. The odds ratio (OR) for an increased probability of developing hearing loss with each unit increase of MA was 2.44 (95 percent CI = 1.01 to 5.89). Morata *et al.* (2002) concluded that styrene exposure affects the human auditory system. A limitation of this study is that the styrene-only group was exposed to higher solvent levels and had higher lifetime styrene exposures, so it may not be directly comparable to the styrene-plus-noise group. Styrene-related hearing loss may be a high-dose effect; the low styrene exposure levels (exposures <20 ppm, and most were <12 ppm) experienced during the time of this study by these workers may also have limited the study's potential to detect a styrene-related hearing loss.

Immunotoxicity

Immunologic alterations (*e.g.*, altered phenotypic profiles among lymphocyte subsets, decreased natural killer cell activity, and decreased chemotaxis) have been observed after styrene exposure, but the limited data prevent quantitative interpretation (Bergamaschi *et al.*, 1995; Governa *et al.*, 1994).

Mutti *et al.* (1992) looked for changes in the balance of immune effector and regulator cells among 32 styrene-exposed workers. The workers were separated into high (>50 ppm, 8 hr TWA) and low (<50 ppm) exposure and compared to 19 unexposed individuals. Flow cytometry analysis of subsets of lymphocytes revealed a reduction in T-helper cells and a relative increase in T-suppressor cells with increasing styrene exposure. There was an inversion of the helper/suppressor ratio in the high exposure

group. The proportion of natural killer (NK) cells also increased. No changes were noted in B cells.

Bergamaschi *et al.* (1995) reported immune system alterations in reinforced plastics industry (RPI) workers. Thirty-two women and 39 men (average age = 32 years, average exposure duration = 7 years) were compared with 65 non-styrene exposed factory workers and matched for age, sex, tobacco use, and ethanol consumption. The factory air styrene levels varied between 10 and 50 ppm. Individual worker exposure was measured by urinary metabolites (mandelic acid + phenylglyoxylic acid) the morning after the last shift (15 hours post-exposure). Among all the styrene-exposed workers (median exposure = 16 ppm calculated according to Guillemin *et al.* (1982)), the proportions of lymphocyte cells in 12/18 lymphocyte subsets and the prevalence of abnormal values of immunologic phenotypes for 11/18 subsets were statistically different from the non-exposed controls ($p < 0.001$ to < 0.05). When the workers were placed into three exposure groups (0, < 25 ppm, and > 25 ppm styrene), dose-response relationships were observed for the prevalence of abnormal responses for four lymphocyte subsets; in the case of two subsets, abnormal responses were observed in the group exposed to < 25 ppm styrene. Natural killer (NK) cell activity, a lymphocyte function, measured in a different group of workers, was decreased compared to unexposed worker controls. The median exposure was calculated as 21 ppm based on Guillemin *et al.* (1982). Exposure of these workers to air styrene levels below 50 ppm, and probably at levels near 25 ppm, resulted in alterations of the immune system.

Governa (1994) found a significant reduction of chemotaxis *in vitro* in PMN sampled from workers (*ex vivo*) exposed to a low level of styrene and having no other biochemical or clinical alteration. PMN chemotaxis returned toward normal by 3 weeks after removal from exposure.

Tulinska *et al.* (2000) examined immune and hematological parameters in 29 hand laminators and sprayers exposed to styrene for an average of 14 years and in 19 in-factory unexposed worker controls. The average styrene air level was 139.5 mg/m^3. The mean blood styrene concentration of exposed workers was 945.7 μg/L and the mean styrene in exhaled air was 38.8 μg/L. Multifactorial analysis of variance indicated a significant decrease in exposed workers in the proliferation of lymphocytes stimulated by the mitogen concanavalin A, but not by pokeweed mitogen (PWM). Proliferative response to PWM was significantly correlated with styrene levels in blood. Styrene-exposed workers had increased levels of complement component C4. Levels of the C3-component were positively correlated with exposure duration. Exposed workers had a significant elevation in the percentage and number of monocytes and a significantly decreased number of lymphocytes. Styrene concentrations in both blood and exhaled air were associated with a decreased percentage of large granular lymphocytes.

Somorovska *et al.* (1999) reported that the proliferative response of T-lymphocytes stimulated with concanavalin A was significantly suppressed in 44 hand laminators exposed to styrene.

Biro *et al.* (2002) studied the immunotoxicity of styrene (and of benzene, polycyclic aromatic hydrocarbon exposure, and mixtures) to investigate any correlation between immunological and genotoxicological parameters. Ten workers exposed only to styrene

(level not stated) were compared to 25 unexposed controls. The styrene exposed workers showed a statistically significant increase in the CD4 positive lymphocyte subpopulation and in CD45RO positive/CD4 positive memory cells.

Neurotoxicity

Acute Neurotoxic Effects

Immediate eye and throat irritation, increased nasal mucus secretion, listlessness, impairment of balance, and drowsiness followed by unsteadiness, muscle weakness, and depression were reported in a study of two human volunteers exposed to 800 ppm styrene for 4 hours (Carpenter *et al.*, 1944). Other symptoms include a feeling of being "lightheaded" or "drunk" (Lorimer *et al.*, 1976). Sensory irritation and neurological impairment occur in acute human studies above 100 ppm (Stewart *et al.*, 1968).

Stewart *et al.* (1968) found eye and throat irritation in 3 of 6 volunteers exposed to 99 ppm (416 mg/m³) styrene for 20 minutes. No symptoms were reported in 3 subjects after exposure to 51 ppm for 1 hour. Exposure to 376 ppm (1,579 mg/m³) styrene for 25 minutes resulted in nausea, significant discomfort, and an abnormal Romberg test, indicative of cerebellar dysfunction. Significant decrements were noted in 3/5 subjects in other tests of coordination and manual dexterity at 50 minutes. Exposure to 216 ppm or less for up to 1 hour did not cause measurable impairment of coordination and balance. The study yielded a 1 hour NOAEL of 51 ppm (OEHHA, 1999). OEHHA considered it the most sensitive acute study and used it to develop an acute inhalation Reference Exposure Level (REL) of 21,000 $\mu g/m^3$ for styrene (OEHHA, 1999; Collins *et al*., 2004).

The neurotoxic effects mediated by styrene consist of slower sensory (but not motor) nerve conduction velocity and CNS depression (Cherry and Gautrin, 1990). Gamberale and Hultengren (1974) reported that reaction time was significantly impaired in 12 men exposed to 350 ppm styrene for 30 minutes, but not at 250 ppm or lower. No effects on perceptual speed or manual dexterity were detected. In 12 workers exposed during the workday to 26 ppm styrene, Edling and Ekberg (1985) measured simple reaction time before and after work and found no significant differences. Other non-CNS symptoms were reported in a neuropsychiatric questionnaire completed by the subjects. The CNS depressant effects of acute exposures to high styrene levels are probably mediated by the direct effect of the lipophilic, unmetabolized styrene on nerve cell membranes.

Abnormal electroencephalograms were correlated with urinary levels of 700 mg/L MA or higher in workers exposed to styrene (Harkonen *et al.*, 1978).

Chronic Neurotoxic Effects

Chronic exposures to styrene result in central nervous system (CNS) and peripheral nervous system (PNS) effects, although the latter are not as pronounced (ATSDR, 1992; Rebert and Hall, 1994; Murata *et al.*, 1991). Long-term effects of styrene exposure may result from the action of one or more styrene metabolites (Savolainen, 1977; Mutti *et al.*, 1988). In humans, styrene metabolism is initiated by cytochrome P450 (P450)-mediated oxidation of styrene to a reactive metabolite, styrene oxide, in liver and, to a minor extent, in lung (Nakajima *et al.*, 1994). The P450 enzymes responsible for the epoxidation of styrene to styrene oxide are found in human brain, but the brain isozymes

have not been tested specifically with styrene as a substrate (Bhamre *et al.*, 1993). Styrene may also be oxidized to styrene oxide by enzymes which share specific iron and porphyrin components with P450 and those that utilize active oxygen species (Belvedere *et al.*, 1983; Tursi *et al.*, 1983; Miller *et al.*, 1992).

Female workers in the reinforced plastics industry (RPI) were studied for levels of substances with neuroendocrine function (Mutti *et al.*, 1984a**).** Serum prolactin, thyroid stimulating hormone, human growth hormone, follicle stimulating hormone, and luteinizing hormone were measured in 30 women who were between the 5th and 15th day of the menstrual cycle. Exposure was based on MA+PGA levels in venous blood samples taken the next morning before the start of work. The authors estimated that the average styrene TWA/8 hr was about 130 ppm. Controls consisted of women factory workers living in the same area as the styrene-exposed women, not knowingly exposed to styrene. After controlling for age and exposure time, the increased prolactin and thyroid stimulating hormone levels were correlated with the concentration of next-morning urinary MA+PGA; only the increased prolactin levels were statistically significant. Bergamaschi *et al.* (1995, 1996) found significantly elevated prolactin levels in styrene-exposed male and female workers in RPI.

Several occupational studies have noted CNS disturbances in styrene-exposed workers. Decreased manual dexterity, increased reaction times, and/or abnormal vestibuloocular reflex (ability to track moving objects) were observed by Gotell *et al.* (1972), Gamberale *et al.* (1975), Lindstrom *et al.* (1976), Mackay and Kelman (1986), Flodin *et al.* (1989), Moller *et al.* (1990), and Cherry and Gautrin (1990) for air styrene levels of about 12 ppm to more than 100 ppm. However, in each of these studies, there were difficulties in quantifying the effect. The difficulties included small sample size, unknown exposure duration, lack of concurrent control group, lack of dose-response data, and either unknown ethanol consumption or lack of adjustment for ethanol consumption. In the Cherry and Gautrin (1990) investigation, however, the authors determined that accounting for ethanol consumption did not reduce the correlation between increased reaction time and exposure.

Decrements in other CNS functions were observed among workers in the well-controlled studies of Fallas *et al.* (1992), Chia *et al.* (1994), and Mutti *et al.* (1984b). Fallas *et al.* (1992) studied 60 male workers (average age = 29.5 years, average air styrene = 24.3 ppm). The styrene-exposed population was compared to non-exposed workers and matched for age, intellectual level, and ethnic origin. The results from a standardized test battery showed decrements in the aiming response; 22 of 60 styrene-exposed workers (37 percent) exhibited increased reaction times compared to 7 of 60 controls (12 percent) ($p < 0.01$ by Fisher's exact test). Acquired color vision loss (dyschromatopsia) was observed in the styrene-exposed workers. Chia *et al.* (1994) observed decrements in CNS function as defined by altered visual retention, audio-digit recognition, and digit recognition. However, a dose-response relationship did not exist. These workers also exhibited a statistically non-significant, dose-dependent dyschromatopsia.

In the most comprehensive occupational study, Mutti *et al.* (1984b) assessed memory and sensory/motor function in 50 male styrene-exposed workers (average exposure = 8.6 years) and in a control group of 50 manual workers. Subjects were matched for age, sex, and educational level; a vocabulary test was included to match for general intelligence.

Eligibility criteria included absence of metabolic, neurological, or psychiatric disorders, limited ethanol intake, and limited cigarette usage. All subjects were instructed to avoid intake of alcohol and drugs for two days prior to testing. Styrene exposure was assessed from urinary MA+PGA levels the morning after the last workday in the week, followed immediately by eight neuropsychological CNS function tests: reaction time, short and long term logic memory, short and long term verbal memory, digit-symbol association, block design, and embedded figures. The mean $\pm$ 2 SD of the control group values was set as the normal range limit for each test. Expressed as continuous data, styrene-exposed workers exhibited significantly poorer performances than controls on all except the digit-symbol test. Urinary styrene metabolite concentration and duration of exposure were significantly correlated with the scores on several tests. As a subgroup, workers with metabolite levels of up to 150 mmoles MA+PGA/mole creatinine (mean = 75 $\pm$ 33 [SD] mmoles/mole creatinine, equivalent to 15 ppm styrene) appeared to have no significant effects. This level of urinary metabolites corresponds to a mean daily 8-hour exposure to 25 ppm styrene. Significantly poorer performances in four or more neuropsychological tests were recorded in the three subgroups with greater urinary excretion of styrene metabolites (150-299, 300-450, and > 450 mmoles MA + PGA/mole creatinine).

Mutti *et al.* (1984b) expressed the quantal data as the fraction of tested subjects who responded abnormally to ≥ 1, ≥ 2, and ≥ 3 tests (Table 23). Positive dose-response relationships existed between intensity of styrene exposure and abnormal scores, whether it was expressed as abnormal responses in at least one, at least two, or at least three tests. The chi-square test and validity calculations were performed by constructing 2 x 2 tables selecting different levels of urinary excretion of MA and PGA as a cut-off point. The highest values for chi-square and predictive validity were found when the cut-off of 150 mmol/mol creatinine was chosen, suggesting that the quantal isolation of the low dose subgroup from the next subgroup is appropriate. When the quantal data for the low dose subgroup were analyzed by OEHHA using Fisher's Exact Test, a significant level of abnormal responses was observed for ≥ 1 ($p = 0.005$) and ≥ 3 ($p = 0.04$) tests. The abnormal responses for ≥ 2 tests were marginal ($p = 0.06$). For each of the higher exposure groups, the p-values were <0.05. The quantal results suggest that the low dose subgroup represents a LOAEL, and that a NOAEL is not available. Mutti *et al.* (1984b) also expressed the data in a quantal three-way representation including prevalence (number of respondents for at least one, two, or three abnormal tests), duration (years at work), and intensity (metabolite level). This representation revealed a positive correlation of neuropsychological deficits with duration as well as exposure intensity.

A linear relationship, based on Guillemin *et al.* (1982), exists for converting urinary metabolite concentrations to ppm styrene in air (4.97 mmoles MA+PGA/mole creatinine is equivalent to 1 ppm styrene). In Table 23 the mean styrene concentrations per group are 0 (controls), 15, 44, 74, and 115 ppm. OEHHA used the data in the table to develop a chronic inhalation Reference Exposure Level (REL) of 900 $\mu g/m^3$ by a benchmark concentration approach (OEHHA, 2000; Rabovsky *et al*., 2001). This involved modeling the dose-response data to calculate the 95 percent lower confidence limit of the dose resulting in a 5 percent incidence of three or more abnormal tests, defined as the $BMCL_{05}$. The $BMCL_{05}$ was determined to be 1.7 ppm, which is equivalent to a continuous inhalation exposure to 0.61 ppm (1.7 ppm x 10/20 x 5/7).

Gobba and Cavalleri (1993) and Campagna *et al.* (1995) also reported dyschromatopsia among styrene workers in the reinforced plastics industry (RPI). Workers (n = 36) exposed to an average of 16 ppm styrene exhibited significantly greater dyschromatopsia than controls, matched for age, ethanol consumption, and tobacco smoking (Gobba and Cavalleri, 1993). Among the study population, only 1 of 36 styrene-exposed workers (compared to 16 of 36 controls) performed the test with 100 percent accuracy. In a different group of workers, those exposed to > 50 ppm styrene exhibited greater dyschromatopsia than those exposed to ≤ 50 ppm, and within this group, a subset exhibited a similar decrement after returning from a one-month vacation. Campagna *et al.* (1995) gave the test for dyschromatopsia to 81 RPI workers (79 male and 2 female) exposed to 4.6, 10.1, and 88.8 ppm styrene (first quartile, median, and third quartile, respectively). No control group was used. Statistical analysis revealed a correlation of color vision loss with exposure to styrene, age, and ethanol consumption.

Table 23. Subjects Classified Positive on Neuropsychological Tests as a Function of Styrene Exposure[1]

MA + PGA (mmoles/mole creatinine)[a]	Styrene Concentration (ppm)[b]	Abnormal Tests[c]		
		≥ 1	≥ 2	≥ 3
Controls	--	4/50	2/50	0/50
< 150 (75 ± 33)	15	6/14 p = 0.005	3/14 p = 0.065	2/14 p = 0.045
150 - 299 (216 ± 45)	44	6/9 p = 0.0003	5/9 p = 0.0005	3/9 p = 0.0026
300 - 450 (367 ± 49)	74	10/14 $p = 5\times10^{-6}$	7/14 p = 0.0002	5/14 p = 0.0003
> 450 (571 ± 108)	115	11/13 $p = 2\times10^{-7}$	8/13 $p = 1\times10^{-5}$	6/13 $p = 2\times10^{-5}$

[1] Data from Mutti *et al.*,1984b.
[a] "Next-morning" styrene urinary metabolites. Mean ± SD in parentheses.
[b] Calculated by OEHHA (2000). Assumes that 4.97 mmoles MA + PGA/mole creatinine is roughly equivalent to 1 ppm styrene, following Guillemin *et al.* (1982). Levels in parentheses are associated continuous exposure concentrations, assuming a five-day workweek and that 10 m^3 air are breathed in a workday (of a 20 m^3 total/day).
[c] Number of individuals with abnormal test divided by the number of subjects tested. Results from Fisher Exact Tests for dosed and control groups are included on the second line.

Toppila *et al*. (2006) investigated the effects of styrene on balance in employees of 16 fiberglass-reinforced plastic boat manufacturers in Finland. They measured postural stability using static and dynamic measurements on a custom-made tilting force platform. Individual breathing zone exposure to styrene was measured. The authors analyzed 88 age-matched pairs of laminators (exposed to an estimated average level of 108 mg/m^3 styrene) and non-laminators (exposed to an average level of 21 mg/m^3 styrene). There were no unexposed controls. The postural stability of laminators was statistically

significantly aggravated compared with non-laminators in two dynamic tests. The impairment was observed in young workers and worsened with age.

Viaene *et al.* (2001) carried out a cross-sectional study of polyester boat builders, 3 years after the main activity ceased. Workers exposed to a lower concentration of styrene (n = 27) and formerly exposed workers (n = 90) were compared with matched control workers (n = 64). Currently and formerly exposed workers laminated at mean levels of 148 and 157 mg/m^3 styrene, respectively. For the period before operations ceased, currently and formerly exposed workers reported more complaints than controls, which related well with the mean level of airborne styrene. Most complaints disappeared after exposure ended, but the equilibrium category score of NSC-60 and the number of workers reporting diminished sense of smell remained increased in formerly exposed workers ($p \leq 0.05$). Test results for symbol-digit substitution and for digit span forwards were worse in currently and formerly exposed workers ($p \leq 0.01$). In the combined group of currently and formerly exposed workers, the symbol-digit substitution and color-word vigilance results related well to duration of exposure ($p < 0.01$ and $p = 0.03$) and microsomal epoxide hydroxylase (mEH) phenotype activity ($p = 0.01$ and $p = 0.05$). Most subjective symptoms were reversible but some persisted after the end of styrene exposure. Dysfunction of visuomotor performance and perceptual speed appeared to persist. Duration of exposure at lamination tasks and the interaction, concentration times duration of exposure (C x t), were the best predictors of worsening visuomotor and perceptual speeds. Activity of the mEH phenotype may modulate styrene neurotoxicity. Less than 10 years exposure to styrene at a mean level of 155 mg/m^3 may result in persistent neurotoxic effects.

Behari *et al. (*1986) reported the case of a man who had been working for 5 years with a photostat process that used styrene and was diagnosed with peripheral neuropathy. However, in occupational studies, the relationship between exposure to styrene and PNS effects has been inconsistent (Triebig *et al.*, 1985; Cherry and Gautrin, 1990). A major difficulty is the choice of an appropriate surrogate for dose that may lead to PNS disturbance (Murata *et al.*, 1991). One study used chronic exposure indices including work method, years at work, time spent laminating (the source of high exposure), styrene air concentration, and end-of-shift urinary MA (Matikainen *et al.* (1993). Numbness in the extremities increased with the exposure index, although with marginal significance ($p < 0.1$). The styrene TWA/8 hr was 32 ppm for the 100 subjects. The irritant and central nervous system (CNS) depressant effects of styrene in humans are consistent with the acute effects observed in experimental animals (Bond, 1989).

Moller *et al.* (1990) gave an otoneurological test battery to 18 workers with long-term exposure to styrene at levels well below the (then) Swedish occupational limit of 110 mg/m^3 (25 ppm) and compared the results to a reference group. Disturbances in the central auditory pathways were found in seven workers (39 percent). Tests reflecting central processing of impulses from different sensory equilibrium organs were abnormal for 16 workers (89 percent).

Morioka *et al.* (1999) measured the upper limit of hearing (frequency = 8 kilohertz) in 93 men exposed to organic solvents including styrene in factories producing plastic buttons or baths. Medical exams, environmental monitoring of breathing zone air, and biological monitoring were carried out. The solvent levels were generally lower (styrene: geometric

mean = 8 ppm; maximum = 91.6 ppm) than the occupational exposure limit (styrene = 50 ppm). Six workers exceeded the occupational limit. The upper limit of hearing was reduced in workers who were exposed for 5 or more years and was dose-dependently related to styrene levels in breathing-zone air and to mandelic acid levels in urine. Individuals with normal medical exams also had a reduced upper limit of hearing.

One postulated styrene toxicity mechanism is binding of the highly reactive styrene oxide to nervous tissue. Another postulated mechanism is alteration in the levels of circulating catecholamines (e.g., dopamine) due to the binding of PGA to these biogenic amines (Mutti, 1993; Mutti *et al.*, 1984a; Checkoway *et al.*, 1994) and changes in physiological functions that are under biogenic amine control. Checkoway *et al.* (1994) described a cross-sectional study of 59 male boat plant workers exposed to <1 to 144 (mean = 37.2) ppm styrene. Monoamine oxidase B (MAO-B) activity in platelets was measured as an indicator of catecholamine metabolism. When the styrene-exposed workers were divided into quartile exposures, a dose-dependent decrease in MAO-B activity was observed after adjustments for age, smoking, alcohol, and medications. Long-term exposures to styrene are associated with decrements in physiological functions.

Kolstad *et al.* (1995) estimated excess deaths due to four major non-malignant disease groups for 53,847 male workers in the Danish RPI. Low and high styrene exposures were based on companies with less than 50 percent (low) and those with 50 percent or more (high) employees involved with reinforced plastics. An internal comparison was made with workers unexposed to styrene to account for more similar activities and lifestyles. Excess deaths due to pancreatitis and degenerative disorders of the myocardium were statistically significant ($p < 0.05$), but the excess deaths due to degenerative diseases of the nervous system were not significant.

Benignus *et al*. (2005) performed a meta-analysis which quantified the relationship between exposure estimated from biomarkers and effects on reaction time and color vision using the studies described above and several others. Data were pooled by end point and transformed to a common metric, percent of baseline. Styrene concentrations were estimated from biomarkers of exposure and linear least-square equations were fitted to the data to derive dose-effect relationships. Statistically significant relationships were found between cumulative styrene exposure (ppm-years) and increased choice reaction time as well as increased color confusion index. Eight work-years of exposure to 20 ppm styrene was estimated to (1) increase choice reaction time by 6.5 percent, an amount that significantly increases the probability of automobile accidents, and (2) increase the color confusion index as much as 1.7 additional years of age in men.

Styrene Oxide Neurotoxicity

In vivo, styrene is metabolized to styrene oxide. Dare *et al.* (2002) exposed human neuroblastoma cells (and primary rat cerebellar granule cells) to 0.3-1 mM styrene-7,8-oxide *in vitro*. The treated cells showed apoptotic morphology, chromatin condensation, DNA cleavage into large fragments, and activation of class II caspases (proteases involved in the apoptotic cascade). Pre-incubation with a caspase inhibitor reduced the styrene oxide-induced damage. The data indicate that induced apoptosis is a possible mechanism for styrene-induced nerve damage.

Chronic Toxicity in Humans

Respiratory Effects

Obstructive lung changes were observed in four of 21 workers exposed to styrene for approximately 10 years (Chmielewski *et al*., 1971, cited in ATSDR, 1992). Upper respiratory system irritations were reported among workers exposed to about 140 ppm styrene (Pokrowskij, 1961, as reported in Ohashi *et al.*, 1985).

Cardiovascular Toxicity

Some occupational studies have suggested elevated risks of heart disease mortality among workers exposed to styrene (Matanoski *et al*., 1990; Bond *et al*., 1992; Wong *et al*., 1994), often in those with short employment duration. Matanoski and Tao (2002, 2003) examined the association between styrene exposure and risk of ischemic heart disease (IHD) (ICD-8 codes 410-414) in male workers employed between 1943 and 1982 in two U.S. plants manufacturing styrene-butadiene rubber. The study population (n = 1,424) included workers who had died from IHD (n = 498) along with a 15 percent random sample of a larger cohort. Job histories were tabulated for all subjects. Exposure levels for the jobs were based on measurements when available; for jobs with no exposure measurements, estimates were based on similar jobs in other plants and the measurement distribution parameters of the study plant. Both acute and chronic IHD mortality were examined using Cox proportional hazard regression analyses of time-weighted average (TWA) exposure and cumulative exposure in models stratified by plant and adjusted for race, birth year, and duration of employment, with age as the time scale.

Matanoski and Tao (2002, 2003) found that death from acute IHD among active workers was significantly associated with styrene exposure (TWA) in the most recent two years of employment, with a relative hazard of 3.26 (95 percent CI = 1.09-9.72) for all active workers which increased to 6.60 (95 percent CI = 1.78-24.54) among active workers employed at least five years. Although cumulative exposure to styrene was also significantly associated with acute IHD in active workers, risks were low compared to those associated with exposure intensity (1.04 to 1.08-fold for each increase of one ppm-year). No models for risks of chronic IHD showed a significant association with styrene exposure. Matanoski and Tao calculated that each increase of one ppm in styrene concentration during the most recent two years of employment was associated with a 5.86-fold increased risk of death from acute IHD. Matanoski and Tao acknowledged a need for further research to examine the suggested risk of styrene exposure and acute IHD in relation to incident events, rather than mortality.

Liver Toxicity

Indicators of hepatic dysfunction included increased levels of certain serum enzymes at exposure levels greater than one ppm styrene, relative to controls. Among workers exposed to one to 100 ppm styrene for one to 20 years, exposure-dependent hepatic injury was suggested by a correlation between serum ornithine carbamyltransferase or alanine aminotransferase and air level of styrene. In a study of 493 workers exposed to five to 20 ppm of styrene for up to 20 years, high serum levels of γ-glutamyltransferase were observed after adjustment for alcohol intake (Lorimer *et al*. 1978).

Brodkin *et al*. (2001) investigated whether hepatocellular necrosis, cholestasis, or altered hepatic clearance of bilirubin occurs with low to moderate styrene exposure. Two cross sectional studies compared serum hepatic alanine aminotransferase (ALT) and aspartate aminotransferase (AST), cholestatic alkaline phosphatase (AP) and gamma glutamyl transpeptidase (GGT)), and bilirubin in 47 workers in fiberglass reinforced plastics (styrene exposure = 21.8 ± 3.5 ppm (mean ± SEM) for 3.7 ± 0.7 yr) and in 21 boat and tank fabricators (styrene exposure = 24.1 ± 5.4 ppm for 5.5 ± 1.4 yr). Each group had its own unexposed controls (14 and 26, respectively). Exposure was assessed by personal breathing zone passive dosimetry and venous blood by headspace analysis. Biochemical variables were assessed across strata of exposure to styrene defined as above or below 25 ppm in air, or 0.275 mg/L in blood, with adjustment for age, sex, body mass index, and ethanol consumption. Both studies showed a significant trend for increasing direct bilirubin and direct/total bilirubin ratio with increasing styrene. Pooled regression analyses showed a significant linear association between ALT and AST and styrene exposure; there was an increase in AP of about 10 IU/ml in workers exposed above 25 ppm styrene in air in pooled analyses from both studies. Workers had evidence of diminished hepatic clearance of conjugated bilirubin and associated cholestasis, consistent with mild hepatic injury and metabolic dysfunction.

Kidney Toxicity

Minor kidney effects were noted in two studies on styrene-exposed workers (24-53 ppm) although increased urinary enzyme levels were not observed (Viau *et al.,* 1987; Vyskocil *et al*., 1989). Among styrene-exposed workers, increased levels of urinary lysozyme (muramidase), considered to represent an effect on renal tubules, were reported by Franchini *et al.* (1983), who noted a marginal increase in the overnight urinary retinol binding protein and end-of-shift albumin (corrected for urinary flow) among styrene workers with an estimated mean exposure of 27 ppm and a mean exposure duration of 13 years. The authors suggested that the marginal increase in urinary albumin could reflect a glomerular or tubular effect. The results of a historical cohort mortality study on styrene workers (Welp *et al.,* 1996) suggested increased mortality due to all nonmalignant diseases of the genitourinary system and to nephritis (inflammation) or nephrosis (degeneration of the renal tubular epithelium).

Hematologic Effects

Workers exposed to styrene experienced little or no changes in hematologic parameters. The presence of lowered erythrocyte counts, hemoglobin, platelets and neutrophils and slightly higher corpuscular red cell volumes among workers in a styrene-butadiene rubber manufacturing plant was difficult to interpret because of the mixed chemical exposures (styrene, butadiene, benzene and toluene) (Checkoway and Williams, 1982).

Carcinogenicity in Humans

Direct human evidence on the potential carcinogenic effects of styrene comes from occupational studies; more than 20 such studies are reviewed here in detail. Greatest emphasis is on: 1) lymphohematopoietic cancers (LHCs), the cancers that have most consistently been found to be associated with styrene exposure, 2) other cancer sites with

elevated rates in exposed workers, and 3) lung and other respiratory cancer sites, of interest because of findings in animal studies. Reviews of these studies are organized according to the type of industry and exposure.

Styrene Monomer and Polystyrene Manufacturing

Following recognition in 1974 of the human cancer risk posed by vinyl chloride, attention was directed to other widely used plastics in which monomer exposure to workers could be of significance. Styrene (vinyl benzene) was a focus because of its widespread use and its vinyl side-chain. Manufacture of polystyrene involves potential exposure to styrene monomer, as well as to other chemicals (Nicholson *et al.*. 1978). In some plants, styrene monomer is first produced from ethylbenzene (combined with superheated steam and iron oxide), and this crude styrene is purified to remove unreacted ethylbenzene, benzene, toluene, and xylene. Exposure to benzene in some styrene monomer manufacturing jobs occurred at levels up to 15 ppm TWA. In polystyrene production, purified styrene is reacted with water, catalysts, and various agents to produce polystyrene beads; these may be impregnated with pentane to form an expanded polymer, or extruded and formed into sheets.

Styrene exposure in polystyrene manufacturing varies by task performed, and most studies have had only crude measures of exposure. Exposure of workers involved in maintenance in these plants, potentially occurring at times of mechanical breakdown or leakage of various systems, may have been quite high. In other jobs, exposure may be somewhat lower and less variable. Average levels of styrene in monomer and polymer production facilities rarely exceed 20 ppm (85 mg/m^3); exceptions are exposures due to leakages and other malfunctioning equipment (IARC, 1994a). The highest average levels found in a plant in the U.S. were in polymerization, manufacturing, and purification areas; mean levels ranged from 8 to 35 ppm (IARC, 1994a). Batch polymerization produced concentrations up to 88 ppm.

In the studies described below, some facilities also manufactured styrene-butadiene latex, and workers in these processes were included in the studies (Nicholson *et al.*, 1978; Ott *et al.*, 1980; Hodgson and Jones, 1985). The results of all of the mortality studies from styrene exposure in this industry sector are presented in Table 24.

A mortality study of workers in Germany (n = 1,960), exposed to styrene during manufacture of styrene and polystyrene, was conducted by Frentzl-Beyme *et al.* (1978). Follow-up was relatively complete for those workers who were German (93 percent), and much less for those who were not (29 percent); the small number of deaths (n = 74) limited the power of the study. Information on exposure was not available for individuals. Reported as proportional mortality ratios (PMRs), the results of the study show no elevated mortality from all malignant neoplasms in the cohort of styrene-exposed workers as compared to the general population (PMR = 81). The PMR for mortality from all lymphohematopoietic cancers was 108, based on a single case. There were two deaths from pancreatic cancer (0.7 expected, PMR 286, $p>0.1$).

Table 24. Mortality Studies of Styrene-exposed Workers in Facilities Manufacturing Styrene and Polystyrene

Study Authors and Design	Mortality rates	Data Analysis: Excess Cancer	Comments
Frentzl-Beyme *et al.* 1978 (Germany) Mortality study of 1,960 men. Follow-up from 1931-1976.	**Cause of Death** PMR: Malignant neoplasms 81 All LHCs 108 Pancreatic cancer 286	No analyses reported.	Small number of deaths limited the study power. No exposure information for individuals.
Nicholson *et al.*, 1978 (U.S.) Retrospective cohort of 560 men employed at least five years. Followed from 1940s through 1975.	**Cause of Death** Obs/Exp: Malignant neoplasms 17/ 21.01 Lung cancer 6/ 6.99 Leukemia 1/ 0.79 Lymphoma 1/1.25	**Cancer Mortality** Obs/Exp: *High styrene* Maintenance 10/8.05 Production & Polymerization 4/8.17 *Low styrene* Service & Utilities 3/4.79	Small cohort size and limit of 5 years employment limited study power. When those with < 5 years were included, 5 of 104 deaths were due to leukemia or lymphoma.
Ott *et al.*, 1980 (U.S.) Retrospective cohort of 2,904 men employed at least one year during 1937 to 1970 in Dow Chemical facilities and followed through 1975.	**Cause of Death** SMR: Malignant neoplasms 76 Leukemia 176 LHC other than leukemia 132 **LHC Incidence** Obs/Exp: Lymphatic leukemia 7/1.64[a] Multiple myeloma 4/1.55 Hodgkin's disease 4/2.73	**LHC Incidence** Obs/Exp: *Exposure subgroup 1* Lymph. leukemia 5/0.26[a] *Exposure subgroup 2* Hodgkin's disease 1/0.18	Exposure subgroup 1: extrusion fumes and polymer dusts; Vapors 1 or 3; direct colorant exposure. Exposure subgroup 2: Vapor 1 only. Extrusion fumes and polymer dusts, and Vapors 1 and 3 denote styrene exposure. Bond *et al.* used the same exposure groupings, but naming the primary agents.
Bond *et al.*, 1992 Same cohort as Ott *et al.* (1980), with mortality updated through 1986. Average follow-up: 30.9 years.	**Cause of Death** SMR: Malignant neoplasms 81[a] All LHCs 144 Hodgkin's 222 Non-Hodgkin's 117 Multiple myeloma 184 Leukemia 118	**15+ Years Follow-up** SMR: All LHC 160[a] **Internal Comparisons** RR: Malignant neoplasms 0.87 All LHCs 1.39 Hodgkin's 2.43 Non-Hodgkin's 1.09	Internal comparisons are with Dow employees who were not exposed to styrene but may have had other chemical exposures.

Study Authors and Design	Mortality rates	Data Analysis: Excess Cancer	Comments
		Multiple myeloma 2.45[a] Leukemia 1.18	
Hodgson and Jones, 1985 (England) Retrospective cohort of 622 men exposed to styrene at least 1 year. Employed 1945 to 1974, followed through 1981.	**Cause of Death** SMR: Malignant neoplasms 90 Lymphoma 540[a] **Cancer Incidence** SRR: Lymphoma 375 Total LHC 250 Laryngeal cancer 600 Lymphatic leukemia 500	**Lymphoma Deaths** Age at death Obs/Exp: 15 - 44 years 2/0.27[b] 45 - 54 years 0/0.15 55 - 64 years 1/0.08 >65 years 0/0.03	No specific information on exposure levels was available.

Abbreviations: LHC = lymphohematopoietic cancer; SMR = standardized mortality ratio; Obs = Observed; Exp = Expected; SRR = standardized registration ratio.
[a]$p<0.05$; [b]$p<0.10$

Nicholson *et al.* (1978) studied mortality of 560 workers who had been employed in a plant manufacturing styrene and polystyrene. Exposures included styrene, benzene, and ethylbenzene, among other materials. Individuals were included only if they had been employed at least five years and were male; an additional criterion of living at least ten years since they were first hired was applied to those who had worked at the plant prior to the time of the study. Exposure categories were based on the major work activity within the plant. These were grouped into "high" (5 to 20 ppm) and "low" (<1 ppm) styrene exposure groups, although wide excursions from these ranges occurred at specific locations. Follow-up was complete, and information on those who had died (n = 83) included death certificates, clinical information, radiographs, and other relevant data.

Overall mortality and cancer mortality were lower than expected based on the general population, while cancer mortality was elevated in one of the high exposure groups (maintenance workers, 10 observed, 8.05 expected), but not the other (production and polymerization workers, 4 observed, 8.17 expected), based on small numbers of cases. Analyses by calendar year of employment and time since first employment showed some subgroups with elevated mortality but no clear trends, based on small numbers of cases. Only one death in the cohort was directly attributed to leukemia, and a second member had leukemia at the time of his death. However, when deaths (n = 21) among workers who did not have five years of employment as of May 1, 1960 were included, the total rose to five leukemia or lymphoma deaths out of 104 deaths. Later follow-up identified additional deaths from lymphohematopoietic cancers (LHCs) (12 LHCs in 444 deaths). Nicholson *et al.* (1978) indicated that, while not definitive, the data were suggestive of an excess risk for leukemia and lymphoma deaths, and suggested that the possibility of benzene exposures should be investigated in this cohort.

Ott *et al.* (1980) studied male workers (n = 2,904) employed for one or more years in the period 1937 through 1970 in Dow Chemical facilities. Work histories were obtained for all employees, and vital status was determined for 97 percent. For the 303 workers who had died, cause of death was obtained from death certificates. The largest group (n = 1,325) was involved in polymerization, coloring, and extrusion of styrene-based products; others were involved in research (n = 783), monomer manufacturing and finishing (n = 410), and styrene-butadiene latex production (n = 391). Some employees worked at least one year in two or more areas. Ott *et al.* (1980) categorized exposure based on the multiple agents in the environment, the clustering of these agents in certain work environments, and average intensity. Some had benzene exposure (Vapors 2), while others did not, and mortality results were examined separately among these in Ott *et al.* (1980). Styrene exposure occurred in three different agent groups (Vapors 1, Vapors 3, and Extrusion fumes and polymer dusts); in each of these, co-exposure with ethylbenzene was present.

For the overall cohort, mortality from malignant neoplasms was not elevated (SMR = 76, no CI reported). Elevated mortality was seen for leukemia (SMR = 176, no CI reported) and for lymphohematopoietic cancer other than leukemia (SMR = 132, no CI reported). When mortality was examined based on clusters of exposure groups, no clear pattern was seen, based on relatively small numbers of cases (e.g., for one grouping that had excess mortality overall, there were nine total deaths from all causes).

Ott *et al.* (1980) followed up to examine LHC incidence in addition to mortality, and a total of 21 incident cases were identified, eight more than in the mortality analyses. In the overall cohort, some LHCs were elevated (e.g., multiple myeloma, 4 observed, 1.55 expected; Hodgkin's disease, 4 observed, 2.73 expected); lymphatic leukemia was significantly elevated (7 observed, 1.64 expected; SMR = 427, $p<0.05$). Ott *et al.* (1980) also observed a statistically significant increase in lymphatic leukemia (5 observed, 0.26 expected; $p<0.05$) among a subgroup of workers who had been exposed to styrene, as well as to other polymer extrusion fumes, solvents, and colorants. Five of the seven workers in the cohort who had lymphatic leukemia had worked in the same general area during 1947-1948, and a sixth had worked in that area in 1952. While noting the possibility of a contagion etiology, the authors suggested that "[i]f this adverse effect were environmentally induced, the causative agent may well be a single entity present during the mid to late 1940s rather than a combination of agents."

Bond *et al.* (1992) reanalyzed the mortality of the cohort with an additional 11 years of follow-up. Individuals with research jobs were analyzed separately because they were considered not to have classifiable exposure. The number of deaths available for analysis increased from 320 to 687, with less than 1 percent of workers lost to follow-up. Only mortality (and not incidence) of LHCs was analyzed. Comparisons were made with the U.S. white male population and with Michigan-based Dow employees from a mix of occupations, including some who had had occupational exposures to other potentially hazardous materials.

In external comparisons, mortality in the cohort from all malignant neoplasms was statistically significantly lower than expected (SMR = 81, 95 percent CI = 69-95). In contrast, mortality was elevated from all LHCs (SMR = 144, CI = 95-208) and from each specific LHC (Hodgkin's: SMR = 222, CI = 71-518; Non-Hodgkin's: SMR = 117, CI =

47-240; multiple myeloma: SMR = 184, CI = 74-380; leukemia and aleukemia: SMR = 118, CI = 54-224), but the increase was not statistically significant for any of these endpoints. Significantly increased rates of LHC mortality were seen among workers with at least 15 years of follow-up (SMR = 160, CI = 102-238). In internal comparisons with the other Dow employees, the cohort had a decreased risk of malignant neoplasms, but the difference was not significant (RR = 0.87; CI = 0.74-1.03). Risk of LHCs were elevated in comparison to the other Dow workers, based on relatively small numbers of deaths (all LHCs, 28 deaths), and was statistically significant for multiple myeloma (all LHCs: RR = 1.39, CI = 0.92-2.08; Hodgkin's: RR = 2.43, CI = 0.94-6.28; Non-Hodgkin's lymphoma: RR = 1.09, CI = 0.48-2.49; multiple myeloma: RR = 2.45, CI = 1.07-5.65; and leukemia: RR = 1.18, CI = 0.58-2.39).

Hodgson and Jones (1985) studied a cohort of 622 men who had worked at a single chemical manufacturing site where styrene monomer was produced, polymerized, and processed during the period 1945 through 1974. These men were identified from among all workers as those who had been exposed to styrene for at least one year, based on the job they held. A group of manual workers at the plant who had variable chemical exposure but no specific occupational exposure to styrene (n = 3,072) was treated as a reference group. Follow-up was complete, except for a small proportion of the cohort (2 percent) who had emigrated, with date of death information for all those who died up to the end of 1978, and for cancer registrations through the end of 1981.

Deaths from all malignant neoplasms were less than expected in the cohort of Hodgson and Jones (SMR = 90); the same ratio was seen in the unexposed reference group of manual workers. Lymphoma mortality was significantly elevated in the cohort (SMR = 540, one-tailed p = 0.02; based on 3 cases). In contrast, in the unexposed population, lymphoma mortality was less than expected, occurring in the same ratio as all malignant neoplasms (SMR = 90). A case of lymphatic leukemia also occurred in the cohort, which was not listed as the cause of death. Thus four incident cases of LHC were observed (1.6 expected based on local registration rates; SRR = 250, 95 percent CI = 67-640).

Although no specific information on exposure was available, the authors note that styrene exposure would have been "well below the hygienic standard of 100 ppm (420 mg/m^3) with isolated excursions to higher levels" associated with certain tasks. Length of service of the four LHC cases in the cohort was examined in relation to individually matched referents and no apparent association was found between length of service in styrene-exposed jobs and LHC incidence. Hodgson and Jones (1985) noted that length of service is "probably a rather poor indicator of individual [styrene] exposure." Each of the four LHC cases had other potential exposures of concern, such as styrene-butadiene rubber production, acrylamide, and benzene. All four cases were engaged in jobs involving styrene contact during one specific time period (November 1960 to March 1961).

Hodgson and Jones (1985) reported that laryngeal cancer occurred in unexpected numbers (3 observed, 0.5 expected, SRR = 600), especially for the youngest age group (<45 years, 2 observed, 0.1 expected; SRR = 2000). This was based on cancer registrations and not mortality for this site. Based on small numbers, elevated mortality was observed for cancer of trachea, bronchus, and lung (5 observed, 4.2 expected; SMR = 119), although this was not statistically significant.

In summary, four studies, two from the U.S. and one each from Germany and England, of workers exposed to styrene in the manufacture of styrene monomer and polystyrene were available (Frentzl-Beyme *et al.*, 1978; Nicholson *et al.*, 1978; Ott *et al.*, 1980; Hodgson and Jones, 1985) (Table 24). Workers in all four studies had styrene exposure combined with exposure to multiple other chemicals, such as benzene, ethylbenzene, or 1,3-butadiene, as well as other potentially carcinogenic agents. One study (Ott *et al.*, 1980; Bond *et al.*, 1992) examined LHC incidence in subgroups of workers with similar exposure histories in an attempt to reduce the variability of exposures and to look for cancer mortality patterns in the cohort. Overall, conclusions were limited by the mixed nature of the exposures and the relatively small numbers of cases.

Although all four studies found suggestions of excess LHC mortality, only the later U.S. study (Ott *et al.*, 1980; Bond *et al.*, 1992) and the study in England (Hodgson and Jones, 1985) reported statistically significant findings. The U.S. study provided the most robust dataset for the examination of LHC incidence in workers engaged in manufacture of styrene and polystyrene, with a cohort of almost three thousand men (687 deaths) followed up to 50 years. Ott *et al.* (1980) separately examined subsets of the cohort with respect to exposure to specific chemicals, devising a scheme for identifying groups of workers based on exposure patterns. In the follow-up study, Bond *et al.* (1992) not only included twice as many deaths for analysis, but also provided detailed mortality comparisons with an internal group of employees. The internal comparisons show a similar pattern to that seen in the external comparisons: overall cancer mortality was lower than expected, but LHC mortality was consistently higher, although most results were not statistically significant.

Based on small numbers of cases, Hodgson and Jones (1985) found that lymphoma mortality was significantly increased, and incidence of laryngeal cancer was elevated, with the elevated mortality for both endpoints concentrated in those who died before the age of 45. Length of service (as a surrogate for styrene exposure) of cases compared with matched referents from the cohort did not indicate an increased risk with increased service, and other exposures experienced by the cases studied by Hodgson and Jones (1985) suggested possible alternative explanations for the cancers seen.

An interesting aspect of the results of two studies (Hodgson and Jones, 1985, which had early deaths from lymphoma; and Ott *et al.*, 1980, which had five cases of lymphatic leukemia), was that the excess mortality occurred in men who had worked during one specific time period in the plant under study. This suggests that particular conditions present at that time in that place may have increased the risk of cancer induction.

Fabrication of Glass-Reinforced Plastics

Styrene is the essential co-reactant and solvent in unsaturated polyesters used in reinforced plastic fabrications (IARC, 1994a). This is the most extensive occupational setting in which styrene exposure occurs with respect to numbers of workers and exposure levels, involving fabrication of objects from glass-reinforced polyester composite plastics such as boats, corrosion-resistant tanks and pipes, bath and shower units, and automotive parts (IARC, 1994a). Based on recent Toxics Release Inventory (TRI) information (Scorecard, 2000), 140 U.S. facilities were engaged in production of glass-reinforced plastic products using styrene as of 1997, of which 19 were located in

California. The five glass-reinforced plastics manufacturing facilities in California with the largest releases emitted 846,280 pounds of styrene into air in 1997 (Scorecard, 2000).

Styrene serves as a solvent and a reactant for the unsaturated polyester resin, in which it is about 40 percent by weight. Both open and closed mold processes are used. In an open mold process, a releasing agent is usually applied to the mold, a first coat containing pigments (gel coat) is applied, then successive layers of chopped and/or woven fiberglass are deposited manually or with a chopper gun at the same time as the resin is sprayed or brushed on, and then the surface is rolled. During lamination and curing, about 10 percent of the styrene may evaporate into workplace air (IARC, 1994a). Exposure to styrene has been extensively documented. Air concentrations in personal monitoring zone measurements vary in different countries and during different time periods, with highs of >4000 mg/m^3, and average values of 100-200 mg/m^3 or more.

Several factors influence the level of styrene in the air. The manufacture of objects with large surface areas (such as boats, truck parts, baths, and showers) by the open–mold process results in the highest exposure. Data from 28 plants in the U.S. showed that the average exposure to styrene in open-mold processes was two to three times higher than that in press-mold processes: 102-350 mg/m^3 (24-82 ppm) versus 47-111 mg/m^3 (11-26 ppm). In a survey of 12 plants making fiberglass in Washington State, 40 percent of the 8-hour samples contained more than 426 mg/m^3 (100 ppm) styrene. Chopper gun operators had the highest exposure, followed by laminators and gel-coat operators; boat-building involved higher exposures than any other sector.

Exposures have decreased gradually over the decades since 1970, according to measurements summarized by Kogevinas *et al.* (1994) as part of a study of reinforced plastics workers in several European countries. Because appropriate respiratory protective equipment can significantly decrease (although not eliminate) styrene exposure, measurement of biological indicators of exposure complements air sampling. Unfortunately, the only available epidemiologic study of cancer incidence or mortality in the reinforced plastics industry, which used biological indicators for assessing styrene exposure, was limited by small numbers of workers (Anttila *et al.*, 1998).

Other substances may be found in workplace air in plants producing reinforced plastics. NIOSH (1983) reported that measurable quantities of a number of substances, in addition to styrene, were found in air samples at worksites producing reinforced plastics, including acetone, methylene chloride, fibrous glass, toluene, methyl ethyl ketone, methyl isobutyl ketone, silica, xylene, amines, naphtha, benzene, ethyl benzene, perchloroethylene, and styrene oxide, among others. In some of these workplaces (e.g., Coggon *et al.*, 1987), asbestos exposure also occurred. Acetone is the major concurrent exposure, according to IARC (1994a), who also noted that benzene was not reported in any of the air samples (n = 2528) in a fairly recent (1990) study in the Danish work environment.

Okun *et al.* (1985) examined cause of death patterns among 5,201 workers, including 682 women, employed between January 1959 and September 1978 at two reinforced plastic boat-building facilities in Washington State. These NIOSH investigators conducted in-depth industrial hygiene surveys to classify jobs and departments, and identified 2,060 workers as having high styrene exposure. Exposure measurements showed a difference in the high styrene exposure levels (company A, mean = 42.5 ppm, range 12.0 to 84.7

ppm; company B, mean = 71.7 ppm, range 10.4 to 183.0 ppm). The majority (75 percent) of the workers employed in high styrene exposure jobs were employed for ≤1 year. The person-years at risk were mostly in the later calendar years of the study, as the bulk of the workforce had been first employed during the late 1960s and early 1970s. Only 37 percent of the high exposure group was observed for greater than ten years from the date of first employment, and less than one percent of individuals more than 20 years, which limits the power of this study to detect a carcinogenic effect of styrene.

Among the high styrene exposure group, 47 deaths were observed; all were white males. No elevated mortality for malignant neoplasms was seen in this group (SMR=86), nor were any cases of lymphohematopoietic cancers seen in the entire cohort (total deaths = 176). Additional follow-up of the cohort revealed one lymphoma death (in 1981), in an individual employed for 10 years in the minimal styrene exposure group. As conducted (Okun *et al.*, 1985), the power of the study to detect a significant excess at a relative risk of 2.0 was 14 percent for leukemia and 15 percent for lymphoma.

Ruder *et al*. (2004) updated mortality for the above styrene-exposed boat builders through 1998. SMRs and 95 percent CIs used Washington State and U.S. death rates. Overall, 860 deaths occurred (SMR = 1.09, CI 1.02-1.17). Excess mortality was recorded for esophageal cancer (12 observed, 5.2 expected; SMR = 2.30, CI 1.19-4.02) and prostate cancer (24 observed, 14.0 expected; SMR = 1.71, CI 1.09-2.54). Urinary tract cancer (6 observed, SMR 3.44, CI 1.26-7.50) was significantly elevated among 2,062 highly exposed workers. Urinary tract cancer SMR increased with duration of employment. No excess leukemia or lymphoma mortality was found. The authors believe that the excess urinary tract cancer mortality could be due to chance.

In a study of 7,949 workers in eight glass-reinforced plastics facilities in Great Britain, Coggon *et al.* (1987) examined mortality in relation to styrene exposure. Four exposure categories were described which all included some styrene exposure (background, low, moderate, high). Follow-up varied for the different plants, half of which had operated for less than 20 years. Results for the single facility that made boats were analyzed separately, because the personnel records for this company were incomplete and thus a lower proportion (62 percent) was available for the analyses. Workers at two facilities had asbestos co-exposure. Although a reasonably large proportion of the cohort had held high exposure jobs (such as hand lamination) at some time (n = 3,494), the power of the study to detect an effect among these workers was limited; the authors note that after allowing for a latency of 20 years from first exposure, only five cancer deaths would have been expected among hand laminators exposed at least one year.

Coggon *et al.* (1987) report an elevated risk of lung cancer among workers in these plants. Lung cancer risks increase in relation to exposure to some extent (Table 25), though none were statistically significant. Excess lung cancer risk was apparent at facilities with no concomitant asbestos exposure. Overall mortality of the cohort from respiratory disease was well below that of the national population (SMR = 70, 95 percent CI = 53-90), which might indicate lower smoking rates in the cohort, or may be due to the miscalculation of person-years at risk, discussed below. When expected mortality rates were corrected to the local areas, the total number of expected lung cancer deaths was, according to the authors, “virtually unchanged.” Lung cancer rates were not increased in relation to time since first exposure and were highest in those members of

the cohort first exposed after 1969 (Table 25), the opposite of that expected based on styrene levels, which were higher before 1970.

Fewer lymphohematopoietic cancers (LHCs) were observed than expected based on national rates (for all LHCs, six observed, 14.9 expected). An additional eight such cancers were registered among members of the cohort still alive at the end of follow-up or who had died from other causes. Mortality may be a poor choice of endpoint for the study of these cancers, since LHCs are often not the immediate cause of death and thus may be missed in mortality studies. The one subject with high styrene exposure among the LHC deaths was a hand laminator from the boat-making facility, who died of non-Hodgkin's lymphoma (SMR = 311). No information was provided on the eight individuals with LHC who were not included in the mortality analyses. Rates of specific LHCs were not elevated in the cohort as a whole, and the number of cases was small; thus, subgroup analyses provide little indication of any pattern of risk.

Of the total cohort, 1311 were women. Although not statistically significant, mortality rate ratios were slightly elevated for cancers of the female reproductive organs, and, based on a small number of cases, appeared to be greater for those with high exposure (SMR = 196, cervix; SMR = 282, ovary) compared to those with background exposure (SMR = 77, cervix; SMR = 105, ovary); no cases were seen in the other exposure groups.

Table 25. Mortality Studies of Styrene-exposed Reinforced Plastics Workers

Study Authors and Design	Mortality Rates[1] (95 percent CI)	Data Analysis: Excess Cancer	Comments
Okun *et al.*, 1985 (U.S.) Retrospective cohort of 5,201 workers employed from 1959 to 1978 at 2 facilities.	**Entire Cohort** SMR: All causes 90 Malignant neoplasms 103 All LHC -- Male repro cancer 224 Female repro cancer 268 **High Styrene** SMR: All causes 113 Malignant neoplasms 67 All LHC --	None.	Workers with high styrene exposure identified based on industrial hygiene measurements. Authors note that due to short length of observation and young age of most cohort members, study had little power to detect mortality excess.
Ruder *et al.*, 2004 (U.S.) Update of Okun *et al.*, 1985	**Entire Cohort** SMR : All causes 109 Esophageal ca. 230 Prostate ca. 171 **High Styrene** SMR : Resp. Disease 254 Urinary tract ca. 344	No excess leukemia or lymphoma mortality. Esophageal and prostate cancer were significantly elevated in the entire cohort. In the High Styrene group urinary tract cancer was elevated (6 obs; 1.7 exp) and increased with duration of employment.	

Study Authors and Design	Mortality Rates[1] (95 percent CI)	Data Analysis: Excess Cancer	Comments
Coggon *et al.*, 1987 (Great Britain) Retrospective cohort of 7,949 men and women workers at eight facilities from 1947 to 1984.	**Entire Cohort** SMR: All causes 83[a] (77-89) All neoplasms 80[a] (69-93) Respiratory disease 70[a] (53-90) Lung ca. 112 (89-139) Cervical ca. 107 (13-386) Ovarian ca. 149 (41-381)	**Lung Cancer** Exposure level SMR: Background 100 Low 104 Moderate 150 High 120 Length of exp SMR: <1 year 96 1-9 years 154 ≥10 years 89 Time since 1st exp SMR: ≤9 years 125 10-19 years 131 ≥20 years 113 Period of 1st exp SMR: <1960 114 1960-69 88 >1969 213 **Female Repro Cancer** Cervical cancer SMR: Background exp. 77 High exposure 196 Ovarian cancer SMR: Background exp. 105 High exposure 282	Lung cancer included cancer of the lung, pleura, and mediastinum (codes 162-164 of ICD, 9th revision). The study had limited power to detect an effect on long latency cancers: only 17 deaths from all neoplasms occurred in those whose first exposure began ≥20 years before end of study. 'High' exposure category was estimated as 8-hour TWA of 40-100 ppm. Reported rates exclude workers from one facility that had substantially different follow-up.

Study Authors and Design	Mortality Rates[1] (95 percent CI)	Data Analysis: Excess Cancer	Comments
Kolstad *et al.*, 1994 (Denmark) Retrospective cohort study of 36,525 men working at 386 companies during the early 1960s to 1988. Kolstad *et al.*, 1995 (Denmark) 36,610 men working in reinforced plastics at 386 companies and 14,293 workers from similar industries without styrene exposure from 1970 to 1990.	**Entire Cohort** SIRs: All cancers 102 (97-107) NonHodgkin's 133 (96-180) Leukemia 122 (88-165) Hodgkin's 108 (62-176) **Exposure Potential** *1-49% styrene* SIR: Non-Hodgkin's 165 (115-228)[a] Leukemia 115 (77-167) Hodgkin's 92 (42-174) All LHC 124 (99-154) *50-100% styrene* SIR: Non-Hodgkin's 62 (23-135) Leukemia 138 (75-232) Hodgkin's 141 (57-291) All LHC 109 (74-155) *Unclassified* SIR: All LHC 171 (89-299) SIRs: All solid cancer 99 Salivary gland 174 Pancreas 120 Nasal cavities 184 Pleura 178 Ext. male genital 160 Other endocrine 166	Yrs since 1st employ SIR: *Non-Hodgkin's* <10 168[a] ≥10 112 *Leukemia* <10 71 ≥10 157[a] *Hodgkin's* <10 121 ≥10 87 *Mult myeloma* <10 141 ≥10 76 *All LHCs* <10 119 ≥10 120 First year of employ SIR: *Leukemia* 1964-1970 154[a] 1971-1975 100 1976-1988 51 *Non-Hodgkin's* 1964-1970 128 1971-1975 119 1976-1988 164 *All LHC* 1964-1970 132[a] 1971-1975 112 1976-1988 97 Regression analysis IRR: *Pancreatic cancer* All RFP 1.4 Low styrene 1.1 High styrene 2.2[a] 1st empl >1970 1.4 1st empl ≤1970 2.0 <1 year empl 2.5 ≥1 year empl 1.8 High <1 yr empl 3.1 High ≥1 yr empl 3.4[a] *Urinary bladder* All RFP 1.2 ≥1 yr empl/low exp 1.6 ≥1 yr empl/high exp 2.1[a]	Incidence rather than mortality was examined in the study of LHCs. Exposure assignment was on a company-wide basis. Monitoring data were available for a subset of the companies.
Kogevinas *et al.*, 1994 [European (composite)] Retrospective cohort of 34,560 men and 6,128 women. Follow-up ranged generally from late 1940s or mid-1950s	**Entire Cohort** SMR: All causes 92 (88-95)[a] All neoplasms 87 (81-94)[a] Circulatory 92 (87-97)[a] **Laminators** SMR: All causes 88 (81-96)[a] All neoplasms	**Internal Comparisons** *≥20 years since 1st expos.* RR: Esophag. 5.82 (1.0-33.9) Pancreas 2.05 (0.58-7.29) *LHCs* Time since 1st exp RR: <10 yrs 1.0 10-19 yr 2.90 (1.29-6.48)[a] ≥20 yrs 3.97	Mortality rates calculated based on rates in each country. Job information used to identify exposure categories was only available for individuals in some countries. The category called "laminators" (n =

Study Authors and Design	Mortality Rates[1] (95 percent CI)	Data Analysis: Excess Cancer	Comments
through late 1980s or early 1990s. *Denmark*:15,867 [39%] *Finland*: 2,087 [5%] *Italy, Lig.*: 1,438 [4%] *Italy, E.R.*: 5,818 [14%] *Norway*: 2,035 [5%] *Sweden*: 3,667 [8%] *UK-1*: 7,971 [20%] *UK-2*: 1,807 [4%]	91 (78-106) Esophagus 181 (87-334) Small int 227 (6-1266) Pancreas 148 (76-258) Cervix uteri 112 (3-626) Ovary 261 (71-669) Thyroid 227 (6-1266) All LHC 81 (43-139) Non-Hodgkin's 140 (56-288) Hodgkin's 133 (27-388) **Unspecified Tasks** SMR: All causes 106 (100-112) All neoplasms 99 (88-112) Pancreas 117 (68-188) Accident/Violence 128 (113-145)[a] All LHCs 119 (80-170) Hodgkin's 107 (22-312) Multiple myeloma 193 (78-398) Leukemia 140 (79-228) Myl. leuk 142 (65-269)	(1.30-12.1)[a] (linear trend p = 0.012[a]) *LHCs – avg. exposure* ppm RR: <60 1.0 60-99 1.68 (0.59-4.79) 100-119 3.11 (1.07-9.06)[a] 120-199 3.08 (1.04-9.08)[a] ≥200 3.59 (0.98-13.1) (linear trend p = 0.019[a]) *LHCs – cum. Exp* ppm-yrs RR: <75 1.0 75-199 0.98 (0.43-2.26) 200-499 1.24 (0.57-2.72) ≥500 0.84 (0.35-2.20) **Excluding Denmark** *LHCs – cumulative exp* ppm-yrs RR: <75 1.0 75-199 1.7 200-499 2.2 ≥500 1.5	10,629), assumed to have the highest exposure, did not include any of the workers exposed in Denmark or Finland, as well as some in Sweden. These workers, or "workers with unspecified tasks" (n = 17,952), were more than 50% of the cohort, and included laminators in these countries, and others who may have had some high exposures. Short-term workers (<2 years exposed) made up 81% of the Danish cohort. Exclusion of the Danish workers led to a finding of increased risk with cumulative exposure not seen in the analysis of the full cohort.
Anttila *et al.*, 1998 (Finland) Retrospective cohort of 2,580 men and women.	**Cancer Incidence** SIR: All sites 80 Rectum 311[a] Pancreas 166 Nervous system 161 Hodgkin's lymph 189	**Cancers 10+ years Since First Measure** SIR: Rectum 349 Pancreas 364 Nervous system 311	Data on a styrene metabolite, urinary mandelic acid, was collected following styrene exposure of cohort members during 1973 to 1983

Abbreviations: CI = confidence interval; LHC = lymphohematopoietic cancer; ppm = parts per million; RR = risk ratio; SMR = standardized mortality ratio; SIR = standardized incidence ratio; exp = exposure; TWA = time-weighted average.
[1] SMRs, SIRs, etc., are not statistically significant unless marked as such.
[a] $p<0.05$

The report failed to provide details on how person-years at risk were calculated. The finding that observed numbers of deaths were 20 percent less than expected for all of the mortality categories raises concerns that the investigators somehow miscalculated the expected numbers. An additional problem was the use of the 9th revision of the ICD for coding deaths in cohort members, which occurred during times when revisions 5 through

9 of the ICD were used to code the comparison (national) deaths. Also the "all cancer" analysis included ICD codes 140-239, but within that range 210-239 are not malignant neoplasms.

Analyses of workers from 30 reinforced plastics plants in the U.S. have been reported (Wong, 1990; Wong *et al.*, 1994). Loss to follow-up was considerable in the earlier analysis (16.1 percent) and less problematic in the later one (3.5 percent). Extended mortality follow-up in the later study (through 1989) gave it more power than the earlier study, but both studies relied on exposure information collected for the original study, including employment history data (through 1977). In our summary of the first paper, we describe the overall methods used; for the summary of the second, only differences are included. Although both looked at mortality, the two studies conducted different analyses with respect to styrene exposure.

Wong (1990) used company and social security records to identify deaths through 1977 in a cohort of 15,908 individuals, men (75.6 percent) and women (24.4 percent), who had worked at least six months from 1948 to 1977. The mortality of the cohort was compared with that of whites in the general U.S. population. Exposure to styrene was estimated for individuals based on an industrial hygiene survey conducted for the study; both average and peak time-weighted averages (TWA) were developed based on a set of consolidated job titles. Almost half of the cohort (46 percent) had worked for less than two years at the participating plants, and 31.9 percent had worked two to five years. At the end of the observation period (1977), more than 80 percent of the cohort was living, and only 3.1 percent (499) were deceased. Person-years for the subjects lost to follow-up were counted up to the date of the last contact (e.g., date employment terminated).

The notable finding from the Wong (1990) study was an excess of laryngeal cancer (all males, SMR = 360, $p>0.05$), that was greater for those with a longer time since first exposure to styrene (20-29 years, SMR = 732, $p>0.05$) and was significantly increased for those with higher average exposure (>12 ppm, SMR = 941, $p<0.01$). Slight elevations were seen for Hodgkin's disease, based on three deaths that occurred in individuals whose average styrene exposure was >12 ppm. Leukemia mortality ratios differed by estimated styrene exposure (<12 ppm, SMR = 33.8; >12 ppm, SMR = 259.6), but were not statistically significant. These analyses were not repeated in the Wong *et al.* (1994) study.

A detailed review of work history, undertaken for a nested case-control analysis, brought out one of the problems with Wong (1990). More than half of the workers were "not exposed" directly to styrene (25 of 40 cases and 58 of 102 controls). Wong (1990) found no association of respiratory cancer with styrene exposure, but did report an association with smoking (RR = 7.33, $p = 0.04$). The work history review indicates that exposure misclassification occurred in the other analyses, in which all subjects were grouped as having either >12 ppm or <12 ppm exposure to styrene; a substantial proportion was actually unexposed. Other problems with this study include the high proportion of workers lost to follow-up and the insufficient follow-up period (the average was 7.7 years, according to Kogevinas and Boffetta, 1991) for the evaluation of cancer risks. These combine to make Wong (1990) a poor quality study.

The second study (Wong *et al.*, 1994) extended the mortality follow-up to the end of 1989, and reduced the percent of the cohort with unknown vital status. Wong *et al.* (1994) considered styrene exposure based not on styrene level as done earlier, but on exposure duration (the number of years from 1948-1977 during which exposure occurred to each worker), and on cumulative exposure (calculated by multiplying duration of exposure by the estimated TWA exposure level). These values were based on the same data used in the Wong (1990) analyses, that is, no updated exposure information was collected. Thus, duration was based on employment only through 1977, and exposure of cohort members who were still working during part or all of 1977-1989 was not included.

In the overall cohort, mortality from all cancers was significantly increased (SMR = 115.5, 95 percent CI = 104.8-127.1), as was mortality from respiratory cancer (SMR = 139.3, CI = 119.0-162.0), and cancer of the bronchus, trachea, and lung (SMR = 140.6, CI = 119.8-164.0). Laryngeal cancer mortality was not elevated. Esophageal cancer was significantly increased (SMR = 191.7, CI = 104.8-321.7). For cancer of the reproductive organs, cervical cancer was significantly increased (SMR = 283.5, CI = 135.9-521.3), as was "cancer of other female genital organs [besides the uterus]" (SMR = 201.6, CI = 107.4-344.8). Kidney cancer was elevated but did not reach statistical significance (SMR = 175.2, CI = 98.1-289.0). Rates of LHC mortality were not elevated (SMRs<100).

When mortality was examined based on "latency," the time since first exposure to styrene, increasing mortality with increasing latency was observed for all cancers and for cancer of the bronchus, trachea, and lung, with statistically significant increased rates for the longest time periods (≥20 years). Rate ratios of specific LHCs were elevated in different ways in relation to latency, with elevations at the longest latency for lymphosarcoma (≥20 years, SMR = 162.6) and "cancer of all other lymphopoietic tissues" (≥20 years, SMR = 150.4), and elevations at the shortest latency for Hodgkin's disease (<10 years, SMR = 128.5) and leukemia (<10 years, SMR = 110.9). All were based on small numbers, and none was statistically significant.

Examination of mortality based on duration of exposure did not indicate an increasing cancer risk with increasing duration or with cumulative exposure; for many cancer sites, the subset of the cohort with less than one year of styrene exposure had the highest rates. The great majority of deaths (69 percent) occurred among those with less than five years of exposure, and analyses of the groups with longer duration were based on very small numbers of cases. As noted by IARC (2002), Wong *et al.*'s use of both cumulative exposure and exposure duration, two correlated exposure indices, in the regression models may have artificially reduced the coefficients of both.

Kolstad *et al.* (1994) examined lymphohematopoietic malignancies in a cohort of all reinforced plastics workers in Denmark: 36,525 male employees of 386 companies in operation beginning in the early 1960s (whenever the work began at each company) and extending through 1988. Exposure status was assigned based on company; specific exposure information was not available for individuals. Based on two raw materials dealers' classification of an initial list of companies, those that had more than 50 percent of their workforce employed in reinforced plastics manufacture (n = 12,837) were considered separately from those with less than 50 percent doing this work. Estimates of employment duration were based on the amount paid for each individual each year into the pension fund by the employer, rather than by date of first and last employment.

These dates did not correlate well with information on employment duration obtained in a questionnaire sample of 671 employees of eight companies; in this sample, 40 percent of workers classified as short-term on the basis of pension fund payments were classified as long-term on the basis of the questionnaire. Monitoring data were also available for a subset of the companies for portions of the study period, allowing changes in styrene levels in air over time to be estimated.

Kolstad *et al.* (1994) reported standardized incidence ratios (SIRs) based on comparison to national rates of lymphohematopoietic cancers (LHC). Use of incidence strengthens the study; long survival times after LHC diagnosis can lead studies based on mortality to miss cases. The authors used several approaches to assess exposure-disease relationships, in each case stratifying by time since first employment. These approaches include considering: 1) the two groupings of companies based on the percentage (<50 percent, ≥ 50 percent) of workforce producing reinforced plastics; 2) the time period of first employment; 3) employment duration; and 4) measured styrene levels.

When LHC incidence was considered by company groups, a statistically significant increased SIR was reported among employees of companies with <50 percent reinforced plastics workers for non-Hodgkin's lymphoma (SIR = 165, 95 percent CI = 115-228). Higher rates were seen among those with <10 years since first employment (SIR = 235, CI = 142-367), and these were also statistically significant. Among this same set of companies, leukemia rates were elevated but not significantly for workers with ≥10 years since first employment. For the set of companies identified as having had 50-100 percent of their workforce producing reinforced plastics, SIRs were elevated for Hodgkin's disease and leukemia, but this did not approach statistical significance. Considering the cohort as a whole, rates of non-Hodgkin's lymphoma and leukemia appeared somewhat elevated but were not statistically significant.

Since styrene exposures were considerably higher in the 1960s, Kolstad *et al.* (1994) examined the LHC incidence with respect to the year of first employment. Among members first exposed during 1964-1970, the rates were statistically significant for all LHCs (SIR = 132, CI = 102-167) and for leukemia (SIR = 154, CI = 104-219). Among those first employed during these early years, statistically significant increased rates were observed for leukemia among those followed ≥10 years after first employment (SIR = 169, CI = 109-249) but not in the first ten years after initial employment (SIR = 106, CI = 35-248). For those first employed during a middle period (1971-1975) or the latest period (1976-1988), non-Hodgkin's lymphoma rates were elevated overall or for subgroups based on time since first employment, but none were statistically significant.

Despite inaccuracy in the estimates of employment duration as identified in the questionnaire sample, Kolstad *et al.* (1994) examined a subset of the cohort (those first exposed after 1970) by length of employment. Rates for overall LHCs were the same regardless of length of employment for the first ten years after initial employment. For those followed ≥10 years after first employment, the overall LHC rate (SIR = 165, CI = 118-226) and the leukemia rate (SIR = 243, CI = 143-361) were significantly elevated only for those employed <1 year. Among those employed longer, neither the overall LHC nor the leukemia rate was elevated.

In the subpopulation for which styrene measurements were available, employees of companies with a mean styrene level above 50 ppm had an elevated rate of Hodgkin's disease (SIR = 248, CI = 80-578), which was greater than the rate of those working in companies with a lower average styrene (SIR = 137, CI = 17-495), but the confidence intervals were wide and overlapped. The SIR values for other LHCs were not increased in this subpopulation and did not correlate with styrene level.

In a second report on this cohort, Kolstad *et al.* (1995) considered incidence of solid tumors. Incidence of all solid cancers was similar to the general population of Denmark (SIR = 99, 95 percent CI = 93-105). Kolstad *et al.* (1995) used Poisson regression models to assess the non-LHC cancer incidence in the cohort, compared with men employed in similar industries that do not have styrene exposure (calculating incidence rate ratios, IRRs). The models included variables for exposure probability, age, year of first employment, duration of employment, and time since first employment. Because of concern about confounding by social class in mortality studies of this cohort of mainly unskilled workers, Kolstad *et al.* (1995) considered these regression analyses to be more valid than comparisons with the general population. Analyses that compare workers with workers are generally more valid due to their intrinsic adjustment for bias from the healthy hire effect, a component of the healthy worker effect.

Incidence of pancreatic cancer was slightly elevated in reinforced plastics workers overall (IRR = 1.4, 95 percent CI = 0.8-2.6), and was statistically significantly increased in those who had high exposure probability (IRR = 2.2, CI =1.1-4.5). Urinary bladder cancer was elevated among reinforced plastics workers employed more than one year (IRR = 1.7, CI = 0.9-3.0), with some indication of a dose effect based on exposure probability (low, IRR = 1.6, CI = 0.9-2.8; high, IRR = 2.1, CI = 1.1-4.1). Rates of lung cancer were somewhat elevated in reinforced plastics workers, but varied little based on exposure probability. Based on year of first employment, increased lung cancer risks were seen in those employed in the higher exposure period (before 1970) (≤1970, IRR = 1.6, CI = 0.9-2.5), but did not reach statistical significance.

Kolstad *et al.* (1995) noted the well-documented high styrene exposure levels that occurred in the reinforced plastics industry during the 1960s, and suggested that calendar year may be the most valid indicator of exposure. Lynge *et al.* (1997) noted that average styrene exposure measured among persons employed in reinforced plastics production during 1964-1970 was 767 mg/m^3 (180 ppm). The lack of information on individual exposure for this cohort is an important limitation, although Kolstad *et al.* (1995) refer to styrene's high volatility and the small companies (≤5 workers) dominating the industry in arguing that employees had few opportunities to avoid styrene exposure.

Reinforced plastics industry workers (n = 40,688) from six countries [Denmark, Finland, Italy, Norway, Sweden, United Kingdom (UK)] were included in the study by Kogevinas *et al.* (1994). It included 34,560 men and 6,128 women ever employed in this industry (Table 24). The percentage of workers employed less than two years varied from a low of 9 percent in Finland to a high of 81 percent in Denmark. Overall, approximately 47 percent of the non-Danish portion of the cohort were short-term workers. Close to half of the cohort was first employed after 1975. Danish workers included in the Kogevinas *et al.* (1994) cohort were the subset of the Kolstad *et al.* (1994) cohort who worked in companies considered to have had at least half of their workers engaged in reinforced

plastics manufacture. The cohort reported by Coggon *et al.* (1987) was also included in the Kogevinas *et al.* (1994) cohort, with a few additional years of follow-up (through 1990 rather than 1984).

Exposure characterization of individuals varied by country. In some countries, exposure status was based on job titles and monitoring data, including both environmental measurements and urine samples (collected in the late 1980s). However, for a substantial proportion of the cohort, individual job titles were not available or workers had unspecified tasks. Nevertheless, Kogevinas *et al.* (1994) distinguished five groups of workers, and indicated that these groupings reflected workers' exposure potential. Of these, the laminators (n = 10,629) were assumed to have had the highest exposure potential. However, all the subjects in the Danish and Finnish subsets of the cohort, as well as a significant portion of the Swedish subset, were grouped together in a category as "workers with unspecified tasks" (n = 17,952). Kogevinas *et al.* indicate that, on average, these workers had lower exposures than laminators, but that some may have had very high exposures (i.e., they were laminators and others working in small companies).

Measured exposure information for early time periods (before 1970) was available only for Denmark, which indicated that average styrene exposure was higher before 1970 than after. Recorded average exposure in 1965 (in Denmark) was 205 ppm, compared to average exposures in 1975 (in all countries in the cohort) of around 100 ppm, decreasing to near or below 40 ppm by 1990. Kogevinas *et al.* (1994) developed two different models for estimating early exposures. Model A assumed that all countries had exposures at the levels measured in Denmark, and model B assumed that styrene levels first measured in each country adequately reflected levels in all previous periods. All results reported here reflect the first exposure assumption, which appeared to be well-justified, based on the similarity in production processes and materials, the similarity in time trends, and some limited data on earlier periods in countries outside Denmark. Cumulative (ppm-years) and average (ppm) exposure were estimated for individual cohort members on the basis of job records (longest job held) and country- and time period-specific exposure estimates using both models.

National mortality rates for each gender were used for external comparisons. Rates were not adjusted for socio-economic status or regional variation; wide social or regional variation has been recorded in some European countries for specific neoplasms such as lung cancer, but that little evidence exists for such variation in rates of lymphomas and leukemias. Regression analyses were conducted using internal comparisons limited to exposed cohort members, and focused *a priori* on neoplasms of the lymphohematopoietic tissues. All regression models included age, calendar period (four levels), country, gender, and time since first exposure. Exposure categories were based on cut-off points chosen so that each level included an approximately equal number of LHC cases and none of the levels were restricted to a narrow exposure range.

Based on external comparisons, mortality from malignant neoplasms was lower than expected based on national rates (SMR = 87, 95 percent CI = 81-94). Among laminators, non-LHC malignant neoplasms showed elevated risks for cancer of the esophagus (SMR = 181, CI = 87-334) and pancreas (SMR = 148, CI = 76-258).

Among laminators, elevated risks were seen for non-Hodgkin's lymphoma (SMR = 140, CI = 56-288). Workers with unspecified tasks, who represented more than 40 percent of the deaths (including all of those from Denmark), had elevated (though not statistically significantly) mortality rates for all LHCs (SMR = 119, CI = 80-170), multiple myeloma (SMR = 193, CI = 78-398), leukemia (SMR = 140, CI = 79-228), and myeloid leukemia (SMR = 142, CI = 65-269). Among all exposed workers, those with two or more years exposure duration and at least 20 years since first exposure had elevated risks for all LHC (SMR = 173, CI = 70-357) and leukemia (SMR = 194, CI = 40-566), but these were not statistically significant.

Results for Poisson regression models provide the most valid findings. In these internal comparisons, the main findings with respect to LHC were that cumulative exposure was not associated with all LHC or with leukemia, whereas average exposure was positively associated. A clear linear trend was seen for all LHC, with a relative risk (RR) of 3.6 (95 percent CI = 1.0-12.1) for those exposed to ≥200 ppm TWA. For leukemias alone (ICD 204-208, 8th revision), elevated risks are observed for each TWA exposure level >60 ppm, as compared with <60 ppm. Similar results were observed for malignant lymphomas (ICD 200-202, 8th revision). Each of these outcome groups showed an increase with increasing time since first exposure. Analyses by duration of exposure were heavily influenced by the Danish results, and mirror those reported by Kolstad *et al.* (1994). Risk for pancreatic cancer increased with increasing cumulative exposure, although the risks were not statistically significant (<75 ppm-years, RR = 1.0, reference group; 100-199 ppm-years, RR = 1.44; 200-499 ppm-years, RR = 1.90; ≥500 ppm-years, RR = 2.56, 95 percent CI = 0.90-7.31; p for trend = 0.07).

An issue of concern with LHC mortality is the impact of the patterns seen in the Danish subset on the overall cohort; workers from this country represented 39 percent of the cohort. Regression analyses conducted by Kogevinas *et al.* (1994) considered only exposed workers; of the 50 exposed LHC deaths, 24 were Danish. Although there was no indication in the overall cohort of a trend for increasing risk of LHCs with increasing cumulative exposure, an increase was observed when the Danish workers were excluded from the analyses.

As noted above, 80 percent of the Danish workers were employed less than two years, and thus analyses by duration of exposure were heavily influenced by the Danish results and mirror the results reported by Kolstad *et al.* (1994). However, analyses of LHC risk in relation to time since first exposure (latency), which were not sensitive to the length of employment, show a statistically significant trend of increasing risk with increasing latency (<10 years [reference group], RR = 1.0; 10-19 years, RR = 2.90, 95 percent CI = 1.29-6.48; ≥20 years, RR = 3.97, 95 percent CI = 1.30-12.13; p for trend = 0.012).

Anttila *et al.* (1998) examined cancer incidence in Finnish workers for whom biological measurements had been made during 1973-1983 for occupational exposure to several solvents. For styrene exposure, the job monitored was "usually lamination of reinforced plastics products." Approximately 34,300 person-years at risk were accrued for styrene-exposed workers (n = 2,580). Post-shift samples of MA in the urine (U-MA) were taken when Finnish standards were changing, and styrene levels were decreasing. However, no overall temporal decrease was seen in U-MA concentrations, nor was any clear difference seen by mean U-MA levels in risk estimates for any of the primary cancer sites.

Incidence of rectal cancer among styrene-exposed workers was statistically significantly elevated relative to the general population (6 cases, SIR = 311, 95 percent CI = 114-677). Incidence of pancreatic cancer was elevated among those diagnosed more than 10 years after the first measurement (3 cases, SIR = 364, CI = 75-106), as was cancer of the nervous system (4 cases, SIR = 311, CI = 85-795), but neither was statistically significant. To address the possibility that socio-economic factors might have influenced the results, the authors computed relative risk estimates versus workers without styrene exposure who had been monitored for blood lead (using only those whose blood lead was not elevated above the general population). Using conditional logistic regression in a case-control design matching for gender, birth year, and age at diagnosis, the risk estimates were not affected (actual values were not reported); the authors concluded that social class-related factors were not an explanation for the increased cancer incidence.

Because workers in the reinforced plastics industry have higher styrene exposures and lower likelihood of concomitant exposures to other carcinogens, these workers provide the cleanest data with which to examine the potential for styrene exposure to cause cancer. In examining the data set as a whole, patterns of increased risk in relation to styrene exposure emerge for several cancers.

LHCs were examined in detail in most studies because of *a priori* concern for these outcomes. The smaller studies and those with less follow-up (Okun *et al*., 1985; Coggon *et al*., 1987; Wong, 1990; Wong *et al*., 1994) lacked the power to observe statistically significant elevated risks. The larger studies (Kolstad *et al*., 1994; Kogevinas *et al*., 1994) were limited by exposure characterization. Nevertheless they observed statistically significant increased LHC risks related to styrene exposure. Kogevinas *et al*. (1994) provided the only internal comparisons, with adjustment for several variables.

LHC risks were increased in relation to exposure during early time periods (1964-1970) associated with higher exposures (Kolstad *et al.*, 1994), time since first exposure (Kolstad *et al.*, 1994; Kogevinas *et al.*, 1994), and average level of styrene exposure (Kolstad *et al.*, 1994; Kogevinas *et al.*, 1994). Kolstad *et al.* (1994) found statistically significant elevated overall LHC and leukemia incidence among cohort members first exposed during 1964-1970, when average styrene exposures were highest. Internal comparisons in Kogevinas *et al.* (1994) indicated that LHC risk increased linearly with time since first exposure (p trend = 0.012) and average exposure (p trend = 0.019). Risks were also elevated for leukemia and malignant lymphomas for average styrene exposures above 60 ppm (Kogevinas *et al.*, 1994). Kolstad *et al.* (1994) found an elevated rate of Hodgkin's disease among employees of companies with a recorded average styrene level above 50 ppm (in a subpopulation for which styrene measurements were available).

Analyses of cumulative exposure were less conclusive. Kogevinas *et al.* (1994) found LHC risk increased with cumulative exposure (<75 ppm-years [reference group], RR = 1.0; 75-199 ppm-years, RR = 1.7; 200-499 ppm-years, RR = 2.2; ≥500 ppm-years, RR = 1.5) when the Danish subcohort, made up primarily of short-term workers, was excluded. As noted above, 80 percent of the Danish workers were employed less than two years; thus, analyses of cumulative exposure, which rely on duration of exposure, were heavily influenced by the Danish results. Nevertheless, rather than excluding those from one country, exclusion of all short-term workers from all the countries would have been a

more appropriate analysis. As noted by IARC (2002), accuracy in estimating duration of employment was a problem, and thus cumulative exposure estimates were problematic.

Elevated cancer rates were found in multiple studies for non-LHC sites including respiratory cancer (Okun *et al*., 1985; Coggon *et al*., 1987; Wong, 1990; Wong *et al*., 1994; Kolstad *et al*., 1995), female reproductive cancers (Okun *et al*., 1985; Coggon *et al*., 1987; Wong, 1990; Wong *et al*., 1994; Kogevinas *et al*., 1994), kidney cancer (Wong *et al*., 1994; Kogevinas *et al*., 1994), and gastrointestinal cancers (Wong *et al*., 1994; Kolstad *et al*., 1995; Kogevinas *et al*., 1994), although not all of these were statistically significant.

Lung cancer was elevated in the U.S. study (Wong *et al.*, 1994), which reported a statistically significant increased risk of cancer of the bronchus, trachea, and lung for both genders combined (SMR = 141, $p<0.05$). Kolstad *et al.* (1995) reported an increased incidence of cancer of the pleura and a very slight elevation in lung cancer incidence when compared with the general population. However, Kolstad *et al.* (1995) pointed out that exposures to smoking or asbestos may be reasons for the elevated lung cancer rates in the Danish cohort, as internal comparisons with workers in similar industries that lack styrene exposure showed similar incidence in the two groups.

In the smaller number of women workers, reproductive organ cancers were consistently elevated. The early reinforced plastics studies reported elevated rates based on small numbers of cases (Okun *et al.*, 1985; Coggon *et al.*, 1987). In the more recent studies, which had more power to detect an effect (Wong *et al.*, 1994; Kogevinas *et al.*, 1994), similar results were found. Wong *et al.* (1994) reported statistically significant elevated rates of mortality from cancer of the cervix uteri (SMR = 283.5, $p<0.01$) and of female genital organs other than the uterus (SMR = 201.6, $p<0.05$). Kogevinas *et al.* (1994) found statistically nonsignificant increased rates of ovarian cancer, with the highest rates found in the high exposure group (laminators).

Kidney cancer showed some elevation (SMR = 175.2, 95 percent CI = 98.1-289.0) in the U.S. study (Wong *et al.*, 1994) and increased with cumulative exposure in the multi-country European study (Kogevinas *et al.*, 1994), based on relatively few cases (<100 ppm-years [reference group], 1.0; 100-199 ppm-years, RR = 4.4; 200-499 ppm-years, RR = 3.3; >500 ppm-years, RR = 6.0). The Danish cohort (Kolstad *et al.*, 1995) found no indication of elevated kidney cancer risks (SIR = 93; CI = 65-128).

All three of the larger studies reported increased risks of esophageal cancer, pancreatic cancer, or both. Wong *et al.* (1994) found esophageal cancer significantly increased in the overall U.S. cohort (SMR = 191.7, 95 percent CI = 104.8-321.7). Kogevinas *et al.* (1994) reported elevated esophageal cancer risks among laminators (considered to have the highest exposure) (SMR = 181, CI = 87-334); esophageal mortality rate ratios in the overall European cohort increased with cumulative styrene exposure to a RR of 1.8 among those with >500 ppm-years. Kolstad *et al.* (1995) found that incidence of pancreatic cancer in the Danish cohort was significantly increased in those who had high exposure probability (IRR = 2.2, 95 percent CI = 1.1-4.5) compared with workers lacking styrene exposure; elevated risks were also seen in those first employed in reinforced plastics before 1970, when exposures were generally higher. Kogevinas *et al.* (1994) found elevated rates of pancreatic cancer in the multi-country European cohort.

Although this study includes some of the Danish (Kolstad *et al.*) cohort, increased mortality from pancreatic cancer was also found among laminators (SMR = 148, 95 percent CI = 76-258), an exposure category which does not include the Danish subcohort. Based on internal comparisons, Kogevinas *et al.* (1994) found that mortality rates for pancreatic cancer in the overall cohort increased with cumulative styrene exposure. A recent small study (Anttila *et al.*, 1998) of cancer incidence in Finnish laminators found that pancreatic cancer incidence was elevated among those diagnosed more than 10 years after the first measurement of urinary MA.

Despite the relatively large number of workers engaged in production of reinforced plastics and the existence of several study cohorts, the number of cancer cases available for subcategory analysis in most studies (e.g., to assess latency or high exposure categories that better define risk) was quite small. The early studies (Okun *et al*., 1985; Coggon *et al*., 1987) had very limited power to detect an effect. The Wong (1990) cohort had a small number of total deaths, and high loss to follow-up. The second report (Wong *et al*., 1994) decreased these limitations somewhat, by updating mortality to include more complete follow-up and more deaths, but failure of these investigators to update the exposure information or to repeat the analyses of Wong (1990) that included categories based on estimated TWA exposure reduces the value of the later analyses.

Kolstad *et al.* (1994) had a much larger cohort, and thus greater power to observe increased risks; also, by examining incidence rather than mortality, it was better designed for the study of lymphohematopoietic malignancies. The primary limitations were the lack of individual exposure information and the high proportion of workers with short-term employment in the industry.

Kogevinas *et al.* (1994) assembled the largest group of reinforced plastics workers by drawing on cohorts from several countries, including two (Coggon *et al.*, 1987; Kolstad *et al.*, 1994) also studied separately. Analyses of this heterogeneous group (Kogevinas *et al.*, 1994) had more power in terms of sheer numbers of cases, but were limited by uneven exposure characterization in the different countries, making groupings across subsets difficult or arbitrary. For example, laminators from countries that had information on job titles were grouped together, while laminators in other countries were grouped with all of their co-workers. Analyses that combine these subgroups have greater numbers but cannot avoid the dilution of the highest exposure (laminators) group with those less exposed. Nevertheless, this study was the most informative.

Misclassification of exposure occurred in three ways. First, some of the studies did not distinguish workers based on their job activities. In the reinforced plastics industry, certain tasks (e.g., hand lamination) involve much greater exposure potential than others. Kolstad *et al.* (1994) did not have information on individual job titles, and exposure classification was based on the company at which the person worked (all the individuals in any company were grouped in the same exposure category). Similarly, Kogevinas *et al.* (1994) grouped all the reinforced plastics workers from Denmark, Finland, and those from half of the Swedish companies in a single category, “workers with unspecified tasks,” combining laminators with all other reinforced plastics workers in these countries.

Second, when exposure was classified based on job titles, groupings may have combined individuals both with and without exposure. For example, Wong (1990) examined a

subset of his cohort, collecting more detailed information on workers included in a case-control analysis of respiratory cancer. He found that 25 of 40 cases and 58 of 102 controls were "not exposed" directly to styrene. However, in the cohort analyses, these same workers had been included in the "lower average TWA" exposure category, together with workers exposed to up to 12 ppm TWA styrene; these workers who were "not exposed" were also included in analyses of risk of exposed workers based on duration of employment as an exposure surrogate.

Third, a common approach in these studies is to develop an estimated cumulative exposure value for each individual based on an estimated average exposure multiplied by exposure duration, which is usually based on employment duration. This approach is problematic not only because exposure levels changed significantly over time, but also because the effect of intensity of exposure is lost. Because different detoxification pathways may be utilized at higher exposure levels, intensity of styrene exposure may be an important determinant of risk. Approaches to exposure classification that factor in intensity, such as calendar-period of exposure, or maximum TWA exposure, may provide better measures of differential exposure and risk.

Use of cumulative exposure creates an additional problem in this data set because of the large number of short-term workers in the industry. Short-term workers exposed to high average levels and long-term workers exposed to low average levels can end up grouped in the same cumulative exposure category, when the risks faced by workers exposed under these two conditions may be quite different. Also, short-term workers have higher mortality rates regardless of exposure level (Boffetta *et al.*, 1998; Kolstad and Olsen, 1999), and their inclusion in the low cumulative exposure category may distort the exposure-risk relationship.

The largest cohorts of reinforced plastics workers included a sizable proportion of short-term workers (usually defined either as <1 or <2 years of employment). Concerns have been raised that the dose-response relationship may be miscalculated when a large proportion of workers are short-term and cumulative exposure is used as the dose. Some of the same authors (Boffetta *et al.*, 1998; Kolstad and Olsen, 1999) who conducted the studies of reinforced plastics workers have examined the implications of the inclusion of short term workers in occupational cohort studies by conducting further analyses, assessing the effect of duration of employment on workers' mortality and the role of pre-employment health status on duration of employment. Health status is often related to duration of employment; this is known as the healthy worker survivor effect (Arrighi and Hertz-Picciotto, 1993, 1994, 1996; Hertz-Picciotto *et al.*, 2000).

Boffetta *et al.* (1998) examined mortality in short-term workers, using the cohort studied by Kogevinas *et al.* (1994) as well as a cohort of workers in the man-made vitreous fiber (MMVF) industry. There were differences between short- and long-term workers in both industries in SMRs for major causes of death, with short-term workers consistently at higher risk. For malignant neoplasms, the differences were small and the confidence intervals were overlapping. Internal comparisons indicated that employment status exerted a confounding effect. Part of the increased mortality could be attributed to a healthy worker survival effect, that is, quitting employment because of poor health. Kolstad and Olsen (1999) investigated this issue in detail, focusing on the Danish cohort of reinforced plastics workers. They found that the number of hospitalizations prior to

employment predicted the length of employment. Pre-employment hospitalization related to alcohol abuse was the strongest predictor of a short length of employment.

Boffetta *et al.* (1998) found that short-term workers had slightly higher average estimated styrene exposure than did long-term workers, mainly because they tended to be employed in earlier periods, when exposure was higher. They also noted that when the Danish cohort was excluded, there was no overall excess mortality among short-term workers (those with less than one year of employment) in the reinforced plastics industry. (In the Kogevinas *et al.* (1994) analyses of these workers, short-term workers were defined as those with less than two years employment.) Boffetta *et al.* (1998) identified a strong trend in overall mortality according to duration of employment ($p<0.01$), where workers with less than one month of employment had the highest mortality.

The finding that healthy workers have a higher likelihood of remaining at work has implications for mortality studies, especially with respect to selection of reference groups for comparative studies of cumulative exposure (Kolstad and Olsen, 1999). Analyses of dose-response relationships may be biased by inclusion of short-term workers because they contribute substantially to the groups with low cumulative exposure and may be critical in the assessment of the shape of the dose-response curve (Boffetta *et al.*, 1998). An overestimation of risk in the low exposure group due to increased mortality of short-term workers, which may be the result of factors other than occupational exposure, could cause an underestimation of the unit risk (Boffetta *et al.*, 1998). These authors suggest that short-term workers be analyzed separately from long-term workers.

In summary, the pattern of risk in reinforced plastics workers suggests that styrene exposure increases the risk of cancer. Subgroups of workers who could be clearly identified as having higher exposure to styrene, either because of their work tasks (i.e., laminators) or because of the time period when they were exposed (i.e., prior to 1970 in the Danish cohort), have elevated risks, especially for LHC. In addition, a trend of increasing cancer risk with increasing cumulative exposure is apparent in the large European cohort study for both LHCs (excluding the Danish cohort) and kidney cancer (Kogevinas *et al.*, 1994). The analytic difficulties posed by the large number of short-term workers in these cohorts have not been entirely solved by the approaches taken (e.g., excluding the Danish subcohort; stratifying by employment duration). However, a linear increase in LHC risk seen with average exposure in the largest study indicates that, despite the impact of the healthy worker survivor effect, risks are increased by exposure. In addition, time since first employment, which is not sensitive to length of employment and has been used as a surrogate for latency, consistently indicates an increased cancer risk for those exposed to styrene (e.g., LHC risk in the Kogevinas *et al.* cohort), strengthening the plausibility of the cause-effect relationship.

Styrene-butadiene Rubber Manufacturing

One type of synthetic rubber, styrene-butadiene rubber (SBR), is produced through an emulsion polymerization reaction of aqueous styrene and gaseous butadiene. Workers in the SBR manufacturing industry are often exposed to both styrene and butadiene. Sulfuric acid is used to coagulate the polymer latex, and other chemicals added, depending on the intended end use of the product (Lewis, 1999).

The National Institute for Occupational Safety and Health (NIOSH, 1983) measured a number of substances, in addition to styrene and butadiene, in air samples at worksites producing or curing SBR, including several with carcinogenic potential: benzene; 4-vinyl-1-cyclohexane; 4-isopropyl-1-methyl-cyclohexane; ethylbenzene; 1,5-cyclooctadiene; 1,5,9-cyclododecatrienes; formaldehyde; acrolein; aromatic amines; and acrylonitrile. Benzene is a known human leukemogen (IARC, 1982b). Few cancer epidemiology studies of the industry have examined benzene co-exposure. Recently, dithiocarbamates have been suggested as an important co-exposure in this industry (Irons and Pyatt, 1998).

There is sufficient evidence of butadiene carcinogenicity in experimental animals (U.S. EPA, 1985; NTP, 1993). Butadiene induces cancer in rats and mice at multiple sites, although the sites and the magnitude of response differ between the two species. Tumorigenic effects were observed at the lowest exposure level studied (6.25 ppm; NTP, 1993; Melnick and Huff, 1993). Malignant lymphomas, induced in inhalation studies of mice, were observed as early as week 23 (NTP, 1993). A dose-rate effect was also apparent for lymphomas: at comparable cumulative exposures, the incidence was greater after exposure to a high concentration for a short time than to a lower concentration for a longer period (Melnick and Huff, 1993). This association of increased cancer risk with increased exposure intensity in animals has a parallel in the results of a large cohort study of workers engaged in butadiene monomer production (Divine *et al.*, 1993).

Epidemiological studies of workers exposed to butadiene include those exposed during monomer production and during SBR production. IARC (1994) found the human carcinogenicity evidence from these studies to be “limited”. More recent studies (e.g., Macaluso *et al.*, 1996; Matanoski *et al.*, 1997) indicate an association of butadiene exposure with leukemia mortality in cohort studies conducted in North America. Two butadiene monomer production studies of reasonably large cohorts found increased risks of lymphosarcoma and reticulosarcoma (Divine *et al.*, 1993; Ward *et al.*, 1995); the third study (Cowles *et al.*, 1994), with a very small number of deaths (total n = 24), found no excess mortality.

One of the two studies was “consistent with the notion that the incidence of lymphatic and hematopoietic malignancies is highest in groups with the heaviest occupational exposure to butadiene” (Landrigan, 1993). The cohort has been the subject of several reports (Downs *et al.*, 1987; Divine, 1990; Divine *et al.*, 1993; Divine and Hartman, 1996). Excess mortality from lymphosarcoma and reticulum-cell sarcoma was concentrated among workers with fewer than five years employment (SMR = 286, 95 percent CI = 104-622) and those who were first employed before 1946 (SMR = 254, CI = 102-523) (Divine *et al.*, 1993). A subgroup defined as having routine (daily) exposure to butadiene had the highest rates of elevated mortality from lymphosarcoma, with an SMR = 452 (CI = 165-984). Divine *et al.* (1993) found that SMRs decreased with increasing length of employment, and interpreted these findings as indicative of a lack of support for causality. However, cumulative exposure, as estimated by number of years worked, did not account for heavy exposure in the early years of plant operation (prior to 1946).

Toxicokinetic interactions between styrene and butadiene have been investigated (e.g., Filser *et al.*, 1993). Both compounds require metabolic activation to DNA-reactive metabolites to induce tumors in animals. The P450IIE1 isozyme may play a major role in

activation of both compounds (Filser *et al.*, 1993). The metabolism of butadiene in rats may be partially inhibited by styrene, whereas the metabolism of styrene appears not to be affected by butadiene (Laib *et al.*, 1992). This ability of styrene to inhibit the metabolic activation of butadiene may have important implications for interpreting the findings of studies of workers exposed to both in the SBR manufacturing industry. A physiologically-based pharmacokinetic (PBPK) model developed to simulate such interactions predicted that a significant inhibition of butadiene metabolism by styrene would occur in humans exposed to both, even at relatively low concentrations (e.g., 20 ppm styrene, 5 ppm butadiene) (Filser *et al.*, 1993). Others have predicted less pronounced effects at such concentrations (Bond *et al.*, 1994).

The U.S. government had foreseen shortages of natural rubber in the early years of World War II, and financed the construction of 15 SBR plants, as well as production facilities for butadiene and styrene monomers (NIOSH, 1976). Draft deferments were given for people working in these plants, and people tended to stay working there for many years. After the war, these plants were sold to private companies, which continued to operate them (NIOSH, 1976). In the late 1960s and early 1970s, reports began to appear that workers in these plants suffered excess mortality from various cancers, including leukemia, other lympho-hematopoietic cancers, and lung cancer (Andjelkovic *et al.*, 1976, 1977; Mancuso *et al.*, 1968; McMichael *et al.*, 1974, 1975). Industrial hygiene inspections of these plants by NIOSH (1976) found numerous problems, including: poor housekeeping; vessel entry to clean out reactors conducted by workers with no respiratory protection; workers eating in areas where toxic materials were handled; and, as described in one report, "[in] several areas…it's more of an open system and there are opportunities for exposures."

Epidemiological studies of worker cohorts in these SBR plants were subsequently published (Table 26). In each study, the SMRs for overall mortality indicated a healthy worker population (SMRs ≤80). These studies followed men who had worked for many years in these facilities, and most had held a variety of jobs. All of the studies found associations with cancer and specific work areas. In the only study (McMichael *et al.*, 1976) in which exposure to styrene-butadiene was specified, an association was found between lymphatic and hematopoietic malignancies and work in the SBR manufacturing department (RR = 6.2, 99.9 percent CI = 4.1-13).

IARC (1982a) concluded that there was: 1) sufficient evidence for excess occurrence in rubber workers and causal association with occupational exposures for bladder cancer and leukemia (presumed agent: aromatic amines and solvents, respectively); 2) sufficient evidence for excess occurrence in rubber workers for stomach and lung cancer with limited evidence for an association with particular job categories (agents not identified); and 3) limited evidence for excess occurrence in rubber workers for colon and prostate cancers and for lymphoma, with inadequate evidence for an association with any agent or job category.

Table 26. Cancer Mortality Associated With Work in Specific Areas of Styrene-Butadiene Rubber Plants: Early U.S. Studies

Study Author & Cohort	Cancer Mortality	Work Areas[1] Associated with Excess Cancer	Comments
Monson & Nakano, 1976 Cohort of 13,571 white males followed from 1925 to 1939 and 1940 to 1974 (two analyses).	**Entire Cohort** SMR:[2] Stomach 190 Large intestine 130 Lung 160 Bladder 310 Brain 190 Lymphatic 160 Leukemia 128	Processing Processing Tire curing All workers Tire building Tire building All workers	Factors that influenced risk included: 1) starting work before 1935 2) working 25 to 34 years in plant 3) being over age 74 at death.
McMichael *et al.*, 1976 6,678 males from 1964 to 1973; 99% with greater than 10 years experience in plant prior to 1964.	**Cancer Site** RR: [3] Stomach 2.3 (1.7-3.6) 2.2 (1.4-4.3) Colorectal 2.2 (1.6-3.4) Respiratory 2.1 (1.4-4.3) 2.3 (1.6-3.9) Bladder 9.6 (1.6-4.0)(*sic*) 2.5 (1.8-4.1) Lymphatic & hematopoietic 6.2 (4.1-12.5) Lymphatic leukemia 3.2 (2.4-5.0) 3.7 (2.8-5.3) 3.9 (2.6-8.0) 2.2 (1.8-2.9)	Extrusion, tread cementing Synthetic plant Extrusion, tread cementing Mill mixing Reclaim Milling Reclaim Synthetic plant[4] Extrusion, tread cementing Inspection, finishing, repair Synthetic plant Janitoring, trucking, etc.	Confidence intervals for RR are based on within-cohort cases "exposed" to specific work areas compared to a subset of the entire cohort. More black workers were in areas with high exposures, but no race-specific analysis was done due to lack of comparison data. The authors conjecture that lymphoma cases in low exposure areas may have been transferred to low exposure after becoming ill in higher exposure job.
Andjelkovic *et al.* 1976; 1977	**Cancer site** SMR:[5] Stomach		Work area analysis is based on "most representative

Study Author & Cohort	Cancer Mortality	Work Areas[1] Associated with Excess Cancer	Comments
8418 white males 1964 to 1973; more than 98 percent with ≥10 years experience in plant prior to 1964.	479 369 Large intestine 629 Trachea, bronchus & lung 434 Leukemia 246	Compound & mixing Milling Special products Synthetic latex General service	department." In-depth analysis of subset showed average time in most representative department was 50% (range of 10 to 100%).

Abbreviations: SMR = standardized mortality ratio; RR = relative risk; CI = confidence interval.
[1] Work area as reported by study author.
[2] No confidence intervals were reported.
[3] Confidence intervals are reported as 99.9 percent. Reported RR for bladder cancer is above upper bound, so either upper bound or RR is an error.
[4] Synthetic plant is identified as a location in which styrene-butadiene rubber is made.
[5] Reported as significant ($p<0.05$), without confidence intervals. Based on small numbers.

Studies of SBR industry workers published in the 1980s and early 1990s are summarized in Table 27. These include a NIOSH study of two SBR facilities in Texas (Meinhardt *et al.*, 1982) and retrospective cohort and case control studies of workers at eight facilities in the U.S. and Canada (Matanoski *et al.*, 1990, 1993; Santos-Burgoa *et al.*, 1992). These studies attempted to assess exposure to specific chemicals, in particular, styrene and butadiene. However, the accuracy of exposure estimates was limited because they were based on specific job classifications, exposure measurements were sparse, and they were only available for certain time periods. Process changes during the long time span of these studies which would likely have affected styrene exposure were not adequately addressed. In addition, individuals moved between jobs in each facility, and exposure estimates for most of the studies were based on the job held for the longest time.

Subsequent studies summarized in Table 28 attempted to address the limitations of the earlier studies. Two groups of researchers published analyses of overlapping sets of workers from the U.S. and Canada: 1) the Johns Hopkins University (JHU) group (Matanoski *et al*, 1990, 1993; Santos-Burgoa *et al.*, 1992) published an updated study (Matanoski *et al.*, 1997) that further refines the examination of lymphohematopoietic cancers (LHC) in their cohort; 2) the University of Alabama at Birmingham (UAB) group has created a combined cohort of 15,649 workers at seven of eight plants previously studied by investigators at JHU with those studied by NIOSH (Meinhardt *et al.*, 1982) at a two-plant complex and published a series of studies examining exposure issues as well as various causes of mortality (Delzell *et al.*, 1996, 2006; Macaluso *et al.*, 1996; Sathiakumar *et al.*, 1998, 2009; Sathiakumar and Delzell 2007, 2009). Other studies

examined mortality in a new SBR cohort in Germany (Weiland *et al.*, 1996; Straif *et al.*, 1998), without any specific information on exposure.

Table 27. Cancer Mortality in Styrene Butadiene Rubber Plants in the U.S. and Canada: Studies from 1982 to 1993

Study Author & Design	Mortality Rates (95 percent CI)	Excess Cancer Seen in Subgroup Analyses (95 percent CI)	Comments
NIOSH Study Meinhart *et al.*, 1982 (Texas) Retrospective cohort included workers employed for ≥6 months (total 53,929 person-yrs at risk). **Plant A** 1662 white men 1943-1976 (252 deaths) **Plant B** 1094 white men 1950-1976 (80 deaths)	**Plant A** SMR: All causes 80[a] (70-90) All LHC 155 (71-295) Lymphosarcoma 181 (NR) Hodgkin's 115 (NR) Leukemia 203 (NR) **Plant B** SMR: All causes 66[a] (NR) All LHC 78 (NR) Lymphosarcoma 132 (NR) Hodgkin's 0 Leukemia (NR)	**Mortality Cause** For those employed from Jan. 1943 to Dec. 1945, during operation of a batch process (201 total deaths). SMR: All causes 83[a] All LHC 212[b] (97-402) Lymphosarcoma 224 (NR) Hodgkin's 213 (NR) Leukemia 278[b] (NR)	**Monitoring** TWA environmental samples (ppm) taken at time of study. **Plant A** Mean (Range) Styrene 0.94 (0.03-6.46) Butadiene 1.24 (0.11-4.17) Benzene 0.10 (0.08-0.14) **Plant B** Mean (Range) Styrene 1.99 (0.05-12.3) Butadiene 13.5 (0.34-174) Benzene (no data)
JHU Study Matanoski *et al.*, 1990; 1993 (U.S. and Canada) Retrospective cohort of 12,110 men workers in eight plants from 1943 to 1982 (follow-up varied by plant).	**Entire Cohort** SMR: All cancers 85 (78-93) No significant excess cancer at any specific site for overall cohort.	**Black Production Workers** SMR: All LHC 510 (190-1,100) Leukemia 660 (140-1,900)	Air samples in 5 of the 8 plants for 1978-83 found average styrene exposure of 3.53 ppm (0.29 to 6.66 ppm). Cohort included workers employed ≥1 year (total 251,431 person-years at risk).
JHU Study Santos-Burgoa *et al.*, 1992 (U.S) Case-control (from Matanoski *et al.* 1990; 1993 cohort), includes 59 LHC cases and 193 controls.	Not applicable.	**Leukemia Mortality** *Exposure to butadiene only* OR: 9.4 (2.1-23) *Exposure to styrene only* OR: 3.1 (0.84-11) *Exposure to butadiene and styrene* Butadiene OR: 7.4 (1.3-41) Styrene OR: 1.1 (0.23-5.0)	Exposure in each job was estimated by a panel of rubber industry experts who ranked jobs from low to high. Controls were matched based on plant, age, year and employment duration.

Abbreviations: CI = confidence interval; LHC = lympho-hematopoietic cancer; OR = odds ratio; SMR = standardized mortality ratio; NR = not reported; ppm = parts per million; TWA = time-weighted average; JHU = Johns Hopkins University.
[a]$p<0.05$; [b]$p<0.10$

In the first four reports published by the UAB group, the first related LHC mortality to process groups (Delzell *et al.*, 1996); the second and fourth related cumulative exposure estimates to leukemia mortality (Macaluso *et al.*, 1996; Delzell *et al.*, 2001); and the third provided further data on cancer mortality patterns in relation to process groups (Sathiakumar *et al.*, 1998). The cohort includes 15,649 men employed for at least one year at any of nine SBR plants. Follow-up was for the period 1943 to 1991, with 3,976 deaths observed. National mortality rates were used for the comparisons on most causes of death.

Table 28. Cancer Mortality of Workers in Styrene-Butadiene Rubber Manufacturing Plants: Epidemiological Studies from 1994 to 1998.

Study Author & Design	Mortality Rates (95 percent CI)	Excess Cancer Seen in Subgroup Analyses (95 percent CI)	Comments
UAB Studies Delzell *et al.*, 1996; Sathiakumar *et al.*, 1998 (U.S. and Canada) Retrospective cohort of 15,649 men employed for ≥ 1 year at eight styrene butadiene rubber plants from 1943 to1991.	**Entire Cohort** SMR: All cancers 93 (87-99) Leukemia 131 (97-174) Non-Hodgkin's 91 (61-132) Large intestine 97 (78-120) Lung 101 (91-112)	**Subgroup 1**[a] SMR: All cancers 96 (87-106) Leukemia 224 (149-323) Non-Hodgkin's 137 (77-226) Large intestine 100 (NR) Lung 100 (NR) **Subgroup 1**[a]**/White** SMR: Non-Hodgkin's 133 (not SS) **Large intestine 23/30** (NR) Lung 99 (NR) **Subgroup 1**[a]**/Black** SMR: Non-Hodgkin's 172 (not SS) **Large intestine 12/5.2** ($p<0.05$) Lung 105 (NR) **Subgroup 2**[a]**/Black** SMR: Lung 167 (110-243)	Areas with potential for relatively high exposure to butadiene or styrene monomers (polymerization, maintenance and laboratories) had SS elevated SMRs for leukemia. Process groups analyses included subjects from all plants, despite missing information from two plants. The excess of large intestine cancer in black men was concentrated in groups hired ≥1950. The lung cancer analyses are based on state rather than national rate comparisons. The authors note that these are closer to the null (about 10% lower) than those based on U.S. rates.

Study Author & Design	Mortality Rates (95 percent CI)	Excess Cancer Seen in Subgroup Analyses (95 percent CI)	Comments
UAB study Macaluso *et al.*, 1996 (The same cohort was reported on by Delzell *et al.*, 1996, 1998, Sathiakumar *et al.*, 1998, and Graff *et al*., 2005.) Mortality study with both internal and external comparisons. Workers from eight styrene butadiene rubber plants from 1943 to 1991.	**Leukemia Mortality** *Styrene exposure* (ppm-yrs) RR: SMR: 0 1.0 89 <5 0.9 63 5-9 5.4 161 10-39 3.4 136 40+ 2.7 235 *Butadiene exposure* (ppm-yrs) RR: SMR: 0 1.0 76 <1 2.0 41 1-19 2.1 133 20-79 2.4 166 80+ 4.5 264		**Median Cumulative Exposure** (reconstructed based on narratives and modeling) *All subjects* ppm-years Styrene 7.4 Butadiene 11.2 Benzene 2.9 *Leukemia decedents* ppm-years Styrene 22.4 Butadiene 36.4 Benzene 5.5
JHU study Matanoski *et al.*, 1997 (U.S.) (From cohort reported by: Matanoski *et al.*, 1990, 1993 and Santos-Burgoa *et al.*, 1992.) Nested case-control study of a cohort of 12,110 workers, including 58 LHC cases and 1,242 controls, from eight SBR plants in U.S.	**Styrene** *All LHC* OR[b] = 2.20 (1.46-3.33) *Lymphomas* OR[b] = 2.67 (1.22-5.84) *Lymphosarcoma* OR[b] = 3.88 (1.57-9.59) *Lymphoma* OR[b] = 2.62 (0.40-17.2) *Myeloma* OR[b] = 3.04 (1.33-6.96) **Butadiene** *Hodgkin's* OR[b] = 1.73 (0.99-3.02) *Leukemia* OR[b] = 1.50 (1.07-2.10)	Not applicable.	Exposures based on measured values (3,649 styrene, 3,952 butadiene) from NIOSH and monitoring in 7 of the 8 plants studied. Missing values extrapolated. Styrene and butadiene modeled as continuous variables. Step-down unconditional logistic regression models included birth year, hire year and hire age, year of hire, race, and duration, plus chemical variables.

Study Author & Design	Mortality Rates (95 percent CI)	Excess Cancer Seen in Subgroup Analyses (95 percent CI)	Comments
Weiland *et al.*, 1996; Straif *et al.*, 1998 (Germany) Retrospective cohort of 11,633 German workers employed for ≥1 year at five plants producing tires or technical rubber goods; includes 2,719 deaths.		**Site-specific Elevated SMRs by Work Areas** SMR: *Leukemia* Area I: 216 (108-387) Area II: 187 (102-213) *Pharyngeal cancer* Area IV: 486 (101-1419) *Esophageal cancer* Area III: 227 (114-407)	Exposure surrogate based on work histories reconstructed from codes, classified into six categories: 1) preparation of materials; 2) production of technical rubber goods; 3) production of tires; 4) storage and dispatch; 5) general service; and 6) others.

Abbreviations: CI = confidence interval; SBR = styrene-butadiene rubber; SMR = standardized mortality ratio; RR = rate ratio; OR = odds ratio; SS = statistically significant; NR = not reported; LHC=lympho-hematopoietic cancer; UAB = University of Alabama at Birmingham; JHU = Johns Hopkins University.
[a] Subgroup1: subjects who were ever hourly, worked 10+ years and were followed 20+ years since being hired; Subgroup2: ever hourly subjects who worked <10 years, and followed 20+ years since being hired.
[b] Risk associated with time-weighted average exposure at 1 ppm compared to 0 ppm.

Delzell *et al.* (1996) reported that overall cancer mortality in the cohort was lower than expected (SMR = 93, 95 percent CI = 87-99). Leukemia mortality was elevated (SMR = 131, CI = 97-174), and was statistically significant for a subset called "ever hourly subjects" (SMR = 143, CI = 104-191). "Ever hourly" subjects accounted for 3,531 (88.8 percent) of the total deaths observed; 98 percent of black workers were ever hourly, as were 84 percent of all white workers. Specific groupings with higher leukemia risks included ever hourly subjects with 10 or more years worked and 20 or more years since hire (SMR = 224, CI = 149-323) and workers in three areas with reported potential for relatively high exposure to butadiene or styrene: polymerization (SMR = 251, CI = 140-414); maintenance (SMR = 265, 95 percent CI = 141-453); and laboratories (SMR = 431, CI = 207-793). Delzell *et al.* (1996) concluded that "exposures in the SBR industry cause leukemia."

Macaluso *et al.* (1996) conducted a follow-up study of leukemia mortality in this cohort. Exposure estimation consisted of four steps: 1) a process analysis associated work areas and specific operations with job titles and identified historical changes in processes that might have affected exposure; 2) a job analysis determined tasks and task-specific determinants of exposures, along with historical changes, and resulted in job profiles in which all sources of exposure were described; 3) exposure models were used to estimate exposure intensities by job task or work area and time periods, and resulted in job-exposure matrices (JEMs) for butadiene, styrene, and benzene; and 4) linkage of JEMs with employee work histories resulted in calculation of exposure indices for all individual

workers. Macaluso and colleagues estimated the annual number of exposure peaks (short periods of intense exposure) and time-weighted-average (TWA) exposures, but presented results only in relation to cumulative exposure.

Benzene exposure was not associated with leukemia risk in this cohort, most likely due to low benzene levels and few exposed workers. Four of the six plants studied were estimated to have <1 ppm maximum eight-hour TWA exposure, and the two plants with more substantial exposure had a maximal eight-hour TWA of 6.6 and 38 ppm.

Analysis of Mantel-Haenszel rate ratios indicated a monotonic increase in leukemia mortality risk by increasing cumulative butadiene exposure. Although the increase with cumulative styrene exposure was not monotonic overall, joint stratification for butadiene and styrene both showed monotonic increases at the low exposure level of the other compound. Among those with low butadiene exposure, the rate ratio for leukemia associated with cumulative styrene exposure was 1.7 for 10-39 ppm-years, and rose to 7.0 (95 percent CI = 2.2-22) for 40 or more ppm-years, compared with referents having 0.1 to 9 ppm-years of styrene exposure.

Macaluso *et al.* (1996) reported that, among the subjects in the cohort who died from leukemia, 90 percent had styrene exposure and 86 percent had butadiene exposure. All of the leukemia deaths with butadiene exposure also had styrene exposure. The median cumulative styrene exposure of cohort subjects who died of leukemia was about three times that of all SBR workers in the cohort, as was the median cumulative butadiene exposure. Benzene exposure was less frequent among leukemia decedents than among other employees, although the median cumulative exposure was about twice as high.

A dose-response analysis of butadiene-associated leukemia risk, conducted by Sielken and Valdez-Flores (2001) using the UAB group's revised estimates for butadiene exposure (Delzell *et al.*, 2001), provided parameter estimates for cumulative styrene exposure (0 ppm-years, 1.0 [reference group]; 0-10.4 ppm-years, 0.9; 10.4-28.3 ppm-years, 1.5; 28.3-40.6 ppm-years, 3.1; 40.6-98.1 ppm-years, 1.5; >98.1 ppm-years, 1.6. No confidence limits or p-values were included).

Delzell *et al.* (2001) evaluated leukemia mortality in the cohort related to exposure to butadiene, styrene, and dimethyldithiocarbamate (DMDTC). This effort focused on a slightly smaller cohort (13,130 men) for whom they could obtain sufficient information on job assignments to allow for quantitative exposure estimation. The exposure estimation was conducted like that reported by Macaluso *et al.* (1996), although this more recent study provides revised estimates, and includes estimates of DMDTC exposure. Although the original estimation was done without knowing the identity of the leukemia cases or their work areas, the revised exposure estimation was not, introducing "the potential for inadvertent bias.…" The follow-up period was the same (1943 to 1991). The study controlled for age and time since hire, limited to persons whose follow-up continued past age 40 and 10 years since hire; all subjects had at least one year of employment. Poisson regression was used to compare groups in a particular exposure category to other worker groups unexposed or having low exposure to a particular agent. The analyses included cumulative exposure indices as well as measures of exposure intensity for both butadiene and styrene.

Results are based on 59 leukemia cases as recorded on death certificates, 49 of which had been confirmed by review of medical records. Most (85 percent) of the cohort had some styrene exposure, and even more of the leukemia cases (92 percent) did. Intense peaks of styrene exposure (>50 ppm) were experienced by 62 percent of the overall cohort and 81 percent of the leukemia cases. Median cumulative styrene exposure was 17 ppm-years; leukemia cases had median styrene exposure values two times higher. Single agent Poisson regression analyses adjusting for age and years since hire and using unlagged exposure estimates indicated a consistent positive association between styrene ppm-years and leukemia (RRs of 1.0, 1.2 [0.5-3.3], 2.3 [0.9-6.2], and 3.2 [1.2-8.8], respectively, for exposures of 0, >0 - <20.6, 20.6 - <60.4, and 60.4+ ppm-years), with statistically significant increases at the highest exposure level. A positive association was also seen with intense styrene peaks (RRs of 1.0, 1.4 [0.7-3.0], 3.8 [1.8-8.2], and 4.0 [1.8-8.7] for total peaks of 0, >0 - <105.8, 105.8 - <435.0, and 435.0+); the increases at the two highest levels were statistically significant.

In multiple agent analyses, RRs for styrene ppm-years were reduced but remained above the null after controlling for either butadiene or DMDTC. Simultaneous control for both butadiene and DMDTC changed the direction of the association between styrene and leukemia from positive to inverse; however, these exposures were highly correlated, and the results were imprecise. Given the lack of epidemiologic or toxicologic data indicating that DMDTC is a potential carcinogen, IARC (2002) questioned the justification for controlling it in these analyses.

Examining possible interaction between butadiene and styrene exposure (ppm-years), Delzell *et al.* (2001) report that within each specified level of styrene, there was no consistent pattern of increasing risk with increasing butadiene exposure; however, within the highest level of butadiene exposure, there was an increasing risk with increasing styrene exposure (high butadiene and low styrene, no cases; high butadiene and mid-level styrene, RR = 2.6, 95 percent CI = 0.7-9.2; high butadiene and high styrene, RR = 4.1, CI = 2.0-8.4). When leukemia mortality was examined with levels of both agents increasing, increasing RRs were seen (low styrene and low butadiene, RR = 1.0, reference group; mid-level styrene and mid-level butadiene, RR = 2.4, CI = 1.2-5.1; high styrene and high butadiene, RR = 4.1, CI-2.0-8.4.

Sathiakumar *et al.* (1998) reported on causes of death other than leukemia within the same cohort studied by Delzell *et al.* (1996) and Macaluso *et al.* (1996). Elevated SMRs were found for a variety of endpoints in specific subgroups. The small group of "never hourly subjects" (less than 12 percent of the total observed deaths) had lower than expected mortality from most causes of death; "their SMR was imprecisely increased for multiple myeloma and Hodgkin's disease and was below the null for non-Hodgkin's lymphoma." For the subgroup of subjects who were ever hourly with 10 or more years worked and 20 or more years since hire, elevated SMRs were reported for non-Hodgkin's lymphoma among both blacks and whites (SMRs of 133 and 172, respectively, CI unreported). For blacks and whites combined, the SMR was elevated but not statistically significant (SMR = 137, CI = 77-226). Sathiakumar *et al.* (1998) noted that their evaluation of SBR employment and non-Hodgkin's lymphoma was limited by reliance on death certificates to identify cause of death. "Because survival rates after a diagnosis of non-Hodgkin's lymphoma are high…mortality may not be an optimal endpoint for the

study of this form of cancer. Non-Hodgkin's lymphoma may, in later clinical stages, transform into leukemia…" which is then the recorded cause of death.

Other sites examined by Sathiakumar *et al.* (1998) included large intestine, lung, and laryngeal cancer. Statistically significant increases were seen for cancer of the large intestine in black men with 10 or more years worked and 20 or more years since hire (SMR = 231, 95 percent CI = 119-402). Black male production workers had an elevated SMR (348, CI = 127-758) for cancer of the large intestine. Of the 12 black men with large intestine cancer who had worked 10 or more years and died 20 or more years after being hired, seven had worked in maintenance (SMR = 175, CI = 70-360). For lung cancer, statistically significant increases were seen among black men with less than 10 years worked and 20 or more years since hire (SMR = 167, CI = 110-243). State mortality rates were used as the comparison for all lung cancer results, while for all other comparisons, national mortality rates were used; this was apparently an attempt to account for smoking prevalence among subjects, as no individual information was available on this potential confounder. Maintenance workers (black and white combined) had elevated lung cancer mortality (SMR = 124, CI = 103-147). Increased SMRs for laryngeal cancer were seen among white production workers ($p<0.05$), maintenance workers, and workers in "other operations" ($p<0.05$). All of the workers who died from laryngeal cancer had worked in all three of these areas (production, maintenance, and "other operations").

Mortality analyses reported by Sathiakumar *et al.* (1998) did not draw on the styrene and butadiene exposures to workers estimated by their colleagues Macaluso *et al.* (1996). Sathiakumar *et al.* examined mortality related to 12 categories of work area groups (five main process groups and seven process subgroups), some of which potentially entailed high styrene or butadiene exposures (e.g., maintenance). Sathiakumar *et al.* (1998) interpreted the excess mortality from large intestine cancer in black men as due to exposures other than butadiene and styrene: "…the finding of an increase both among maintenance labourers and among workers in process groups or subgroups that did not entail high exposure to butadiene and styrene suggests that exposure to [styrene and butadiene] monomers was not responsible." Lung cancer increases "may be attributable in part to confounding by smoking and in part to an unidentified occupational exposure." They noted that asbestos may have been present at some plants.

A group of investigators at JHU conducted several studies of a cohort of 12,110 workers from eight North American SBR plants (seven U.S. and one Canadian). The earlier studies from this group were summarized in Tables 27 and 28 (Matanoski *et al.*, 1990, 1993; Santos-Burgoa *et al.*, 1992). Follow-up of the cohort included the years 1943 through 1982. Earlier work (Santos-Burgoa *et al.*, 1992) focused on the LHCs, for which excess mortality was seen, particularly among black production workers. A case-control analysis using exposure assignment based upon a ranking scheme found high leukemia risks associated with butadiene exposure (Santos-Burgoa *et al.*, 1992, Table 27).

Another study by the JHU group (Matanoski *et al.*, 1997) focused on the same LHC cases (n = 59) in the cohort in the earlier study (Santos-Burgoa *et al.*, 1992). Workers at seven of eight plants studied by JHU investigators were included in the UAB cohort. This new JHU study confirmed the LHC case diagnoses using hospital records for 95 percent of the cases. Controls (n = 1242), drawn from non-cancer subjects in the overall cohort, were

matched by plant, age and year of hire, duration of work, and survival as long as or longer than the date of death of the index case for all cancer cases in the cohort (Santos-Burgoa *et al.*, 1992). Matanoski *et al.* (1997) included all of the controls but only the LHC cases; thus, many controls had no matched cases. Since controls had to live as long as or longer than cases, their duration of employment (and thus their exposure potential) was slightly longer than that of the case group, leading to possible higher exposure. For that reason, all cumulative exposure models included a variable for "duration worked."

Exposure estimates were based on measurements of styrene (3,649 samples) and butadiene (3,952 samples) from monitoring at seven of the eight plants. Job- and plant-specific exposure levels and job histories of cases and controls were used to calculate both a cumulative exposure in ppm-months and a time-weighted average (TWA) exposure in ppm, based on total cumulated exposure in ppm-months divided by the total time employed for each individual. Data were analyzed using unconditional logistic regression, an unmatched analysis, because many controls had no matched cases. The analyses included TWA and cumulative exposure [respectively expressed as the log (ppm+1) and log (ppm months + 1)] as continuous variables for exposure. In the average exposure analyses, the resulting coefficients (beta values) were converted into odds ratios corresponding to time-weighted average exposure to each chemical at 1 ppm. All of the models included variables for age, race, year of hire, and exposure level. The cumulative dose models also included a variable for duration worked.

The associations between average (TWA) styrene exposure and all LHC, all lymphoma, lymphosarcoma, and multiple myeloma were each statistically significant. The odds ratios (ORs) associated with continuous exposure at 1 ppm TWA styrene (compared to 0 ppm) were: all LHCs, OR = 2.2 (95 percent CI = 1.46-3.33); all lymphomas, 2.7 (CI = 1.3-5.8); lymphosarcoma, OR = 3.9 (CI = 1.6-9.6); multiple myeloma, OR = 3.0 (CI = 1.3-7.0). Hodgkin's disease was associated with butadiene exposure (at 1 ppm TWA, OR = 1.73, CI = 0.99-3.02).

Leukemia risk was associated with TWA exposure to butadiene, and the odds ratio at 1 ppm TWA butadiene (compared to 0 ppm) was 1.50 (95 percent CI = 1.07-2.10). Matanoski *et al.* (1997) also examined risks for all leukemias and lymphoid and myeloid cell types. Average styrene exposure was not associated with all leukemia or with the lymphoid or myeloid subtypes. Myeloid leukemia was associated only with the work area variable, while lymphoid leukemia was associated with average exposure to butadiene. In the cumulative exposure model for leukemia, the association with butadiene was still present but styrene exposure also played a role.

The cumulative exposure models for all LHCs showed an association with cumulative styrene exposure and duration worked. For multiple myeloma, there was an association with cumulative styrene exposure but not duration worked, and no variables were significant for cumulative exposure in the lymphoma and lymphosarcoma final models. "Duration worked" contributed significantly for all LHCs, Hodgkin's disease, and leukemia, and according to the authors, was usually negative. Matanoski *et al.* (1997) suggested that this indicated the risk was higher for those reaching a cumulative dose in a shorter time period, that is, for those with a higher intensity dose. This would be congruent with the pattern of risk seen in animal studies of butadiene exposure.

Matanoski *et al*. (1997) suggested that their use of measured exposure information helped to distinguish the effects of styrene from those of butadiene in this SBR industry cohort. Another advantage of their study was the use of hospital records to validate cases, as contrasted with the majority of studies, which have used death certificates; using medical records to correctly diagnose cases reduced bias. The effect of high-intensity exposures could be examined because the study included an exposure duration measure separate from the dose estimate. However, confidence in the reported results is limited by concerns regarding the type of analyses, as noted in the discussion section below.

Weiland *et al*. (1996) examined the ten year mortality of a cohort of 11,663 German men who were active and retired German rubber industry workers (Jan 1981 - Dec 1991; 2719 decedents). Cohort members had been employed for at least one year in one of five plants. Mortality from all cancers (SMR = 111; 95 percent CI = 103-119) was significantly increased, with significant excess mortality from lung cancer (SMR = 130; CI = 115-147) and pleural cancer (SMR = 401; CI = 234-642). Elevated but not statistically significant SMRs were found for cancers of the pharynx, esophagus, stomach, rectum, larynx, prostate, and bladder, as well as for leukemia. Deaths from liver cancer, brain cancer, and lymphoma were lower than expected. Straif *et al*. (1998) examined the mortality from non-respiratory cancers by work area in the cohort. Work histories were reconstructed from "cost centre codes" and classified into six categories. Significant mortality increases were found for pharyngeal cancer in storage and dispatch (three deaths, SMR = 486, CI = 101-1419), esophageal cancer in production of tires (SMR = 227, CI = 114-407), and leukemia in preparation of materials (SMR = 216; CI = 108-387) and in production of technical rubber goods (SMR = 187; CI = 102-213).

Loughlin *et al.* (1999) reported a mortality study of students who attended a Texas high school near facilities that had been producing SBR since 1943. All former students who attended from the early 1960s to the early 1990s were part of the cohort; 338 deaths were identified through 1995 (241 men, 97 women). Male mortality for all lymphatic and hematopoietic cancer was elevated (SMR = 164, 95 percent CI = 85-287). For women, the SMR for all LHC mortality was lower than unity (SMR = 47, CI = 6-170). An unexpected finding was an excess of deaths from benign brain neoplasms; mortality ratios were significant for combined sexes (SMR = 418, CI = 153-909) and for men (SMR = 629, CI = 204-1463), but not for women (SMR = 156, CI = 4-871).

The oldest members were approximately 50 years old at the time of the study. The number of subjects lost to follow-up was not ascertained. Potential for styrene exposure was not described. The authors do not report results by time period of attendance, which could have been correlated with exposure. Loughlin *et al.* (1999) suggested that the elevated risks of lymphatic and hematopoietic cancer in men and the “deficit” in women may represent random fluctuations, or may be due to occupational exposures in the men after leaving school. Other interpretations include: 1) males may have had higher exposures through outdoor sports activities, and 2) the loss to follow-up was higher for women due to name changes.

In discussing the benign neoplasm results, Loughlin *et al.* (1999) noted an elevated rate of mortality from brain tumors in an occupational study (Divine and Hartman, 1996) of butadiene production workers (6 deaths, 3.8 expected). A similar endpoint was reported in workers in the North American SBR industry study by the UAB group (Sathiakumar *et*

al., 1998); an elevated SMR was reported for mortality from benign neoplasms in the subgroup of white ever hourly workers with ≥10 years worked and ≥20 years since hire (181, no CI reported).

Updated studies (Macaluso *et al.*, 1996; Matanoski *et al.*, 1997; Delzell *et al.*, 2001) shed light on styrene's potential role in increasing cancer risks for workers in the SBR industry, more than two decades after IARC (1982a) identified exposures in rubber manufacturing as causing increased rates of certain cancers. Co-exposure to styrene and butadiene has made the effects of the individual chemicals difficult to untangle, although earlier studies (Meinhardt *et al.*, 1982; Matanoski *et al.*, 1990, 1993; Santos-Burgoa *et al.*, 1992) had identified subgroups with statistically significant elevated risks of LHCs and leukemia associated with areas that had styrene and butadiene exposure. While both exposures have been associated with lymphohematopoietic system cancers, the correlations between these two exposures are often high. Additionally, co-exposure to benzene, a known human leukemogen, has complicated the picture for workers in some plants in these studies.

Two research groups (JHU: Matanoski *et al.*, 1997; UAB: Macaluso *et al.*, 1996) re-examined previously studied North American SBR cohorts, and attempted to distinguish the roles of styrene and butadiene exposures in LHC mortality. The cohorts have substantial overlap: the cohort evaluated by Macaluso *et al.* (1996) included workers at seven of the eight plants studied by Matanoski and her colleagues, and combined these with workers from a two-plant cohort (Table 27) studied by Meinhardt *et al.* (1982). Both groups developed exposure estimates that enabled them to separately examine styrene and butadiene exposures, with one study examining risks of leukemia (Macaluso *et al.*, 1996), and the other including leukemia as well as other LHCs (Matanoski *et al.*, 1997). Both sets of researchers collected information to assess the risk associated with exposure intensity as well as cumulative exposure; however, only the Matanoski *et al.* (1997) report presented results related to exposure intensity.

The two groups' main findings with respect to leukemia are consistent. In both studies, the contribution of average styrene exposure to leukemia risk is statistically nonsignificant. For butadiene, both groups found elevated risks. The RRs calculated by the UAB group (Macaluso *et al.*, 1996) were suggestive of a dose-response effect for cumulative butadiene exposure and leukemia risk. An OR for a comparable cumulative butadiene exposure value was not reported by the JHU group; however, in their cumulative exposure model, both butadiene and styrene exposure played a role, as did exposure duration (Matanoski *et al.*, 1997).

With respect to analyses of LHCs other than leukemia, the UAB group did not estimate risks for styrene and butadiene and all LHCs or any specific LHCs other than leukemia. The JHU group (Matanoski *et al.*, 1997) did analyze other LHCs, and reported statistically significant associations between the continuous measure of styrene exposure (at 1 ppm time-weighted average) and all LHCs combined (including leukemia), all lymphomas, lymphosarcoma, and myeloma. No statistically significant association was found for butadiene exposure with any LHC cause of death other than leukemia (Matanoski *et al.*, 1997), although the OR was elevated for Hodgkin's disease.

The Matanoski *et al.* (1997) results must be regarded cautiously because of concerns regarding the methodology and the analyses. Only LHC cases were used in these analyses, while controls were matched to all cancer cases; thus, there were many controls for which there were no matched cases. For this reason, the control group was treated as unmatched in the analysis. Because controls had to have lived as long as, or longer than cases, the duration of employment in controls as a whole was longer than that of cases, leading to possible higher cumulative exposures. This bias would lead to conservative odds ratios (lower than they should be). Other biases may also have resulted from unmatched analysis of partially matched data. Additional concerns relate to the transformation of exposures. The use of the log transformation in the fitted models imposes a steep dose-response curve at very low exposures, followed by a very shallow one at higher levels. No alternative parameterizations were discussed. The authors did not report data on the distributions of estimated exposures over time, or on the distribution of cumulative or TWA exposures in the cases or controls or overall.

Other limitations of these studies include the exposure estimates. Both approaches, one relying on narratives and modeling (Macaluso *et al.*, 1996) and the other on industrial hygiene monitoring data (Matanoski *et al.*, 1997), may misclassify exposure for individuals and for groups of workers. This misclassification may make it more difficult to identify chemical effects. The concordance of results of these two groups on the one endpoint (leukemia) that both examined provides some reassurance that the estimates are reasonable, and at least relatively accurate. However, the high correlations between butadiene and styrene exposure in these workers limit the ability of any study to determine the contribution of each. The association of styrene exposure and risk of lymphosarcoma seen in the JHU analyses is somewhat unexpected, given that increases in this endpoint have not been seen in previous studies of styrene-exposed workers, but have been found in studies of butadiene production workers (Divine *et al.*, 1993; Ward *et al.*, 1995 and 1996), who did not have styrene exposure. As Matanoski *et al.* (1997) note, "[I]f both agents are carcinogenic for the lymphatic system, it may be difficult to distinguish risks from exposure to each [agent] independently without more precise dose information [than was available on these cohorts of SBR workers]." Finally, from a mechanistic point of view, inhibition of the metabolic activation of butadiene by co-exposure to styrene may influence risks faced by workers exposed to both chemicals. It is not clear what impact this possibility would have on the dose-response relationship.

More recently, Graff *et al*. (2005) and Delzell *et al.* (2006) updated the mortality analysis for synthetic rubber workers, concentrating on the effects in males who had worked at least a year in any of the eight plants between 1944 and 1998. This added 18 percent more person-years to the earlier follow-up. The analysis focused on exposure to butadiene, styrene and dimethyldithiocarbamate (DMDTC), and cancer mortality from leukemia, non-Hodgkin lymphoma, and other lymphohematopoietic cancers (LHCs). Vital status was established for 97 percent of the 18,000 men in the cohort, and cause-of-death for 98 percent of those who had died. Mortality rates were compared with the general population. Exposures to the three chemicals were estimated from work records and interviews, with modeling used to aid in predicting workplace concentrations. Further analytical details are available in Macaluso *et al.* (2004).

Fewer deaths were found than expected compared to the general population from all causes combined and for all cancers except for colorectal, prostate and certain LHCs, which were all higher than expected. Leukemia showed the highest ratio of observed to expected deaths, concentrated among hourly workers, and highest in workers with 20 to 29 years since hire, and in workers with 10 or more years of employment. In single-agent analyses, associations were found between leukemia and cumulative exposures to butadiene, DMDTC, and styrene. Correcting for co-exposures to the other two chemicals, the relationship was retained for butadiene and DMDTC, but lost for styrene. Delzell *et al.* (2006) concluded that the results strengthened the case for human carcinogenicity (leukemia) induced by butadiene, made a weaker case for DMDTC, and provided no evidence to support any carcinogenic effects of styrene.

Sathiakumar and coworkers (Sathiakumar and Delzell, 2007, 2009; Sathiakumar *et al*., 2009) have recently reported on mortality from cancer and other diseases among 4863 women employed at eight SBR plants in North America (181,831 person-years of follow-up, average 37 years). Cause-specific standardized mortality ratios and 95 percent confidence intervals were calculated for multiple cancer types, including leukemia, Hodgkins and non-Hodgkins lymphoma, multiple myeloma, lung, breast, bladder, and ovary cancer, etc. Ever-hourly employed women had more-than expected deaths from lung cancer (47 observed, 30 expected; SMR 159, CI 117-211) and bladder cancer (6 observed, 1.8 expected; SMR 332, CI 122-723). There was no statistically significant excess of any other tumor, including LHCs (34 observed, 36 expected; SMR 95, CI 66-133). Death rates for all causes combined and for all cancers were slightly lower than the rates of general population comparison groups. The authors reported no association of cancer rates with butadiene or styrene exposures in exposure-response analyses from the six out of eight plants with sufficient data.

However, there is actually an increased rate of lung cancer with exposure to both styrene and butadiene. The small number of cases makes it difficult to observe significant trends, but it should be noted that the next-to-highest styrene exposure level has a statistically significant increase on the adjusted RR basis (Table 4 of Sathiakumar and Delzell, 2009). To us, the increases in lung cancer versus styrene exposure appear to reveal a trend, as shown in Table 28A. We calculated the unadjusted relative risks from deaths/PY; the adjusted RRs in the right column (from Sathiakumar and Delzell, 2009) are adjusted for age, years since hire, and ever-hourly status. Providing unadjusted rates is useful as a check against the possibility that overadjustment may obscure a valid association.

Because no increases in lung or bladder cancer were observed among the men at the same plants, and there was no increase in lung cancer (the site with the greatest number of excess tumors) rate with increasing years of service, the authors concluded that the increased tumor types in ever-exposed women only were not likely to have been related to occupational exposures (Sathiakumar and Delzell, 2009). Sathiakumar *et al.* (2009), with a more robust statistical analysis, conclude that there is no causal association between either styrene or butadiene and the increased lung cancer rate in ever-hourly women, and suggest that confounding by smoking accounted at least in part for the observed excess of lung cancer.

Table 28A. Relative Rates (RR) of Lung Cancer Versus Styrene Exposure (Sathiakumar and Delzell, 2009)

Styrene ppm-yrs	Deaths/PY	Unadjusted RR	Adjusted RR (95%CI)
0	60/118,785	1.0	1.0
>0 to <0.8	11/17,046	1.28	1.5 (0.8-3.0)
0.8 to <6.0	11/15,420	1.41	1.3 (0.7-2.6)
6.0 to <21.6	11/7,321	2.98	2.5 (1.3-5.0)
21.6+	11/8,705	2.50	1.7 (0.8-3.5)

Case-control Studies in a Range of Occupational Settings

Five case-control studies examined styrene exposure (along with exposure to other chemicals and agents) in a range of occupational settings (Flodin *et al.*, 1986; Cantor *et al.*, 1995; Gerin *et al.*, 1998; Parent *et al.*, 2000; Dumas *et al.*, 2000). Each found elevated, in some cases statistically significant, cancer risk associated with styrene exposure. However, because of the study design limitations, these studies are not considered in detail.

Flodin *et al.* (1986) examined potential risk factors for acute myeloid leukemia in a case-referent study in Sweden. The primary exposure of interest was background radiation, evaluated by means of a gamma radiation index. By a mailed questionnaire, cases (n = 59) and referents (n = 354) self-reported qualitative data on occupational exposures, in addition to other information. Exposure to styrene was associated with a significantly increased odds ratio (OR = 18.9, 95 percent CI = 1.9-357), based on a small number of exposed subjects (three cases, one referent).

Cantor *et al.* (1995) found an elevated risk of breast cancer mortality in both black and white women in relation to occupational styrene exposure in a case-control study, where exposure probability and level were estimated based on occupation listed on the death certificate. Limitations include lack of control for known breast cancer risk factors, as well as lack of information on actual exposure levels or duration.

A series of reports by Gerin *et al.* (1998), Parent *et al.* (2000), and Dumas *et al.* (2000) from a hypothesis-generating population-based case-control study in Montreal found elevated risks of rectal, prostate, and renal cancer, and of lymphoma, in relation to styrene exposure. Incident cancer cases were identified from area hospitals and interviewed. A detailed exposure assessment was based on experts' translation of lifetime occupational history into specific exposures, and all cases had confirmed histological profiles and information on potential confounders. Comparisons were made to a pool of population controls (n = 533) and a subset of the cancer cases. No multiple comparison corrections were made, although in Gerin *et al.*, 56 different associations were examined. Gerin *et al.* (1998) examined 15 types of cancer (excluding leukemia) and occupational exposure to several monocyclic aromatic hydrocarbons including styrene. The authors estimated that 2 percent of the 4,263 subjects had potential occupational exposure to styrene, 0.1 percent at a high concentration. Risks for non-

Hodgkin's (8 cases, OR = 2.0, 95 percent CI = 0.8-4.8) and Hodgkin's lymphoma (2 cases, OR = 2.4, CI = 0.5-11.6) were elevated (not statistically significant) for those with any occupational exposure to styrene. Rectal cancer risk was significantly elevated in those exposed to medium or high styrene (5 cases, OR = 5.1, CI = 1.4-19.4), as was prostate cancer (7 cases, OR = 5.5, CI = 1.4-21.8).

Dumas *et al.* (2000) examined exposures associated with rectal cancer among cases (n = 257) in the study, of whom five had substantial occupational styrene exposure as defined (>5 years since first exposure, >5 years duration, and at least medium concentration and exposure frequency). Rectal cancer risk was significantly elevated in those with substantial occupational exposure to styrene (OR = 3.9, 95 percent CI=1.2-12.9) in the unconditional logistic regression model that controlled for age, education, smoking, beer consumption, and body-mass index.

In the third report from the Montreal study, Parent *et al.* (2000) reported on analyses of renal cell carcinoma cases (n = 142), of whom ten had exposure to styrene-butadiene rubber, although none had substantial exposure as defined above. For those with any occupational exposure to styrene-butadiene rubber, renal cell cancer risks were elevated (OR = 1.8, 95 percent CI = 0.9-3.7) in the unconditional logistic regression model controlled for both occupational (felt dust exposure) and non-occupational (age, smoking, and body-mass index) risk factors.

Ecological study

Coyle *et al.* (2005) conducted an ecological study of the possible association of the release of styrene (and of 11 other industrial chemicals) with breast cancer incidence in Texas. From 1995 to 2000, 54,487 cases of invasive breast cancer were reported to the Texas cancer registry. The 12 toxicants were: (1) positively associated with breast cancer in earlier epidemiological studies, (2) either U.S. EPA Toxics Release Inventory (TRI) chemicals designated as carcinogens or had estrogenic effects associated with breast cancer risk, and (3) had releases consistently reported to EPA TRI for multiple Texas counties during 1988 to 2000. Univariate and multivariate analyses adjusted for race and ethnicity were used to examine the association of toxicant releases during 1988 to 2000 with the average annual age-adjusted breast cancer rate at the county level. Univariate analysis indicated that styrene (as well as formaldehyde, methylene chloride, tetrachloroethylene, trichloroethylene, chromium, cobalt, copper, and nickel) was positively associated with the breast cancer rate. Multivariate analyses indicated that styrene TRI-reported releases were positively associated with the breast cancer rate in (1) women and men (beta = 0.219, p = 0.004), (2) women (beta = 0.191, p = 0.002), and (3) women ≥ 50 years old (beta = 0.187, p = 0.002). During the period of the study Texas ranked first in the U.S. in industrial emissions of styrene as reported to the TRI.

Burns *et al.* (2006), in commenting on this study, note that the rates of breast cancer in Texas are the lowest in the nation for all races (110 vs. 127/100,000), whites (110 vs. 130), blacks (104 vs. 107), and Hispanics (77 vs. 87). They question why it should be assumed that breast cancer rates are elevated because of styrene exposure when in fact the Texas breast cancer rates are lower than in other areas with lower styrene emissions. Burns *et al.* also comment, "Based on ongoing monitoring, ambient styrene monomer exposures in the Houston area average 0.018 ppb, whereas exposures in reinforced

plastics workers have been 50,000 ppb or greater. In other words, industrial exposures are approximately 3 million fold higher than environmental exposures in the area of Texas. It is not biologically plausible that styrene could increase breast cancer rates among individuals living in areas with extremely low exposure potential without evidence of risk among those highly exposed in the industrial settings."

OEHHA disagrees that there is no evidence of risk for occupational exposures, but thought it worthwhile to investigate whether the small styrene exposures through ambient air could be likely to cause an observable increase in breast cancer in the Texas population. Coyle *et al*. (2005) state that "ambient air in urban locations and indoor air may both be principal routes of styrene exposure for the general population," thus substantiating the relevance of this exposure route to the observed increase in breast cancer rates. Inhalation exposure to 0.018 ppb styrene around Houston (a major industrial area) would result in a dose of 0.015 µg/kg-day for a 65 kg female, assuming a breathing rate of 20 m^3/day and pulmonary retention of 65 percent. At a styrene cancer potency of 0.026 $(mg/kg\text{-}day)^{-1}$ (derived from the data of Cruzan *et al*., 2001), this yields a lifetime cancer risk of 3.9×10^{-6}, or 0.4/100,000. Thus the upper-bound cancer potency yields a projected excess *lifetime* cancer risk of about 0.4 percent of the Texas state-wide *annual* breast cancer rate. Coyle *et al*. report a "median average annual age-adjusted breast cancer rate" of 66.2 in 61 counties with styrene releases reported, versus 59.7 in 193 counties without reported styrene releases ($p < 0.001$ by Mann-Whitney U test; see their Table 3). This difference is more than two orders of magnitude greater than the estimated excess cancer risk from styrene in air in Houston. Styrene releases are predominantly from industrialized, urban counties constituting the larger population centers in Texas. The multiple regression model used by Coyle *et al*. did not account for population variables related to income, occupation, education or other socioeconomic indicators and risk factors which have been shown to be related to breast cancer rates (Robbins *et al*., 1997; Prehn and West, 1998; Baquet and Commiskey, 2000; Albano *et al.*, 2007). Thus there seems little reason to infer that the higher breast cancer rates in industrialized counties in Texas could be related to the styrene releases in those counties.

Summary and Conclusions

In considering the human evidence of styrene carcinogenicity, three main occupational exposure settings and data sets were examined: the manufacture of styrene monomer and polystyrene; the production of reinforced plastics; and the manufacture of styrene-butadiene rubber. Case-control studies conducted in the U.S. and Canada were also briefly reviewed. While each of these data sets provides some evidence of an effect of styrene on cancer risk, the strongest evidence comes from reinforced plastics workers.

Workers exposed to styrene in the manufacture of styrene monomer and polystyrene had exposure to multiple other chemicals, with some work environments including exposure to benzene, ethylbenzene, or 1,3-butadiene, as well as other potentially carcinogenic agents. Styrene exposure levels of workers in this industry were generally lower (mean levels up to 35 ppm) than those in the manufacturing of reinforced plastics. Overall, conclusions from the four cohorts analyzed were limited by the mixed nature of the exposures and the relatively small numbers of cases. In the one study (Ott *et al.*, 1980; Bond *et al.*, 1992) that attempted to address co-exposures and had an internal comparison

group, overall cancer mortality was lower than expected but LHC mortality was consistently higher, although most results were not statistically significant.

The results from the reinforced plastics worker studies are the most informative because of the relative lack of confounding exposures, the high styrene levels, and the large cohorts. The strongest evidence points to an increased risk of LHCs with styrene exposure. This finding is supported by studies of workers in the reinforced plastics industry (Kolstad *et al.*, 1994; Kogevinas *et al.*, 1994), in which leukemias and malignant lymphomas were both found at high rates among workers with exposures above 60 ppm TWA. The suggestive evidence from the SBR manufacturing studies is somewhat consistent with these findings. However, given the relatively low styrene levels in SBR manufacturing compared to those in reinforced plastics production, it is unclear how the SBR studies have been able to observe an effect of styrene exposure; it is possible that residual confounding from butadiene or benzene exposure might have played a role in the SBR findings for styrene.

Of the reinforced plastics studies, both the Kolstad *et al.* (1994) study and the multi-country study of Kogevinas *et al.* (1994) provide evidence that styrene's effects are stronger after a latency period. Analyses of LHC risk in the Kogevinas cohort in relation to time since first exposure (latency) showed a statistically significant trend in the overall cohort of increasing LHC risk with increasing time (Table 29), an effect not seen with cumulative exposure:

Table 29. Relative Risk of Lymphohematopoietic Cancer (LHC) as a Function of Length of Exposure to Styrene

Length of Exposure	Relative Risk (RR)
<10 years [comparison group]	1.0
10 to 19 years	2.90, 95 % CI = 1.29-6.48
≥20 years	3.97, 95 % CI = 1.30-12.13

p for trend test = 0.012

Findings from the reinforced plastics industry on mortality from esophageal and pancreatic cancers are also notable. Relative risks increased with cumulative exposure, and for workers identified as having the higher exposures. Esophageal cancer was elevated in Wong *et al.* (1994), who observed an SMR of 192 (95 percent CI = 105-322), and in Kogevinas *et al.* (1994), who observed an SMR of 181 among laminators (considered to have the highest exposure in the cohort). Kogevinas *et al.* (1994) found that esophageal cancer mortality rate ratios in the overall cohort increased with cumulative exposure (RR = 1.8, CI = 0.42-7.30 among those with ≥500 ppm-years). Wong *et al.* (1994) found statistically significant elevated mortality (SMR = 295, $p<0.05$) for those with 30-99 ppm-years of styrene exposure. Risks were not elevated in the highest category, ≥100 ppm-years, in which only two cases occurred.

Pancreatic cancer risk was elevated in studies in the reinforced plastics industry (Kogevinas *et al.*, 1994, Kolstad *et al.*, 1995). In the Kolstad cohort, incidence of pancreatic cancer was significantly increased in those who had high exposure probability (IRR = 2.2, 95 percent CI = 1.1-4.5), based on internal comparisons to a similar group of workers lacking styrene exposure; in addition, higher rate ratios were seen in those whose first year of employment was before 1970 (when exposures were higher). The multi-country European study (Kogevinas *et al.*, 1994) found elevated, though not statistically significant, rate ratios of pancreatic cancer, highest for those with the longest time since first exposure (≥20 years, RR = 2.05, CI = 0.6 -7.3). Although this cohort included a portion of the Kolstad *et al.* cohort, increased mortality from pancreatic cancer was also found in other subcohorts, namely among laminators (SMR = 148, CI = 76-258), a high exposure category which does not include the Danish subcohort. Kogevinas *et al.* (1994) found, based on internal comparisons, that mortality rates for pancreatic cancer in the overall cohort increased with cumulative styrene exposure (p for trend = 0.07).

In the SBR industry, the effects of co-exposure to styrene and butadiene have been difficult to untangle. Exposure to each has been associated with cancers of the lymphohematopoietic system, and the correlations between these two exposures in the SBR industry are often high. Co-exposure to benzene, a known human leukemogen, has complicated the picture for workers in some plants. From a mechanistic point of view, inhibition of the metabolic activation of butadiene by co-exposure to styrene may influence risks faced by workers exposed to both chemicals. It is not clear what impact this would have on the dose-response relationship. In addition, if both agents are carcinogenic for the lymphatic system, it may be difficult to distinguish risks from exposure to each agent independently without more precise dose information than was available on the cohorts of SBR workers. It may not be possible to obtain accurate risk estimates for either styrene or butadiene from regression models that include both exposure variables, when these exposures are as highly correlated as they are in the SBR workers studied by Matanoski *et al.* (1997) and Macaluso *et al.* (1996). It is unclear whether the styrene concentrations were sufficiently high in the SBR industry to induce a measurable increase in LHC.

Although studies in both reinforced plastics and SBR manufacturing found slight elevations in risk for lung cancer (e.g., Wong *et al.*, 1994; Kolstad *et al.*, 1995; Sathiakumar *et al.*, 1998), the small magnitude of excesses combined with the lack of adjustment for smoking renders these results difficult to interpret. Wong *et al.* (1994) found a statistically significant increased risk of cancer of the bronchus, trachea, and lung in reinforced plastics workers of both genders combined (SMR = 141, $p<0.05$). The report by Kolstad *et al.* (1995) of statistically nonsignificant increased incidences of cancer of the pleura and lung when reinforced plastics workers were compared with the general population, but not when compared with a group of workers in similar industries that lack styrene exposure, suggests that smoking or other factors may account for the results seen. Confounding by exposure to asbestos or silica must also be considered in studies of lung cancer in reinforced plastics workers. Sathiakumar *et al.* (1998) found statistically significant increased lung cancer mortality (SMR = 124, 95 percent CI = 104-143) among a subset of SBR workers in a high exposure job (maintenance). Sathiakumar *et al.* (1998) did not have information on smoking in the workers studied, but attempted to address the concern about confounding by comparisons using state rather than national

mortality rates for lung cancer analyses, apparently assuming that these would more accurately reflect local smoking prevalence.

Among women workers, present in small numbers in the reinforced plastics cohorts, reproductive organ cancers were consistently elevated. The early reinforced plastics studies reported elevated rates based on small numbers of cases (Okun *et al.*, 1985; Coggon *et al.*, 1987), and in the more recent studies which had more power to detect an effect (Wong *et al.*, 1994; Kogevinas *et al.*, 1994), similar results were found. Wong *et al.* (1994) reported statistically significantly elevated rates of mortality from cancer of the cervix uteri (SMR = 283.5, $p<0.01$) and of female genital organs other than the uterus (SMR = 201.6, $p<0.05$). Kogevinas *et al.* (1994) found statistically nonsignificant increased rates of ovarian cancer, with the highest rates found in the high exposure group (laminators).

Case-control studies provided some evidence for elevated risks for acute myeloid leukemia (Flodin *et al.*, 1986), breast cancer (Cantor *et al.*, 1995), prostate cancer (Gerin *et al.*, 1998), and rectal cancer (Gerin *et al.*, 1998; Dumas *et al.*, 2000) associated with a wide range of occupations with styrene exposure. In a case-control study of lung cancer, Scelo *et al*. (2004) looked at 5979 workers (2861 lung cancer cases, 3118 controls) in seven European countries. Exposures to vinyl chloride, acrylonitrile, and styrene were addressed with full adjustment for smoking. For each job (each worker held several jobs in his or her career), experts assessed the exposure to occupational agents on the basis of detailed occupational questionnaires. Information on tobacco consumption and other risk factors was collected. The odds ratio (OR) for lung cancer for ever exposure to vinyl chloride was 1.05 (95 percent CI = 0.68-1.62). A modest but non-significant increased risk for lung cancer was found in the highest exposed subgroup. The OR for ever exposure to acrylonitrile was 2.20 (CI = 1.11-4.36) with a positive dose-response relationship for cumulative exposure. However, no association between exposure to styrene and lung cancer risk was found (OR for ever exposure = 0.70 (CI = 0.42-1-18)).

In a recent review of the epidemiologic literature on styrene and cancer, Boffeta *et al.* (2009) reviewed studies on workers exposed to styrene in manufacturing and polymerization, in the reinforced plastics industry, and in production of styrene-butadiene rubber. They reported that an association was found between average styrene exposure and non-Hodgkin lymphoma (NHL, $p < 0.05$) in a study of reinforced plastic workers, with no trend with increasing duration of exposure. Also, excess mortality from leukemia was reported in the rubber industry, which they conclude to be in line with that expected from exposure to butadiene. There was an association of styrene exposure with increased esophageal cancer in two U.S. studies of reinforced plastic workers, but not in two European studies. They conclude that there is no consistent increased risk of any cancer among workers exposed to styrene, and that "it is not surprising that an increased risk of a few cancers has been occasionally found" in some of the relatively large number of styrene studies. Their overall conclusion is that "The evidence for human carcinogenicity of styrene is inconsistent and weak. On the basis of the available evidence, one cannot conclude that there is a causal association between styrene and any form of cancer."

OEHHA comes to a different conclusion on these data; we believe there is suggestive evidence that occupational exposures to styrene have increased the risk of cancer,

particularly at lymphohematopoietic sites. In addition to the LHCs, the findings of elevated risks for cancers of the esophagus and pancreas are weak but relatively consistent. To achieve conclusive findings for lung cancer, careful studies that collect high quality information on smoking and other potential confounders (*e.g*., exposure to silica or asbestos) would be necessary. Suggestive associations with female reproductive organ, breast, prostate, and rectal cancers deserve further investigation. In summary, we acknowledge that the epidemiological data are inconclusive, but, along with the supporting data described in other sections of this document, these data help justify concern about human carcinogenicity of styrene.

Mechanisms of Carcinogenicity

There are two aspects to the biochemical mode-of-action of styrene in the carcinogenic process. One is the direct action of styrene or a metabolite (e.g., styrene-7,8-oxide) on genetic material that results in the initial step of carcinogenesis; hence, the label of styrene as an initiator. This activity is considered relevant for human LHCs (see previous section on Carcinogenicity in Humans) and mouse lung tumors (Cruzan *et al.*, 2001). The second aspect is an indirect action of styrene on a central nervous system pathway that controls serum prolactin. The indirect mode-of-action may be applicable to the observation of styrene-related rat mammary tumors (Conti *et al.*, 1988).

Direct Evidence

A major metabolite of styrene metabolism is styrene-7,8-oxide, a reactive chemical classified by IARC (1994b) as a probable human carcinogen and by NTP (2001) as "reasonably anticipated to be a human carcinogen." Styrene-7,8-oxide has been detected free or bound to tissue macromolecules in the blood of humans exposed to styrene occupationally or under controlled laboratory conditions (Engstrom *et al.*, 1978; Wigaeus *et al.*, 1984; Lof *et al.*, 1986; Christakopoulos *et al.*, 1993; Korn *et al.*, 1994; Johanson *et al.*, 2000; Tornero-Velez *et al.*, 2001). The metabolite has been detected in blood and other tissues of styrene-exposed rats and mice (Lof *et al.*, 1984; Nordqvist *et al.*, 1985; Mendrala *et al.*, 1993; Osterman-Golkar *et al.*, 1995; Leavens *et al.*, 1996; Cruzan *et al.*, 1998; Boogaard *et al.*, 2000; Cruzan *et al.*, 2001). Human and rodent tissues, including lung and blood, have the capacity to metabolize styrene to styrene-7,8-oxide, and more than one enzyme system may be involved with the bioactivation (Elovaara *et al.*, 1991; Mendrala *et al.*, 1993; Nakajima *et al.*, 1994a,b; Kim *et al.*, 1997; Carlson, 1997; Carlson *et al.*, 1998; Hynes *et al.*, 1999; Carlson *et al*., 2000; Wenker *et al.*, 2001). Hence, humans and rodents have the capacity for *in situ* metabolism of styrene to a carcinogenic metabolite in blood and in the lung.

Mutagenicity has been shown for styrene in studies with bacterial strains that are sensitive to base-substitution in the presence of metabolic activation system (IARC, 1994a). Styrene-7,8-oxide is also a mutagen towards the base-substitution sensitive bacterial strains, but metabolic activation is not required (IARC, 1994b). Mutagenesis is supported by the observation of styrene-7,8-oxide-DNA adducts in styrene exposed workers and in rodent studies (Nordqvist *et al.*, 1985; Cantoreggi and Lutz, 1993; Horvath *et al.*, 1994; Pauwels *et al.*, 1996; Vodicka *et al.*, 1999, 2001; Marczynski *et al.*, 1997; Boogaard *et al.*, 2000; Koskinen *et al.*, 2001; Otteneder *et al.*, 2002). Adduction

was detected at the exocyclic region (Horvath *et al.*, 1994; Pauwels *et al.*, 1996; Vodicka *et al.*, 1999; Otteneder *et al.*, 2002), and such adducts, e.g., O^6- and N^2-guanine, possess mutagenic properties (van Zeeland and Vrieling, 1999; Koskinen and Plna, 2000). Other styrene-derived DNA adducts were alkylated at ring guanine or adenine sites (Nordqvist *et al.*, 1985; Pauwels *et al.*, 1996; Boogaard *et al.*, 2000; Vodicka *et al.*, 2001) and these adducts can form apurinic and/or open ring sites that lead to base-pair changes (van Zeeland and Vrieling, 1999; Solomon, 1999; Koskinen and Plna, 2000).

A role of styrene mutagenesis in carcinogenicity, through the action of styrene-7,8-oxide, is further suggested by the genotoxicity findings. Chromosomal aberrations (CA), sister chromatid exchanges (SCE), micronuclei (MN), DNA strand breaks, gene mutation, and DNA adducts were detected in humans exposed to styrene *in vivo* or *in vitro* (IARC, 1994a; Anwar and Shamy, 1995; Lee and Norppa, 1995; Bigbee *et al.*, 1996; Bonassi *et al.*, 1996; Kolstad *et al.*, 1996; Somorovska *et al.*, 1999; Vodicka *et al.*, 1999; Oberheitmann *et al.*, 2001; Laffon *et al.*, 2002a,b). Several genotoxicity endpoints were detected in styrene-exposed rodents (Vaghef and Hellman, 1998; Vodicka *et al.*, 2001). Among styrene-exposed workers *in vitro* and among rodents *in vivo*, dose-related changes in genotoxicity parameters are noted (Solveig-Walles and Orsen, 1983; Chakrabarti *et al.*, 1993; Kligerman *et al.*, 1993; Walles *et al.*, 1993; Yager *et al.*, 1993; Artuso *et al.*, 1995; Bonassi *et al.*, 1996; Pauwels *et al.*, 1996; Vaghef and Hellman, 1998; Somorovska *et al.*, 1999). Styrene-7,8-oxide was also genotoxic *in vitro* in non-bacterial systems, at lower doses than would be expected for styrene (Lee and Norppa, 1995; Bastlova *et al.*, 1995; Ollikainen *et al.*, 1998; Laffon *et al.*, 2001, 2002a; Laws *et al.*, 2001). Correlations of specific genotoxic actions in humans with styrene exposure are complicated by lack of good information on human polymorphisms associated with particular genotoxic endpoints (Rueff *et al*., 2009).

The putative carcinogenic metabolite, styrene-7,8-oxide, is an exocyclic epoxide. Evidence for the presence of an arene oxide, styrene-3,4-oxide, in styrene exposed humans and rodents was inferred by the presence of its metabolite vinylphenol (Bakke and Scheline, 1970; Pantarotto *et al.*, 1978; Pfaffli *et al.*, 1981). Additional studies showed that styrene-3,4-oxide is a highly reactive compound and a bacterial mutagen (Watabe *et al.*, 1982). Although apparently present in very small quantities, its high mutagenicity suggests that it may play an important role in styrene mutagenicity.

While mode-of-action studies have focused on styrene epoxides, other styrene metabolites have been associated with genotoxicity. The oxidative metabolite 8-hydroxydeoxyguanine was detected in styrene-exposed workers (Marczynski *et al.*, 1997) and in transformed Syrian hamster embryo cells (Zhang *et al.*, 2000). A mercapturic acid styrene metabolite was genotoxic to human lymphocytes in whole blood cultures (Zhang *et al.*, 1993).

The observed mutagenic and genotoxic properties of styrene and of a major styrene metabolite, styrene-7,8-oxide, strongly suggest that styrene is a genotoxic carcinogen that acts by combining with DNA and altering the normal functioning of the genetic material. Other styrene metabolites may possess genotoxic properties; however, their role in the carcinogenic process has not been thoroughly investigated.

Cruzan *et al.* (2002, 2005c) have proposed an alternate hypothesis for the mode of action (MOA) of styrene, based on their conclusion that the respiratory tracts of mice are particularly sensitive to styrene (Cruzan *et al*., 2002). Styrene inhalation leads to cytotoxicity in terminal bronchioles, bronchioloalveolar tumors, and degeneration and atrophy of nasal olfactory epithelium in mice. In rats, terminal bronchioles are not affected, and effects in the nasal olfactory epithelium occur to a lesser degree (and from higher styrene concentrations) than in mice. The authors state that cytotoxicity and tumor formation are not related to blood levels of styrene or styrene oxide measured in chronic studies and that rats and mice metabolize styrene differently (including 4- to 10-fold higher activity of the ring-oxidation and phenylacetaldehyde pathways in mice).

In the mouse respiratory tract, local metabolism of styrene by CYP2F2 results in cytotoxicity. Cells and tissues with high levels of CYP2F2 isoforms produce a high ratio of R-SO to S-SO (at least 2.4:1). Inhibition of CYP2F2 activity with 5-phenyl-1-pentyne prevents the styrene-induced cytotoxicity in mouse terminal bronchioles and nasal olfactory epithelium. The R-SO enantiomer is more toxic to mouse terminal bronchioles than S-SO. Furthermore, 4-vinylphenol (produced by ring oxidation of styrene) is highly toxic to mouse terminal bronchioles and is also metabolized by CYP2F2 (Vogie *et al*., 2004; Carlson, 2004). In human nasal and lung tissues, styrene metabolism to SO is below the limit of detection in nearly all samples; the most active sample of human lung had less than one percent the activity of mouse lung. The PBPK model for styrene of Cruzan and colleagues (Sarangapani *et al*., 2002) predicted that humans do not generate sufficient levels of CYP2F2-generated metabolites in the terminal bronchioles to reach a toxic level. Their postulated mode of action is that tumors arise from cytotoxicity and cell proliferation in the terminal bronchi as a result of these metabolites. They argue that this MOA is specific to the mouse and is unlikely to be significant in humans (Cruzan *et al*., 2005c).

Cruzan *et al*. (2009) further investigated the activity of the CYP2F isoforms in mouse, rat, and human lungs, relative to metabolism of styrene. They concluded that rat CYP2F4 appears to be as active as the mouse CYP2F2 isoform in metabolizing styrene and related chemicals. However, the amount of the enzyme is less in the rat Clara cells, and the resulting levels of metabolites are lower, which they consider insufficient to cause cytotoxicity in rat lung. They found that microsomes prepared from human lungs produced little or no styrene metabolites, and that human lungs contain fewer Clara cells than mice or rats. Cruzan *et al.* also suggest that humans should be much less sensitive than mice to toxicity due to the metabolites, because of differences in the morphology of their Clara cells. They conclude that it is unlikely that chemicals that cause mouse lung tumors via CYP2F2 metabolism will cause lung tumors in humans via the corresponding CYP2F1 isozyme. Other chemicals in this category addressed by Cruzan *et al*. (2009) are coumarin, cumene, naphthalene, benzofuran, ethylbenzene, and alpha-methylstyrene.

Another alternate MOA hypothesis has been proposed by Hoffmann *et al*. (2006) based on the styrene-7, 8-oxide burden in ventilated, perfused lungs of mice and rats exposed to styrene vapor. Hoffmann *et al*. observed that at styrene concentrations up to 410 ppm, the mean styrene oxide levels in mouse lungs were only two-fold higher than that observed in rat lung. The authors postulate that this difference, when translated to *in vivo* conditions, is too small to account for the observed difference in tumorigenicity. They

propose an MOA based on the differential depression of glutathione (GSH) predicted in PBPK modeling of mice and rats (Csanady *et al.*, 2003). The PBPK model estimated metabolism and GSH levels in the alveolar and conducting airways separately. An exposure to 20 ppm styrene for six hours resulted in a higher concentration of styrene oxide in the terminal bronchi of mice than of rats and an even larger depression of tissue GSH, about a 40 percent decrease in mice versus a marginal decrease in rats (about 5 percent based on Csanady *et al*. Fig 5A). Hoffmann *et al.* proposed that repeated styrene oxide perturbation of GSH homeostasis in the terminal bronchi of mice leads to apoptosis and regenerative cell proliferation of Clara cells, leading eventually to tumor formation. Other metabolites in addition to the 7, 8-oxide may also participate in the process.

These are interesting hypotheses for the MOA of styrene-induced tumors, despite being specific to the lung. Aside from the very limited human data supporting the lack of relevant human metabolism (i.e., CYP2F1, etc.), and inadequate characterization of all the ring metabolites involved in addition to 4-VP, OEHHA cannot discount the potential direct role of styrene 7,8-oxide and/or a ring epoxide in a genotoxic MOA of relevance to humans. Also it is unclear how to distinguish between reduced GSH *per se* and increased tumorigenicity due to cytotoxicity and proliferation, as opposed to that due to increased direct genotoxic action of SO on DNA, which is also a likely result of decreased tissue GSH. In situations where more than one MOA may be operative, the genotoxic MOA is likely to dominate at low exposure concentrations.

Recent studies focus on the generation of reactive oxygen species (ROS) by styrene and R-styrene oxide (R-SO) in isolated mouse lung Clara cells exposed *in vitro* or *in vivo*. The Clara cells located in the terminal bronchioles of mice are a main target of styrene metabolism. Clara cells secrete Clara cell 10-kDa protein (CC10), which has anti-inflammatory and antioxidant properties. Treatment *in vitro* with 1 mM styrene or metabolites resulted in significant increases in ROS (using a fluorescence assay) over controls for styrene, R-SO, S-SO, and racemic (RS)-SO (all $p < 0.05$), but not for 4-vinyl phenol (Harvilchuck *et al*., 2009). Similar results were obtained in Clara cells isolated from mice administered styrene (600 mg/kg, i.p.), R-SO, S-SO, RS-SO (all 300 mg/kg, i.p.), or 4-VP (100 mg/kg, i.p.). In addition, Clara cells isolated after i.p. treatments with R-SO showed significant increases in superoxide dismutase (SOD) activity, 8-hydroxydeoxyguanosine (8-OHdG/10^6 2dG), and the ratio of bax/bcl-2 mRNA and protein expressions, all indicating oxidative stress and oxidative effects, and increased caspase 3 activity indicating apoptosis. Harvilchuck *et al*. (2008) and Harvilchuck and Carlson (2009) reported significant decreases in CC10 mRNA and protein expression in isolated mouse Clara cells after i.p. treatment of mice with styrene or R-SO. Chronic decreases in CC10 levels may also contribute to oxidative stress in Clara cells. Oxidative stress and oxidative effects like 8-OH-dG may play a role in mouse lung tumorigenesis.

This styrene risk assessment does not assume tumor site concordance in its interspecies extrapolation. In fact there is typically little tumor site concordance between rodent species. Human epidemiological data on styrene is limited to exposed “healthy” workers in a few industrial settings. Therefore this assessment and the derivation of a health protective PHG are necessarily based on the animal cancer data. The extrapolation of relevance is from cancer in the most sensitive site in animals to *any* cancer in humans with adjustments for pharmacodynamic and pharmacokinetic differences (when no PBPK

model adjustment is available). Thus arguments based on the "species specificity" of styrene-induced lung tumors, while not proven, are also of limited relevance in the overall assessment of human risk.

Structure Activity Considerations

In addition to styrene oxide, other compounds that are structurally related to styrene are carcinogenic. Both ethylene oxide and propylene oxide are mutagenic and carcinogenic. Ethylbenzene, which does not have an unsaturated side chain, is an animal carcinogen. Benzene, naphthalene, and benzo(a)pyrene only contain aromatic rings and are carcinogenic. Table 30 shows related chemicals, their structures, their IARC classification for carcinogenesis, and their potency, if available.

Indirect Evidence

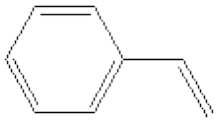
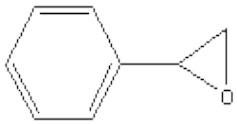
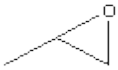
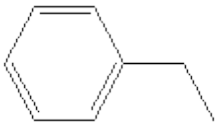
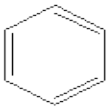
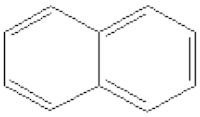
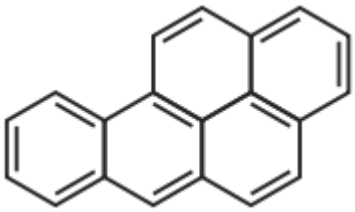
Another potential mode-of-action of styrene carcinogenicity is an indirect one that may operate through a modulation of an endocrine system. Such activity could be important in the development of styrene-related mammary tumors in rat (Conti *et al.*, 1988). Biochemical studies in experimental animals suggest that exposure to styrene may lead to decreased levels of dopamine in the hypothalamus (reviewed by Mutti, 1993). Prolactin secretion is under the control of the hypothalamus through negative feedback, and increased serum levels of this hormone have been observed in styrene exposed workers (Bergamaschi *et al.*, 1997). In rats, increased prolactin levels have been associated with increased spontaneous mammary tumors and an enhanced growth of preformed mammary tumors (spontaneous or carcinogen induced) (Meites, 1980).

At the organ level, the immunochemical evidence suggests that the mouse lung tumor may originate in the alveolar region, in particular from Type II cells (Brown, 1999). Bronchiolar Clara cells appear to be involved with styrene induced bronchiolar hyperplastic tissue, separate from involvement with the alveolar region. Although it is difficult to unequivocally ascribe tumor origin to solely one region of the lung, the absence of Clara cell specific protein in mouse lung styrene-derived adenomas may indicate the involvement of type II cells in styrene lung tumorigenesis. The role of the bronchiolar Clara cells in hyperplasia is consistent with the non-cancer pneumotoxicity of styrene in humans and experimental animals (Coccini *et al.*, 1997; Cruzan *et al.*, 2001).

Summary

The carcinogenicity of styrene can operate through the genotoxic action of one or more reactive epoxide metabolites, e.g., styrene-7,8-oxide and/or styrene-3,4-oxide. These epoxides are present in the blood of styrene-exposed humans and experimental animals and each is a bacterial mutagen. Styrene-7,8-oxide is genotoxic. Exposure to styrene results in styrene-7,8-oxide adducted DNA, and these adducts possess direct or indirect mutagenic activity. Enzymes that metabolize styrene to styrene-7,8-oxide are available in the tissues where styrene-related tumors have been observed, i.e., human blood and mouse lung. While a genotoxic mechanism may be involved in the formation of rat mammary tumors, a non-genotoxic mechanism that involves endocrine dysfunction may also be operative.

Table 30. Structure-activity Comparison of Chemicals related to Styrene

Chemical	**Structure**	**Potency** (mg/kg-day)$^{-1}$	**Prop 65 NSRL** (µg/day)	**IARC Class** (IARC, 2006)
Styrene		0.028 (proposed by OEHHA)		2B (possibly carcinogenic to humans)
Styrene-7,8-oxide		0.16 (OEHHA)	4	2A (probably carcinogenic to humans)
Ethylene oxide		0.31 (OEHHA)	2	1 (carcinogenic to humans)
Propylene oxide		0.24 (U.S. EPA)		2B (possibly carcinogenic to humans)
Ethylbenzene		0.01 (OEHHA)	41 (oral)	2B (possibly carcinogenic to humans)
Benzene		0.1 (OEHHA)	6.4 (oral)	1 (carcinogenic to humans)
Naphthalene		0.12 (OEHHA)	5.8	2B (possibly carcinogenic to humans)
Benzo(a)pyrene		9 (OEHHA)	0.06	1 (carcinogenic to humans)

DOSE-RESPONSE ASSESSMENT

Dose-response evaluations define the relationship between the dose of an agent and a specific adverse effect. A dose-response evaluation usually requires extrapolation from doses administered to experimental animals to the exposures expected from human contact with the agent. When evaluating toxicological effects in animals, it is generally assumed that the animal response to a chemical will be similar to the human response; humans may be more or less sensitive, but are subject to dose-response relationships similar to those in animals. Human exposure data might be useful to better characterize the dose-response relationship of a chemical and its toxic effect or to supplement the information obtained from laboratory experiments.

This section identifies the key studies in animals or humans that provide information on the most sensitive toxicological endpoints. For risk assessment, quantification of the dose levels that cause effects is essential to determine limits of exposure based on risk.

Noncarcinogenic Effects

For risk assessment, chemicals may be considered to exhibit a threshold for toxicity or to have no effective threshold. The basic principle for chemicals that exhibit a threshold is that a specific dose level can be identified below which no toxic effect would be observed; this approach is generally used for non-carcinogenic effects. Risk assessment for chemicals that exhibit a toxicity threshold involves identifying the most sensitive endpoint from all studies, and the highest NOAEL for that endpoint. If no NOAEL can be identified, then the LOAEL is substituted and uncertainty about the actual NOAEL level is accounted for with an uncertainty factor. Alternatively, a benchmark dose (BMD) methodology might be utilized, which uses dose-response models to calculate doses associated with varying effect levels, from the experimental data set. The lower confidence limit on the dose estimated to result in a minimal effect level (below the level of statistical significance) is generally considered to represent the NOAEL equivalent. OEHHA has selected the 5 percent response level as an appropriate benchmark for most circumstances (Fowles *et al*., 1999; OEHHA, 1999, 2000; Collins *et al*., 2004).

The liver, lung, and nervous system are sensitive to the toxic effects from chronic exposure to styrene. Reproductive and developmental toxicity are also observed after exposure to moderate levels of styrene. In this section, the styrene data most pertinent to PHG development are emphasized, typically for experiments exhibiting toxic effects at the lowest dose levels.

Neurotoxicity Dose-Response in Humans

The most comprehensive assessment of CNS disturbances in styrene-exposed workers is the occupational study of Mutti *et al.* (1984b). In this study, memory and sensory/motor function were assessed in groups of 50 styrene-exposed and control workers using a battery of eight neuropsychological tests designed to measure CNS function. Styrene exposure was assessed from urinary MA plus PGA levels the morning after the last workday in the week. The average period of occupational exposure was 8.6 years. Workers were matched for age, sex, and educational level, and a vocabulary test was included to match for general intelligence.

Styrene-exposed workers exhibited significantly poorer performances than controls in all but the digit-symbol test. In addition to reporting data as continuous variables, Mutti *et al.* (1984b) reported the fraction of tested subjects who responded abnormally to at least one, two, or three tests (Table 23). The lowest dose tested was a LOAEL because statistically significant effects (compared to controls) were observed in all dose groups. Mutti *et al.* (1984b) reported a positive correlation of neuropsychological deficits with duration as well as intensity.

In 2000, OEHHA developed a chronic Reference Exposure Level (REL) for inhaled styrene using the data presented in Table 23, applying a BMD approach (OEHHA, 2000; Rabovsky *et al*., 2001). The concentration associated with a 5 percent incidence of three or more abnormal tests (i.e., the "BMC_{05}") was determined to be 1.7 ppm using a probit log dose model in ToxRisk version 3.5. This BMC_{05} is equivalent to a continuous inhalation exposure to 0.61 ppm assuming that the workers breathe half their daily air intake during the workday and work five days/week. Since absorption of styrene by inhalation is approximately 65 percent that by the oral route, 0.61 ppm is equivalent to a dose of 0.48 mg/kg-day by the oral route (0.61 ppm = 2.6 mg/m^3; 2.6 mg/m^3 x 20 m^3 breathed/day ÷ 70 kg = 0.74 mg/kg-day x 0.65 = 0.48 mg/kg-day).

The mechanism for chronic styrene neurotoxicity has not been established. One possibility is binding of the highly reactive styrene oxide to components of nervous tissue. If correct, individuals with higher levels of circulating styrene oxides due to cytochrome P_{450} polymorphism might be at greater risk from styrene neurotoxicity. Another postulated mechanism is an alteration in levels of circulating catecholamines (e.g., dopamine) due to the binding of PGA to these biogenic amines (Mutti, 1993; Mutti *et al.*, 1984c; Checkoway, 1994) and the subsequent changes in physiological functions that are under biogenic amine control.

Respiratory Dose-Response

The few studies on respiratory toxicity in humans did not have adequate data to develop a dose response curve. The Cruzan *et al.* (2001) study in mice is the most sensitive available animal study on respiratory toxicity. Mice of both sexes were chronically exposed via inhalation to 0, 20, 40, 80, or 160 ppm styrene for six hours/day, five days/week (Table 31). At all styrene concentrations, significant changes in lung epithelia were reported: decreased eosinophilic staining of cells in terminal bronchioles, bronchiolar hyperplasia, bronchiolar hyperplasia extending into alveolar ducts, and bronchioloalveolar hyperplasia. Statistically significant differences ($p \leq 0.002$) were detected for both sexes, except for male bronchioloalveolar hyperplasia ($p = 0.2$). Changes in the nasal passages were also reported for all styrene concentrations. Greater changes were reported for olfactory than for respiratory epithelia.

From this study, a LOAEL of 20 ppm is identified for both lung and nasal pathologies, which is equivalent to 3.57 ppm after adjusting for duration and frequency of exposure (3.57 ppm = 20 × 6 hours/24 hours × 5 days/7 days). This is converted to a daily oral human equivalent dose as follows: (0.65 × 3.57 ppm × 4.26 mg/m^3-ppm × 20 m^3/day) ÷ 70 kg = 2.82 mg/kg-day. The factor of 0.65 adjusts for an intake of 65 percent styrene via inhalation, and assumes 100 percent absorption via the oral route. The calculation

also assumes that the effect on respiratory tissue occurs after systemic distribution of the styrene, rather than as a direct effect on tissues at the point of contact.

Cruzan *et al.* (1998) exposed female and male rats for a lifetime to 0, 50, 200, 500, or 1,000 ppm styrene by inhalation for six hours/day, five days/week. Changes in nasal tissue were observed at all doses. Among males, 50 ppm styrene resulted in atrophy and/or degeneration of the olfactory epithelium and "prominent Bowman's glands" in the olfactory epithelium ($p = 7x10^{-6}$ and 0.02, respectively, one-sided Fisher's Exact Test). Among females, significant ($p = 0.04$, one-sided Fisher's Exact Test) atrophy and/or degeneration of the olfactory epithelium were detected. Non-neoplastic changes in the lung were not observed. A LOAEL of 50 ppm (males) was identified, which can be converted from a discontinuous to continuous daily exposure equivalent of 8.92 ppm (50 ppm x 6/24 hours x 5/7 days = 8.92 ppm). Converting ppm to mg/kg-day yields an equivalent human retained dose of 7.02 mg/kg-day (8.92 ppm x 4.26 mg/m^3 per ppm x 20 m^3/day) / 70 kg x 0.65 = 7.02 mg/kg-day). The calculation again assumes that effects are due to the systemically absorbed dose rather than to a direct effect at the point of entry.

Table 31. Bronchiolar Effects of Inhaled Styrene in Mice[1]

Toxicological Effect	Males					Females				
	Styrene (ppm)									
	0	20	40	80	160	0	20	40	80	160
Decreased eosinophilic staining in terminal bronchioles	0/50	29/50	41/50	48/50	49/50	0/50	37/50	46/50	47/50	45/50
Hyperplasia	0/50	10/50	37/50	48/50	46/50	0/50	21/50	39/50	45/50	45/50
Hyperplasia extending to alveolar ducts	0/50	5/50	29/50	35/50	35/50	0/50	9/50	18/50	31/50	40/50

[1] Data from Cruzan *et al.* (2001). Numbers of animals with effect over number examined, after 24 months of treatment in males and 22.5 months in females. All findings are significant by Fisher's exact test at $p \leq 0.002$, except male hyperplasia extending into the alveolar ducts, where $p = 0.03$.

Hepatotoxicity Dose-Response

In humans, increased levels of certain serum enzymes indicate hepatic dysfunction. Among workers exposed to a range of one to 100 ppm styrene for one to 20 years, serum levels of ornithine carbamoyltransferase and alanine aminotransferase were correlated with air levels of styrene (Hotz *et al.*, 1980). In workers exposed to five to 20 ppm styrene for up to 20 years, high serum levels of γ-glutamyltransferase were observed after accounting for alcohol intake (Lorimer *et al.*, 1978). While the human data indicate hepatotoxicity is a concern, they provide limited information on dose-response relationships. For hepatotoxicity, the following animal data support characterization of the dose response.

Srivastava *et al.* (1982) treated male rats (five/dose group) by gavage for 100 days six days/week with 0, 200, or 400 mg/kg-day of styrene. Compared to vehicle controls, significant changes in liver enzymatic activities were reported at both doses. At 200 mg/kg-day microsomal benzo[a]pyrene hydroxylase, aminopyrine-N-demethylase, glutathione-S-transferase, lysosomal β-glucuronidase, and mitochondrial succinic dehydrogenase were changed compared to controls. At 400 mg/kg-day serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and lysosomal acid phosphatase were changed compared to controls. Hepatic benzo[a]pyrene hydroxylase and aminopyrine-N-demethylase activities are associated with the centrilobular area of liver, whereas the two transaminase activities are associated with the periportal area. The authors reported liver histopathological changes at 400 mg/kg-day consisting of "tiny areas of focal necrosis comprised of a few degenerated hepatocytes and inflammatory cells." A LOAEL of 200 mg/kg-day is identified based on changes in activities of five liver enzymes. Correcting for the discontinuous exposure (six days/week) results in a LOAEL of 171 mg/kg-day.

In the subchronic study of Quast *et al*. (1979), four beagle dogs/sex were gavaged with doses of 0, 200, 400, or 600 mg styrene/kg-day in peanut oil for 560 days. U.S. EPA determined a LOAEL of 400 mg/kg-day and a NOAEL of 200 mg/kg-day for red blood cell and liver effects (U.S. EPA, 2004). However, because of the small number of animals per group, OEHHA decided to concentrate on other reports.

Carcinogenic Effects

Cancer Potency Estimates from Human Data

More than 20 studies have examined the potential for styrene exposure to influence cancer risk in human populations. Of these, five epidemiological studies were found that included both quantitative exposure estimates and findings of positive dose-response relationships for specific categories of cancer (Wong *et al.*, 1994; Macaluso *et al.*, 1996; Matanoski *et al.*, 1997; Kogevinas *et al.*, 1994; Delzell *et al.*, 2001). OEHHA found the study by Kogevinas *et al.* (1994) to be the most suitable human study for dose-response risk quantification, considering study designs, confounding exposures, and other factors influencing the results of these studies.

Lymphohematopoietic cancers (LHCs) are most consistently associated with styrene exposure in humans. In the Kogevinas *et al.* (1994) cohort, the relative risk results for all LHCs combined showed a linear increase with average exposure and for time since first exposure. However, for cumulative exposure, risk for the highest category (≥ 500 ppm-years) for all LHCs dropped below the values found for the two other groups exposed above the baseline (75 ppm-years). A positive dose-response was seen with cumulative exposure and malignant lymphoma, one of the LHCs, although the highest cumulative dose category shows some drop-off. The Kogevinas *et al.* data showed a positive dose-response relationship between cumulative exposure and relative risks for pancreatic cancer. A number of factors might contribute to this. First, average exposure may relate more closely to intensity of exposure, and for styrene and LHCs, exposure intensity may be a more important determinant of risk than total dose (cumulative exposure). Total

dose may be more important for other diseases, such as pancreatic cancer. While exposure intensity varies for individuals within all of the cumulative exposure groups, the highest cumulative exposure category may include especially disparate groups: person-time for both those exposed at high levels for short periods (e.g., 250 ppm for 2 years) as well as those exposed at relatively low levels for long periods (e.g., 50 ppm for 10 years). The LHC risks faced by these two groups may be quite different.

A second consideration is that individuals chronically exposed at the highest levels of styrene may suffer other health effects that compete as causes of death. Thirdly, the large percentage of workers (>60 percent) who were short-term (<2 years employment) in the Kogevinas *et al.* (1994) cohort may impact the dose-response observed. Short-term workers may have nonoccupational factors (e.g., alcohol abuse) that affect both their ability to work and their mortality risks (Kolstad and Olsen, 1999). Short-term workers also may be given the jobs with the highest exposure levels. Research by Boffetta *et al.* (1998) suggests that the short-term workers in the Kogevinas *et al.* (1994) cohort had slightly higher average styrene exposures, mainly because they tended to have been employed in the earlier periods (prior to 1970), when exposure levels were higher. Finally, the workers exposed during the earliest time periods of reinforced plastics production not only had high exposure levels but also have had the longest time since first employment, allowing more time for cancer to develop. All of these factors may influence the dose-response curves for LHC mortality data.

A positive dose-response relationship between cumulative exposure and two cancer sites was found in the Kogevinas *et al.* study, pancreatic cancer and malignant lymphoma. OEHHA estimated the slopes of these dose-response relationships assuming a multiplicative biological model (background risk is multiplied by exposure-related risk).

Models for Estimating Cancer Potency from Human Data

Kogevinas *et al.* (1994a,b) studied mortality and exposures among reinforced plastics workers (n = 40,688) from six European countries. The authors constructed a styrene exposure database using approximately 16,500 personal environmental measurements, made between 1955 and 1990, and about 18,500 measurements of styrene metabolites in urine, made in the late 1980s. Cumulative exposure (in ppm-years) was estimated on the basis of individual job records, where available, and of country-, period-, and job-specific exposure estimates. For a substantial proportion of the cohort (>40 percent), individual job titles were not available or workers had unspecified tasks, and thus exposure estimates for these workers were more uncertain. Elevated rate ratios for malignant lymphomas and cancer of the pancreas in relation to cumulative styrene exposure in this cohort (Table 32) provide a basis for cancer potency estimation.

The occupational cohort data presented in Table 32 were used to derive cancer potency estimates for occupational styrene exposure using a relative risk (RR) model that adjusts for estimated uncontrolled confounding bias, such as a healthy worker effect (Arrighi and Hertz-Picciotto, 1994, 1996), and may be described as:

$$RR = \beta_0(1 + \beta_1 d) + \varepsilon \qquad \text{Equation 1}$$

where:

β_0 = ratio of background cancer rate in the population studied to rate in the general population,
β_1 = potency slope for excess relative risk of cumulative exposure,
d = styrene dose (cumulative ppm-years), and
ε = error.

Table 32. Cumulative Exposure and Mortality from Malignant Lymphomas and Pancreatic Cancer[1]

Cumulative Exposure (ppm-yrs)	Exposure Mid-point (ppm-yrs)[2]	Malignant Lymphomas[3]			Pancreas		
		Obs	Exp[4]	Rate Ratio (95% CI)[5]	Obs	Exp[4]	Rate Ratio (95% CI)[5]
<75	37.5	5	-	Comparison group	9	-	Comparison group
75-199	137.5	5	1.9	2.6 (0.7-9.3)	5	3.5	1.4 (0.5-4.3)
200-499	350.0	5	1.7	3.0 (0.8-10.9)	6	3.2	1.9 (0.7-5.5)
500+	650.0	3	1.8	1.6 (0.3-7.8)	10	3.9	2.6 (0.9-7.3)

[1] From Kogevinas *et al.* (1994); exposure was not lagged.
[2] Mid-point of exposure category as determined by OEHHA.
[3] Malignant lymphomas (ICD-8 200-202) include Non-Hodgkin's lymphoma (200, 202) and Hodgkin's disease (201).
[4] Expected numbers of deaths were calculated for the present report based on the relationship that expected = observed/relative risk; original study reported relative risks and observed deaths.
[5] Adjusted by Kogevinas *et al.* using Poisson regression for the effects of age, gender, country, calendar period, and time since first exposure.

To determine parameter estimates for β_0 and β_1, Equation 1 is reformulated such that the dependent variable is the observed number of cancer cases. The number of observed cancer cases is assumed to be a Poisson random variable and hence ε will follow a known distribution. The parameter estimates encompass the error term that is described in Equation 1. The resulting model that is fit to the cohort data is as follows:

$$obs = \beta_0\, expected\, (1 + \beta_1 d) \qquad \text{Equation 2}$$

where:

obs = observed number of cancer cases, and
expected = the expected number of cancer cases (i.e., using the relationship *expected* = *obs*/RR).

Maximum likelihood estimation (MLE) methods were used to determine the parameter estimates in Equation 2 and their corresponding 90 percent profile likelihood-based confidence intervals (i.e., the outline of the likelihood was traced to determine the interval rather than using normal asymptotic theory). Model fitting and computations were carried out using Mathematica software (version 5), to fit the following two models to the data:

Model 1 $obs = \beta_0\, expected\, (1 + \beta_1 d)$

Model 2 $obs = expected\, (1 + \beta_1 d)$

The best fit of the two models to the observed data was then determined for each cancer site. Note that Model 2 is nested within Model 1, i.e., when $\beta_0 = 1$ then Model 1 is equivalent to Model 2. The hypothesis tested was whether the background rate in the study population is the same as that in the general population ($H_0 : \beta_0 = 1$ versus $H_A : \beta_0 \neq 1$). For both pancreatic cancer and malignant lymphomas, Model 2 gave a better fit with fewer parameters (likelihood ratio test: pancreatic cancer, p = 0.35; malignant lymphomas, p = 0.34), as shown in Tables 33 and 34. The model-predicted rate ratios based on Model 2 can be compared with the observed rate ratios in Figures 3 and 4, based on the data of Kogevinas *et al.*, 1994. Figure 3 shows rate ratios for pancreatic cancer mortality and cumulative styrene exposure; the observed rate ratios are indicated by the point estimates with 95 percent confidence intervals, while model-predicted rate ratios lie on the straight line.

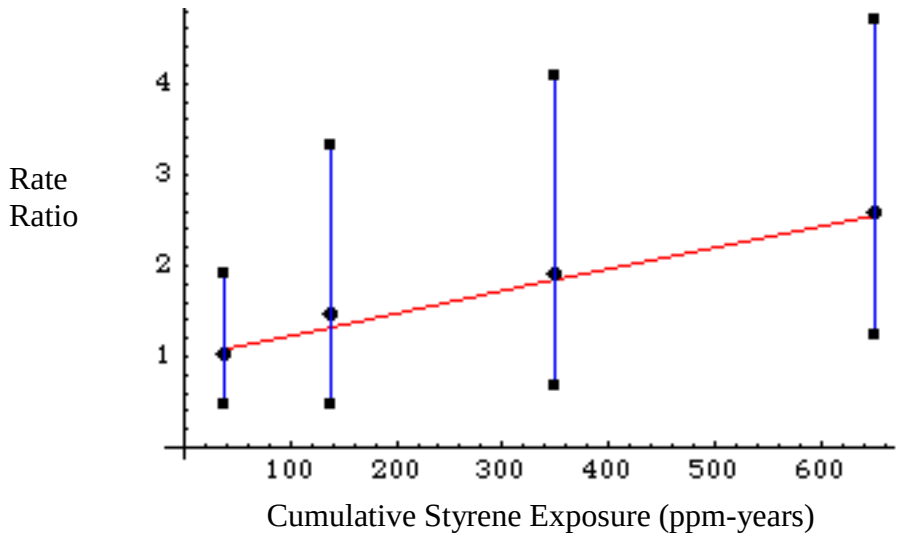


Figure 3. Pancreatic Cancer and Cumulative Styrene Exposure in the Workplace. Observed (± 95 percent CI) and predicted rate ratios using Model 2.

Figure 4 shows observed and predicted based on Model 2 rate ratios for malignant lymphoma mortality and cumulative styrene exposure, depicted as in Figure 3, based on the data of Kogevinas *et al.*, 1994. The model provides an estimate of potency for pancreatic cancer and malignant lymphomas, based on the value of β_1 in Model 2 for each site. The values calculated are 0.0024 for pancreatic cancer and 0.0023 for malignant lymphomas, expressed in units of excess relative risk per ppm-yr.

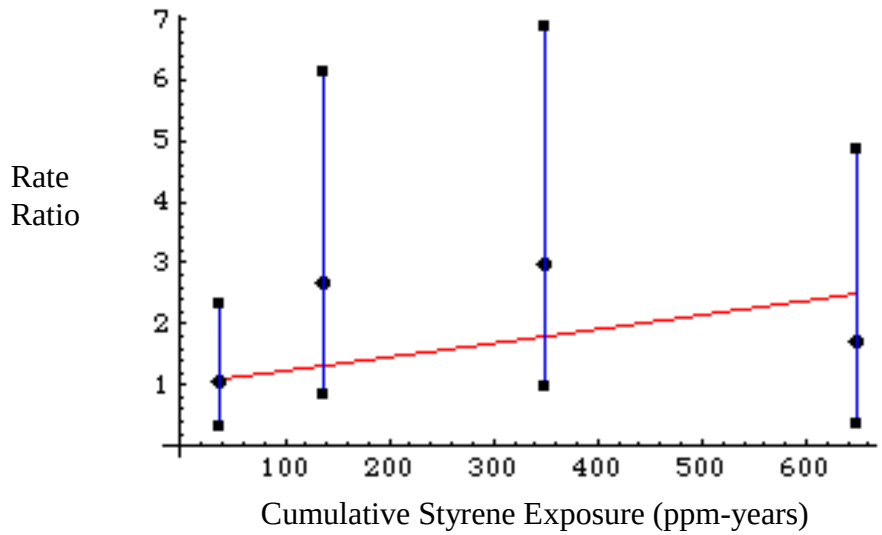


Figure 4. Malignant Lymphomas and Cumulative Styrene Exposure in the Workplace. Observed (± 95 percent CI) and predicted rate ratios using Model 2.

Population Risk Estimates

The cancer potency values calculated above represent risks posed to occupationally exposed individuals, given the source of the data used in the estimation. These values can be adjusted to estimate cancer potency for continuous exposure to styrene throughout life. To calculate the excess lifetime risk per cumulative gram of styrene exposure using the simplified background risk approach, the following formula is used:

$$ELR = \beta_1 * R(0) \qquad \text{Equation 3}$$

where:

ELR = excess lifetime risk per cumulative gram of styrene exposure,

β_1 = cancer potency slope (or 95 percent upper confidence limit of the slope), and

R(0) = background lifetime risk of being diagnosed or dying of specific cancers.

Depending on the units of exposure, the equation would appear as follows:

$$ppm = \beta_1 * R(0) * 70\ yrs * 365/240d * 20/10\ m^3/d \qquad \text{Eq. 3A}$$

[ppm = units of risk per ppm (air concentration)], or

$$mg/m^3 = \beta_1 * R(0) * 70\ yrs * 365/240\ d * 20/10\ m^3/d * (1\ ppm/4.26\ mg/m^3) \qquad \text{Eq. 3B}$$

[mg/m^3 = units of risk per mg/m^3 (air concentration)], or

$$mg/kg\text{-}day = \beta_1 * R(0) * 70\ yrs * 365/240\ d * 20/10\ m^3/d * (1\ ppm/4.26\ mg/m^3) * (1\ d/20\ m^3) * (70\ kg) \qquad \text{Eq. 3C}$$

Table 33. Model Fits (Via Maximum Likelihood Estimation) for Pancreatic Cancer

Cumulative Styrene Exposure (ppm-years)	Observed Pancreatic Cancer Cases (obs)	Model Predicted Pancreatic Cancer Cases
A. Model (1) [obs = 0.93 expected (1+0.0028 dose)] [a]		
37.5	9	9.2
137.5	5	4.5
350.0	6	5.8
650.0	10	10.1
B. Model (2) [obs = expected (1+0.0024 dose)] [b]		
37.5	9	9.8
137.5	5	4.7
350.0	6	5.9
650.0	10	10.0

[a] Chi-square goodness-of-fit: p = 0.97.
[b] Chi-square goodness-of-fit: p = 0.99.

Table 34. Maximum Likelihood Estimation Model Fits for Malignant Lymphomas

Cumulative Styrene Exposure (ppm-years)	Observed Malignant Lymphoma Cases (obs)	Model Predicted Malignant Lymphoma Cases[1]
A. Model (1) [obs = 1.14 expected (1+0.0017 dose)][1]		
37.5	5	6.1
137.5	5	2.7
350.0	5	3.1
650.0	3	4.3
B. Model (2) [obs = expected (1+0.0023 dose)][2]		
37.5	5	5.4
137.5	5	2. 5
350.0	5	3.1
650.0	3	4.5

[1] Chi-square goodness-of-fit: p = 0.15
[2] Chi-square goodness-of-fit: p = 0.23

To convert from an occupational exposure scenario to continuous exposure, the potency estimate is multiplied by an estimated lifetime of exposure (70 years) and by various conversion factors to obtain the desired units. For the calculation in mg/kg-day, a body weight of 70 kg is used. The upper bound of the estimated cancer potency value is used so that, given the uncertainties inherent in the calculation, the estimate will not be too low. The background risk of being diagnosed with the cancer of interest, R(0), is

estimated based on information from the California Cancer Registry. The background lifetime risk of being diagnosed with pancreatic cancer in California for all races combined is 0.0117 in males and 0.0125 in females, and an average of 0.0121 for the two sexes combined (Kwong *et al.*, 2005). Similarly, the lifetime risks for malignant lymphoma are 0.0225 in males and 0.0181 in females, and an average of 0.0203 for the sexes combined. For the cancer potency calculation for lifetime styrene exposure, the combined sex value of R(0) was used. The results are provided in Table 35.

Table 35. Estimates of Excess Lifetime Unit Risk or Cancer Potency for Cumulative Styrene Exposure in California Using the Simplified Background Risk Approach

Cancer Endpoint	Cancer Potency Estimates[1] (β_1, 90% CI)	Background Cancer Risk[2] R(0)	Excess Lifetime Unit Risk or Potency[3]
Pancreatic Cancer	0.0024 (0.00080, 0.0042)	0.0121	0.011 $(ppm)^{-1}$ 0.0025 $(mg/m^3)^{-1}$ 0.0089 $(mg/kg\text{-}day)^{-1}$
Malignant Lymphomas	0.0023 (0.00059, 0.0047)	0.0203	0.020 $(ppm)^{-1}$ 0.0048 $(mg/m^3)^{-1}$ 0.017 $(mg/kg\text{-}day)^{-1}$

[1] Based on modeling the Kogevinas *et al.* (1994) data, using Model (2), in units of excess relative risk per ppm-year.
[2] Lifetime probability of being diagnosed with cancer in California.
[3] Calculated from Equations 3A, 3B, and 3C.

Excess lifetime risk for continuous exposure of the California population can be determined using life tables, which can provide a more accurate representation of the way that risk for developing a particular cancer changes as a person ages. Life tables summarizing the calculation of excess lifetime risk of continuous styrene exposure (to one ppm) in California were constructed for diagnosis with malignant lymphoma (Table 36) or pancreatic cancer (Table 37). The life tables were based on the methods described by Chiang (1984), and have been employed in several OEHHA risk assessments (e.g., cadmium, diesel exhaust, and benzene). A lag time of ten years was used for pancreatic cancer, since it is generally a late-forming tumor. A lag time of zero was conservatively used for malignant lymphomas, since this tumor occurs commonly in childhood and has formed following a short latency period in animal studies of aromatic hydrocarbons (Toth *et al.*, 1963).

Table 36. Life Table Summarizing the Calculation of Excess Lifetime Risk of Continuous Styrene Exposure (1 ppm) in Californians (All Races, Both Sexes) for Malignant Lymphoma.

Estimate cohort potency (adjusted to continuous exposure)* 4.68E-03 ppm-yr

Lag time 0

	Background rates								Rates with exposure									
Age	Average Death rate 1990-1994 (per 10^5) a	q(i) P[dying in ith interval) b	p(i) P(survive) to begin of interval c	L(i) Cum P{survival to age i)	1994-8 CA lymphoma incid. rate (per 10^5) d	d(i) P{death lymphoma given surv to age i} e	Uncon-ditional P[death lymphoma f	Cum '[lymphoma b age i+4)	ave cum dose ppm-yr	Adjusted 1990-4 annu death (per 10^5) g	q(i) P[dying in ith interval)	P(survive) to begin of interval	Cum P[surv to age i}	Adjusted lymphoma Inc rate (per 10^5) h	P{death lymphoma, given surv to age i}	Uncon-ditional P[death lymphoma	Cum P[lymph. by age i+4]	
0-4	189.10	0.0094	0.9906	1.0000	1	0.00005	0.00005	0.00005	2.5	188.11	0.0094	0.9906	1.0000	1.012	5.03E-05	5.03E-05	0.00005	
5-9	19.00	0.0009	0.9991	0.9906	1	0.00005	0.00005	0.00010	7.5	18.04	0.0009	0.9991	0.9906	1.035	5.17E-05	5.12E-05	0.00010	
10-14	24.40	0.0012	0.9988	0.9896	2	0.00010	0.00010	0.00020	12.5	22.52	0.0011	0.9989	0.9897	2.117	1.06E-04	1.05E-04	0.00021	
15-19	90.20	0.0045	0.9955	0.9884	4	0.00020	0.00020	0.00040	17.5	86.53	0.0043	0.9957	0.9886	4.328	2.16E-04	2.13E-04	0.00042	
20-24	108.90	0.0054	0.9946	0.9840	6	0.00030	0.00029	0.00069	22.5	103.53	0.0052	0.9948	0.9844	6.632	3.31E-04	3.26E-04	0.00075	
25-29	117.90	0.0059	0.9941	0.9787	8	0.00040	0.00039	0.00108	27.5	110.93	0.0055	0.9945	0.9793	9.030	4.50E-04	4.41E-04	0.00119	
30-34	156.40	0.0078	0.9922	0.9729	10	0.00050	0.00048	0.00156	32.5	147.92	0.0074	0.9926	0.9739	11.521	5.74E-04	5.59E-04	0.00175	
35-39	206.80	0.0103	0.9897	0.9653	12	0.00060	0.00058	0.00214	37.5	196.91	0.0098	0.9902	0.9667	14.106	7.02E-04	6.78E-04	0.00242	
40-44	270.80	0.0134	0.9866	0.9554	14	0.00070	0.00066	0.00281	42.5	259.58	0.0129	0.9871	0.9572	16.785	8.34E-04	7.98E-04	0.00322	
45-49	362.10	0.0179	0.9821	0.9425	18	0.00089	0.00084	0.00365	47.5	348.10	0.0173	0.9827	0.9449	22.001	1.09E-03	1.03E-03	0.00425	
50-54	530.90	0.0262	0.9738	0.9256	24	0.00118	0.00110	0.00474	52.5	512.80	0.0253	0.9747	0.9286	29.897	1.48E-03	1.37E-03	0.00562	
55-59	810.50	0.0397	0.9603	0.9014	33	0.00162	0.00146	0.00620	57.5	786.38	0.0386	0.9614	0.9051	41.880	2.05E-03	1.86E-03	0.00748	
60-64	1269.70	0.0615	0.9385	0.8656	43	0.00208	0.00180	0.00800	62.5	1239.28	0.0601	0.9399	0.8702	55.578	2.69E-03	2.34E-03	0.00983	
65-69	1897.60	0.0905	0.9095	0.8123	59	0.00281	0.00229	0.01029	67.5	1857.24	0.0887	0.9113	0.8179	77.638	3.71E-03	3.03E-03	0.01286	(70yr)
70-74	2893.80	0.1347	0.8653	0.7388	76	0.00354	0.00261	0.01290	72.5	2843.59	0.1325	0.8675	0.7454	101.787	4.74E-03	3.54E-03	0.01639	
75-79	4537.40	0.2030	0.7970	0.6393	94	0.00420	0.00269	0.01559	77.5	4477.49	0.2006	0.7994	0.6466	128.094	5.74E-03	3.71E-03	0.02010	
80-84	7146.20	0.3004	0.6996	0.5095	105	0.00441	0.00225	0.01784	82.5	7081.74	0.2982	0.7018	0.5169	145.541	6.13E-03	3.17E-03	0.02327	
85+	14746.40			0.3564	93	0.00225	0.00080	0.01864	87.5	14691.48			0.3628	131.084	3.24E-03	1.17E-03	0.02445	(life)

Excess Lifetime Risk (1/ppm)	0.00580
70-year excess risk (1/ppm)	0.00257

1/(mg/kg-d)	0.004768747	life
1/(mg/kg-d)	2.11E-03	70-year

* Adjustments from occupational to continuous exposure scenarios were made in the dose calculations.

a) Annual death rates, all causes, for California 1990-1994 (CDHS, Center for Health Statistics)

b) Probability of dying in the interval, q(i) = 1-exp(-5*death rate(i))

c) Probability of surviving in the ith interval, p(i) = 1-q(i)

d) Annual avearage lymphoma incidence rates, California 1994-1998, all races, both sexes, California Disease Registry

e) Probability of being diagnosed with lymphoma in the ith interval, given survival to the beginning of the interval, d(i) = (lymphoma rate/total death rate)*q(i)

f) Probability of being diagnosed with lymphoma in the ith interval, not conditioned on surviving to the beginning of the interval = d(i)*L(i)

g) Adjusted death rate, all causes, with exposure = total death rate + background lymphoma rate*(potency*(dose - lag time)-1)

h) Predicted lymphoma rate with exposure = background lymphoma rate*(1 + potency*(dose - lag time))

Table 37. Life Table Summarizing the Calculation of Excess Lifetime Risk of Continuous Styrene Exposure (1 ppm) in Californians (All Races, Both Sexes) for Pancreatic Cancer.

Estimate cohort potency (adjusted to continuous exposure)* 4.25E-03 ppm-yr

Lag time 10

	Background rates								Rates with exposure									
Age	Average Death rate 1990-1994 (per 10^5) a	q(i) P[dying in ith interval) b	p(i) P(survive) to begin of interval c	L(i) Cum P{survival to age i)	1994-8 CA pancreas c inc.rate (per 10^5) d	d(i) P{death panc ca, given surv to age i} e	Uncon-ditional P[death pan ca f	Cum P[pan ca by age i+4)	ave cum dose (ppm-yr)	Adjusted 1990-4 annu death (per 10^5) g	q(i) P[dying in ith interval)	P(survive) to begin of interval	Cum P[surv to age i}	Adjusted pan ca Inc rate (per 10^5) h	P{death pan ca, given surv to age i}	Uncon-ditional P[death pan ca	Cum P[pan ca by age i+4)	
0-4	189.10	0.0094	0.9906	1.0000	0	0.00E+00	0.00E+00	0.0000	2.5	189.10	0.0094	0.9906	1.0000	0.000	0.00E+00	0.00E+00	0.00000	
5-9	19.00	0.0009	0.9991	0.9906	0	0.00E+00	0.00E+00	0.0000	7.5	19.00	0.0009	0.9991	0.9906	0.000	0.00E+00	0.00E+00	0.00000	
10-14	24.40	0.0012	0.9988	0.9896	0	0.00E+00	0.00E+00	0.0000	12.5	24.40	0.0012	0.9988	0.9896	0.000	0.00E+00	0.00E+00	0.00000	
15-19	90.20	0.0045	0.9955	0.9884	0	0.00E+00	0.00E+00	0.0000	17.5	90.20	0.0045	0.9955	0.9884	0.000	0.00E+00	0.00E+00	0.00000	
20-24	108.90	0.0054	0.9946	0.9840	0	0.00E+00	0.00E+00	0.0000	22.5	108.90	0.0054	0.9946	0.9840	0.000	0.00E+00	0.00E+00	0.00000	
25-29	117.90	0.0059	0.9941	0.9787	0	0.00E+00	0.00E+00	0.0000	27.5	117.90	0.0059	0.9941	0.9787	0.000	0.00E+00	0.00E+00	0.00000	
30-34	156.40	0.0078	0.9922	0.9729	0	0.00E+00	0.00E+00	0.0000	32.5	156.40	0.0078	0.9922	0.9729	0.000	0.00E+00	0.00E+00	0.00000	
35-39	206.80	0.0103	0.9897	0.9653	1	4.974E-05	4.802E-05	0.00005	37.5	205.92	0.0102	0.9898	0.9653	1.117	5.56E-05	5.36E-05	0.00005	
40-44	270.80	0.0134	0.9866	0.9554	2	9.933E-05	9.490E-05	0.00014	42.5	269.08	0.0134	0.9866	0.9554	2.276	1.13E-04	1.08E-04	0.00016	
45-49	362.10	0.0179	0.9821	0.9425	4	1.982E-04	1.868E-04	0.00033	47.5	358.74	0.0178	0.9822	0.9427	4.638	2.30E-04	2.17E-04	0.00038	
50-54	530.90	0.0262	0.9738	0.9256	9	4.441E-04	4.111E-04	0.00074	52.5	523.53	0.0258	0.9742	0.9259	10.626	5.24E-04	4.86E-04	0.00086	
55-59	810.50	0.0397	0.9603	0.9014	15	7.350E-04	6.625E-04	0.00140	57.5	798.53	0.0391	0.9609	0.9020	18.028	8.84E-04	7.97E-04	0.00166	
60-64	1269.70	0.0615	0.9385	0.8656	25	1.211E-03	1.048E-03	0.00245	62.5	1250.28	0.0606	0.9394	0.8667	30.578	1.48E-03	1.28E-03	0.00295	
65-69	1897.60	0.0905	0.9095	0.8123	37	1.765E-03	1.434E-03	0.00389	67.5	1869.64	0.0892	0.9108	0.8142	46.042	2.20E-03	1.79E-03	0.00473	(70yr)
70-74	2893.80	0.1347	0.8653	0.7388	52	2.421E-03	1.788E-03	0.00567	72.5	2855.61	0.1331	0.8669	0.7415	65.813	3.07E-03	2.27E-03	0.00701	
75-79	4537.40	0.2030	0.7970	0.6393	67	2.997E-03	1.916E-03	0.00759	77.5	4489.62	0.2011	0.7989	0.6428	86.221	3.86E-03	2.48E-03	0.00949	
80-84	7146.20	0.3004	0.6996	0.5095	81	3.405E-03	1.735E-03	0.00933	82.5	7090.16	0.2985	0.7015	0.5136	105.958	4.46E-03	2.29E-03	0.01178	
85+	14746.40			0.3564	94	2.272E-03	8.099E-04	0.01013	87.5	14683.36			0.3603	124.961	3.07E-03	1.10E-03	0.01289	(life)

Excess Lifetime Risk (1/ppm) 0.00275

70-year excess risk (1/ppm) 0.00085

1/(mg/kg-d) 0.00226 life

1/(mg/kg-d) 6.98E-04 70-year

* Adjustments from occupational to continuous exposure scenarios were made in the dose calculations.

a) Annual death rates, all causes, for California 1990-1994 (CDHS, Center for Health Statistics)

b) Probability of dying in the interval, q(i) = 1-exp(-5*death rate(i))

c) Probability of surviving in the ith interval, p(i) = 1-q(i)

d) Annual avearage pancreatic cancer incidence rates, California 1994-1998, all races, both sexes, California Disease Registry

e) Probability of being diagnosed with pancreatic cancer in the ith interval, given survival to the beginning of the interval, d(i) = (pancreatic cancer rate/total death rate)*q(i)

f) Probability of being diagnosed with pancreatic cancer in the ith interval, not conditioned on surviving to the beginning of the interval = d(i)*L(i)

g) Adjusted death rate, all causes, with exposure = total death rate + background pancreatic cancer rate*(potency*(dose - lag time)-1)

h) Predicted pancreatic cancer rate with exposure = background pancreatic cancer rate*(1 + potency*(dose - lag time))

Excess lifetime risks are calculated based on two different time frames: 70-year exposure and full life exposure, which extends beyond 70 years. Annual death rates for all causes, all races, and both sexes (1990 to 1994) were obtained from the California Department of Health Services, Center for Health Statistics. Population statistics for California (1990 to 1994) were obtained from the California Department of Finance, Demographic Research Unit. Annual California incidence data for malignant lymphomas and pancreatic cancer (1994 to 1998) were published by the California Cancer Registry (Kwong *et al.*, 2005).

Results of the life table analyses are provided in Table 38. Results for full life risk (including time beyond 70 years of age) are similar, though not identical, to those obtained using the simplified background risk approach; results using the simplified background risk are also displayed in Table 36 for comparison.

Summary of Human Cancer Potency Estimates

As described above, the cancer potency estimates based on human data for the styrene PHG used a modeling approach for pancreatic cancer and malignant lymphomas in units of excess relative risk per ppm-year. The values used to estimate excess lifetime risk for continuous exposure in California were 0.0089 mg/kg-day $^{-1}$ for pancreatic cancer and 0.017 mg/kg-day $^{-1}$ for malignant lymphomas. Estimates using a life table approach for full life risk were similar.

Table 38. Estimates of Excess Lifetime Unit Risk or Cancer Potency for Cumulative Styrene Exposure, Simplified Background Risk and Life Table Approaches

Cancer Endpoint	Excess Lifetime Unit Risk or Potency		
	Simplified Background Risk Approach[1]	Life Table Approach[2]	
		70-Year Exposure	Full Life Exposure
Pancreatic Cancer	0.011 (ppm)$^{-1}$ 0.0025 (mg/m^3)$^{-1}$ 0.0089 (mg/kg-d)$^{-1}$	0.0025 (ppm)$^{-1}$ 0.00059 (mg/m^3)$^{-1}$ 0.0021 (mg/kg-d)$^{-1}$	0.0081 (ppm)$^{-1}$ 0.0019 (mg/m^3)$^{-1}$ 0.0066 (mg/kg-d)$^{-1}$
Malignant Lymphomas	0.020 (ppm)$^{-1}$ 0.0048 (mg/m^3)$^{-1}$ 0.017 (mg/kg-d)$^{-1}$	0.0077 (ppm)$^{-1}$ 0.0018 (mg/m^3)$^{-1}$ 0.0063 (mg/kg-d)$^{-1}$	0.017 (ppm)$^{-1}$ 0.0040 (mg/m^3)$^{-1}$ 0.014 (mg/kg-d)$^{-1}$

[1] Results as reported in Table 33.
[2] Life table calculations used the same values for β_1 as in the first method (listed in Table 33). Life table calculations used a ten-year lag time between exposure and pancreatic cancer and no lag for malignant lymphomas.

Cancer Potency Estimates from Animal Data

In this section, cancer potencies are calculated from four selected high-quality animal studies (Conti *et al.*, 1988; Beliles *et al.*, 1985; Cruzan *et al.*, 2001; and NCI, 1979). The results of physiologically-based pharmacokinetic modeling (PBPK) are also presented. Although there were additional studies and endpoints demonstrating styrene carcinogenicity in animals, the data presented here are best suited for quantitative risk

assessment and development of a styrene PHG, considering the study quality (number of animals, doses and procedures) and the tumor dose-responses. Table 52 at the end of this section summarizes the cancer potency estimates from five cancer endpoints reported in the four bioassays selected from the available animal data to derive cancer potency estimates. The key features of the four studies are:

1) Conti *et al.* (1988) reported female benign and malignant mammary tumors in Sprague-Dawley rats exposed to styrene via inhalation. Incidence data are presented in Table 20. Rats were exposed to styrene at air concentrations of 0, 25, 50, 100, 200, or 300 ppm air for four hours/day, five days/week. Dosing occurred for 52 weeks and the animals were observed to week 138.

2) Beliles *et al.* (1985) reported increased mammary tumors in female Sprague-Dawley rats exposed to styrene in drinking water for two years. Incidence data are presented in Table 16. Drinking water concentrations of styrene were 0, 112, and 221 mg/L (mean measured levels).

3) Cruzan *et al.* (2001) reported significant increases in the incidence of lung adenoma and carcinoma in male and female CD1 mice exposed to styrene at 0, 20, 40, 80, or 160 ppm in air for six hours/day, five days/week. Exposure duration was 104 weeks for male and 97 weeks for female mice. Incidence data are presented in Table 17.

4) NCI (1979) reported bronchiolar-alveolar adenomas and carcinomas in male $B6C3F_1$ mice exposed to styrene via gavage at dose levels of 0, 150, or 300 mg/kg for 5 days/week for 78 weeks, followed by a 13 week observation period. Incidence data are presented in Table 15.

Both ingestion and inhalation studies are considered applicable for risk assessment of styrene by the ingestion route because systemic tumors (not at the point of contact) have occurred by both routes, i.e., mammary and lung tumors by both exposure routes.

Physiologically-based Pharmacokinetic Analysis of Rodent Data

The rodent bioassay data identified above were analyzed using a PBPK model essentially as described by Csanady *et al*. (1994). The two primary differences are: 1) the addition of physiological and biochemical parameters for metabolism of styrene in the lung (denoted as parameter Sets “1” and “2” in this section) (Hynes *et al*., 1999; Mendrala *et al*., 1993), and 2) division of the lung compartment into alveolar and bronchiolar sub-compartments (Evelo *et al*., 1993).

The Csanady *et al*. (1994) PBPK model is based on a model of 1,3-butadiene by Johanson and Filser (1993). In the model styrene is oxidized to styrene 7,8-oxide in the liver. The intrahepatic first-pass hydrolysis of styrene 7,8-oxide is catalyzed by epoxide hydrolase, and styrene 7,8-oxide is also conjugated with GSH through the action of GST. The body tissues are represented by five compartments: 1) liver; 2) fat; 3) a richly perfused tissue group (vessel rich group, VRG) containing, for example, kidney, brain, and the GI tract; 4) slowly perfused tissues, representing primarily muscle; and 5) lung, composed of alveolar and bronchiolar sub-compartments. While the mass and metabolic capacities of the sub-compartments are approximately equal, the large majority of gas exchange (93 percent) takes place in the alveolar sub-compartment.

Two sets of biochemical parameters are used. Set "1" employs liver parameters from Csanady *et al*. (1994) and lung parameters from Hynes *et al*. (1999) and Mendrala *et al*. (1993) as presented in Tables G-3 and G-4 of Cohen *et al*. (2002). Set "2" employs liver and lung parameters of the "Panel" model as presented in Table G-12 of Cohen *et al*. (2002) and shown in Table 39. The chemical and physiological parameters are essentially the same as Csanady *et al*. (1994), adjusted for bioassay conditions as described below. The models were run using Berkeley Madonna version 8.0.1 software (www.berkeleymadonna.com). Typical one-week simulations of bioassay exposures run in less than 1 second on a standard desktop computer.

Table 39. Biochemical Parameters Used in PBPK Modeling of Rodent Styrene Data

Tissue & Parameters		Rat 1	Rat 2	Mouse 1	Mouse 2
Lung					
P_{450}	V_{max}	1.2E-6	2.4E-6	2.6E-6	1.6E-5
	K_m	1.5E-5	1.5E-5	1.3E-5	1.3E-5
EH	V_{max}	2.2E-6	1.1E-6	2.5E-6	2.5E-6
	K_m	1.3E-4	1.3E-4	9.0E-5	9.0E-5
GST	V_{max}	2.5E-4	1.3E-4	2.9E-4	2.9E-3
	K_m	1.0E-4	1.0E-4	1.0E-4	1.0E-4
Liver					
P_{450}	V_{max}	5.3E-6	1.1E-5	1.1E-5	1.1E-5
	K_m	1.5E-5	1.5E-5	1.3E-5	1.3E-5
EH	V_{max}	1.1E-5	5.5E-6	1.1E-5	1.1E-5
	K_m	1.3E-4	1.3E-4	9.0E-5	9.0E-5
	K_{mh}	1.3E-5	1.3E-5	9.0E-6	9.0E-6
GST	V_{max}	3.7E-4	1.9E-5	2.9E-4	2.9E-3
	K_{m1}	1.0E-4	1.0E-4	1.0E-4	1.0E-4
	K_{m2}	2.5E-3	1.0E-4	2.5E-3	1.0E-4
	K_d	0.2	0.2	0.1	0.1

Abbreviations: P_{450} = microsomal cytochrome P_{450}-dependent oxidation; EH = epoxide hydrolase; GST = glutathione-*S*-transferase; V_{max} = apparent maximum rate in mole/hour/gram tissue; K_m = Michaelis-Menten constant in moles/L; K_{mh} = estimated intrahepatic K_m; K_{m1} = K_m value for GST in relation to styrene oxide; K_{m2} = K_m value for GST in relation to reduced glutathione (GSH); and K_d = GSH degradation rate in hr^{-1}.

[1] Parameter Set "1" based on Csanady *et al*. (1994), Hynes *et al*. (1999), and Mendrala *et al*. (1993); Parameter Set "2" based on Panel recommendations of Cohen *et al*. (2002).

The following discussion presents the results of PBPK modeling for the four studies identified above to be used for cancer potency derivation:

The first study is the rat inhalation study of Conti *et al*. (1988). A lifetime average body weight for a rat of 0.4 kg was assumed because body weight data were not provided by the investigators. Cardiac output and alveolar ventilation were adjusted allometrically from the Csanady *et al*. (1994) values by (body weight)$^{0.74}$. Simulations were conducted for four hours/day, five days/week, for one week and the dose metrics were divided by seven to give average daily values. The dose metrics chosen for evaluation in terms of improved dose-response relations were: 1) the amounts of styrene metabolized in liver, lung, and total; 2) the amounts of styrene oxide metabolized via the epoxide hydrolase path in liver, lung, and total; and 3) the areas under the styrene oxide concentration x time curve (AUC) for blood, lung, and VRG (as a surrogate for mammary tissue). Simulation results for the bioassay doses and an additional very low dose control for the parameter set "1" are given in Table 40. Table 41 gives the values using the parameter set "2." In Tables 40 and 41, the AUC units are (mole/L)(hours/day), the amount metabolized (AMET) is in units of mol/kg-day, and blood AUCs are based on mixed venous blood in the PBPK model.

Table 40. Female Rat Inhalation PBPK Estimates of Dose Metrics for Styrene and Styrene 7,8-Oxide (Conti): Parameter Set "1" [a]

Metric	Concentration (ppm)				
	25	50	100	200	300
AUC (M-hr/day)					
Blood SO	4.0E-6	6.7E-6	1.1E-5	1.6E-5	1.9E-5
Lung SO	8.3E-6	1.5E-5	2.4E-5	3.7E-5	5.1E-5
VRG SO	5.3E-6	9.0E-6	1.4E-5	2.0E-5	2.6E-5
AMET (mol/kg-day)					
ST liver	3.9E-5	7.9E-5	1.6E-4	3.3E-4	4.9E-4
ST Lung	9.2E-6	1.6E-5	2.6E-5	4.0E-5	5.1E-5
ST Total	4.8E-5	9.4E-5	1.9E-4	3.7E-4	5.4E-4
SO EH Liver	1.4E-8	2.9E-8	6.1E-8	1.2E-7	1.9E-7
SO EH Lung	5.9E-8	1.0E-7	1.6E-7	2.5E-7	3.3E-7
SO EH Total	7.3E-8	1.3E-7	2.2E-7	3.8E-7	5.2E-7

Abbreviations: AUC = area under the concentration x time curve; AMET = amount metabolized; VRG = vessel rich group, a surrogate for mammary tissue; ST = styrene; SO = styrene oxide; EH = styrene oxide metabolism via the epoxide hydrolase path; Blood = mixed venous blood.
[a] Data from Conti *et al*. (1988), biochemical parameters from Csanady *et al*. (1994); Hynes *et al*. (1999); and Mendrala *et al*. (1993). Model results are based on seven-day simulations (with the exposure pattern of 4 hours/day x 5 days/week).

Table 41. Female Rat Inhalation PBPK Estimates of Dose Metrics for Styrene and Styrene Oxide (Conti): Parameter Set "2" [a]

Metric	Concentration (ppm)				
	25	50	100	200	300
AUC (M-hr/day)					
Blood SO	1.1E-5	2.0E-5	3.2E-5	4.7E-5	5.7E-5
Lung SO	2.3E-5	4.1E-5	6.8E-5	1.0E-4	1.3E-4
VRG SO	1.5E-5	2.7E-5	4.3E-5	6.3E-5	7.6E-5
AMET (mol/kg-day)					
ST Liver	3.3E-5	7.0E-5	1.5E-4	3.2E-4	4.9E-4
ST Lung	1.4E-5	2.5E-5	4.0E-5	6.0E-5	7.4E-5
ST Total	4.7E-5	9.5E-5	1.9E-4	3.8E-4	5.6E-4
SO EH Liver	5.0E-9	1.0E-8	2.2E-8	4.8E-8	7.3E-8
SO EH Lung	8.2E-8	1.4E-7	2.4E-7	3.5E-7	4.4E-7
SO EH Total	8.7E-8	1.6E-7	2.6E-7	4.0E-7	5.1E-7

Abbreviations: AUC = area under the concentration x time curve; AMET = amount metabolized; VRG = vessel rich group, a surrogate for mammary tissue; ST = styrene; SO = styrene oxide; EH = styrene oxide metabolism via the epoxide hydrolase path; Blood = mixed venous blood.
[a] Data from Conti *et al*., (1988), biochemical parameters from Table G-12 of Cohen *et al*. (2002). Model results are based on seven-day simulations (with the exposure pattern of 4 hours/day x 5 days/week).

For the second study, the two-year drinking water study in rats (Beliles *et al*., 1985), the PBPK model predictions were based on seven day simulations with a pulsed oral input of 1/6 of a daily dose every four hours. Drinking water intake was based on the time-weighted-average lifetime body weight of 0.40 kg for female rats in the study and an allometric relation for water intake vs. body weight ($0.1*W^{0.7377}$). Terminal model values were divided by seven to give the daily dose metrics presented in Table 42. Simulations were conducted with parameter set "1" only.

Table 42. Rat Drinking Water PBPK Estimates of Dose Metrics for Styrene and Styrene Oxide [1]

Metric	Concentration (mg/L)	
	125	250
AUC (M-hr/day)		
Blood SO	6.1E-6	1.2E-5
Lung SO	1.3E-5	2.5E-5
VRG SO	8.0E-6	1.6E-5
AMET (mol/kg-day)		
ST Liver	1.7E-4	3.3E-4
ST Lung	8.4E-6	1.7E-5
ST Total	1.8E-4	3.5E-4
SO EH Liver	2.0E-8	4.1E-8
SO EH Lung	5.4E-8	1.1E-7
SO EH Total	7.4E-8	1.5E-7

Abbreviations: AUC = area under the concentration x time curve; AMET = amount metabolized; VRG = vessel rich group, a surrogate for mammary tissue; ST = styrene; SO = styrene oxide; EH = styrene oxide metabolism via the epoxide hydrolase path; Blood = mixed venous blood.
[1] Data from Beliles *et al*. (1985), biochemical parameters from Csanady *et al*. (1994); Hynes *et al*. (1999); Mendrala *et al*. (1993). Results based on seven-day simulations with pulsed input of 1/6 daily dose every four hours. Drinking water intake based on a time-weighted average body weight of 0.4 kg for female rats in Beliles *et al*. (1985) and allometric relation for water intake vs. body weight (0.1*W^0.7377).

Table 43. Mouse Inhalation PBPK Estimates of Dose Metrics for Styrene and Styrene Oxide: Females Parameter Set "1"[a]

Metrics	Concentration (ppm)			
	20	40	80	160
AUC (M-hr/day)				
Blood SO	1.4E-5	2.3E-4	3.7E-4	5.5E-4
Lung SO	3.1E-5	5.7E-5	9.4E-5	1.4E-4
VRG SO	1.8E-5	3.1E-5	5.0E-5	7.3E-5
AMET (mol/kg-day)				
ST Liver	1.3E-4	2.8E-4	5.9E-4	1.3E-3
ST Lung	3.5E-5	6.3E-5	1.0E-4	1.5E-4
ST Total	1.7E-4	3.4E-4	6.9E-4	1.4E-3
SO EH Liver	1.1E-7	2.2E-7	4.7E-7	1.0E-6
SO EH Lung	3.3E-7	6.0E-7	9.8E-7	1.4E-6
SO EH Total	4.4E-7	8.2E-7	1.4E-6	2.5E-6

[a] Data from Cruzan *et al*. (2001), biochemical parameters are from Csanady *et al*. (1994); Hynes *et al*. (1999); and Mendrala *et al*. (1993). Results use seven-day simulations (6 hours/day x 5 days/week).

Table 44. Mouse Inhalation PBPK Estimates of Dose Metrics for Styrene and Styrene Oxide: Females Parameter Set "2"[a]

Metrics	Concentration (ppm)			
	20	40	80	160
AUC (M-hr/day)				
Blood SO	3.7E-6	7.1E-6	1.3E-5	2.1E-5
Lung SO	6.5E-6	1.3E-5	2.4E-5	4.3E-5
VRG SO	5.0E-6	9.4E-6	1.7E-5	2.8E-5
AMET (mol/kg-day)				
ST Liver	6.2E-5	1.3E-4	2.9E-4	6.9E-4
ST Lung	7.6E-5	1.5E-4	2.7E-4	4.8E-4
ST Total	1.4E-4	2.8E-4	5.7E-4	1.2E-3
SO EH Liver	2.0E-10	4.1E-10	9.4E-10	2.4E-9
SO EH Lung	7.2E-8	1.4E-7	2.6E-7	4.6E-7
SO EH Total	7.3E-8	1.4E-7	2.6E-7	4.6E-7

[a] Data from Cruzan *et al*. (2001), biochemical parameters from Cohen *et al*. (2002). Models use seven-day simulations (6 hours/day x 5 days/week).

Table 45. Mouse Inhalation PBPK Estimates of Dose Metrics for Styrene and Styrene Oxide: Males Parameter Set "1"[a]

Metrics	Concentration (ppm)			
	20	40	80	160
AUC (M-hr/day)				
Blood SO	1.3E-5	2.3E-5	3.7E-5	5.4E-5
Lung SO	3.1E-5	5.5E-5	9.3E-5	1.4E-4
VRG SO	1.9E-6	3.1E-5	4.9E-5	7.3E-5
AMET (mol/kg-day)				
ST Liver	1.5E-4	3.1E-4	6.4E-4	1.3E-3
ST Lung	4.2E-5	7.4E-5	1.2E-4	1.7E-4
ST Total	2.0E-4	3.9E-4	7.6E-4	1.4E-3
SO EH Liver	1.2E-7	2.5E-7	5.2E-7	1.1E-6
SO EH Lung	4.0E-7	7.0E-7	1.1E-6	1.6E-6
SO EH Total	5.2E-7	9.6E-7	1.6E-6	2.7E-6

[a] Data from Cruzan *et al*. (2001), biochemical parameters from Csanady *et al*. (1994); Hynes *et al*. (1999); and Mendrala *et al*. (1993). Models use seven-day simulations (6 hr/d x 5 d/wk). Body weights for increasing dose groups were 44, 43, 42, 40, and 39 g, respectively.

Table 46. Mouse Inhalation PBPK Estimates of Dose Metrics for Styrene and Styrene Oxide: Males Parameter Set "2"[a]

Metrics	Concentration (ppm)			
	20	40	80	160
AUC (M-hr/day)				
Blood SO	3.4E-6	6.9E-6	1.3E-5	2.1E-5
Lung SO	6.2E-6	1.2E-5	2.3E-5	4.2E-5
VRG SO	7.1E-7	9.2E-6	1.7E-5	2.8E-5
AMET (mol/kg-day)				
ST Liver	7.0E-5	1.5E-4	3.2E-4	7.4E-4
ST Lung	9.0E-5	1.7E-4	3.0E-4	5.3E-4
ST Total	1.6E-4	3.2E-4	6.2E-4	1.3E-3
SO EH Liver	2.3E-10	4.8E-10	1.0E-9	2.4E-9
SO EH Lung	8.6E-8	1.6E-7	2.9E-7	5.0E-7
SO EH Total	8.6E-8	1.6E-7	2.9E-7	5.1E-7

[a] Data from Cruzan *et al*. (2001), biochemical parameters from Cohen *et al*. (2002). Model results are based on seven-day simulations (with the exposure pattern of 6 hours/day x 5 days/week). Body weights for increasing dose groups were 44, 43, 42, 40, and 39 grams, respectively.

The fourth study is the NCI (1979) gavage study in mice. Model simulations were run for a 0.030 kg mouse for five days with parameter set "1" only. Cumulative model predictions were divided by seven to give average daily values. Csanady *et al*. (1994) give an oral uptake constant of 0.8 $hour^{-1}$ for oral administration to a 0.025 kg mouse. The model simulations were unstable with 0.8 $hour^{-1}$ and thus uptake constants were reduced to 0.4 $hour^{-1}$ at 150 mg/kg and 0.2 $hour^{-1}$ at 300 mg/kg to allow the simulations to proceed without excessive error. It seems unlikely that these alterations in uptake rate significantly affected model predictions since uptake was still quite rapid and both styrene and styrene oxide were cleared from blood and fat within 24 hours. The results of the model predictions are shown in Table 47.

Table 47. Mouse Gavage PBPK Estimates of Dose Metrics for Styrene and Styrene Oxide: Parameter Set 1[a]

Metrics	Dose (mg/kg-day)	
	150	**300**
AUC (M-hr/day)		
Blood SO	1.9E-4	4.0E-4
Lung SO	4.7E-4	1.0E-3
VRG SO	5.1E-5	1.1E-4
AMET (mol/kg-day)		
ST Liver	1.6E-3	3.4E-3
ST Lung	1.1E-4	2.2E-4
ST Total	1.7E-3	3.6E-3
SO EH Liver	1.6E-6	2.7E-6
SO EH Lung	1.0E-6	2.1E-6
SO EH Total	2.6E-6	4.8E-6
C_{max} (M)		
SO	2.4E-7	2.6E-7

Abbreviations: AUC = area under the concentration x time curve; AMET = amount metabolized; VRG = vessel rich group, a surrogate for mammary tissue; ST = styrene; SO = styrene oxide; EH = styrene oxide metabolism via the epoxide hydrolase path; Blood = mixed venous blood.
[a] Data from NCI (1979), biochemical parameters from Csanady *et al*. (1994); Hynes *et al*. (1999); and Mendrala *et al*. (1993). Model results are based on five-day simulations. Intestinal uptake constant was reduced to 0.4/hour at the low dose and 0.2/hour at the high dose to allow the model to run (compared to 0.8/hour default value from Csanady *et al*. (1994).

Calculation of Cancer Potency

Linear Multistage (LMS) Method

Cancer potency values are derived in this section according to the methods and guidelines described above and in CDHS (1985), U.S. EPA (1986), Anderson *et al*. (1983), Crump (1981), and Crump *et al*. (1977). Air concentrations in ppm were converted to concentration in μg/L with the formula: 1 ppm styrene = 104.15 g/mole × 1 mole/24.45 L × 10^{-6} = 4.26 μg/L. The calculations for cancer potency incorporate the PBPK modeling results described above and also adjustments for human scaling from animal data as described below for each study and endpoint.

Dose calculations from Conti *et al*. (1988) are adjusted for continuous exposure as follows: 100 ppm styrene = 426 μg/L × 4/24 hours × 5/7 days × 52/104 weeks = 25.36 μg/L. To calculate human equivalent doses (HED) from the adjusted air concentrations, a breathing rate of 20,000 L/day and a 70 kg body weight were assumed. The estimated human equivalent doses based on the administered dose levels are: 0, 1.811, 3.622, 7.245, 14.49, and 21.73 mg/kg-day, for the 0, 25, 50, 100, 200, and 300 ppm exposure groups, respectively. From the PBPK analysis, human surrogate doses for VRG using Parameter Set "1" (Table 40) are estimated to be: 0, 1.509, 2.536, 3.925, 5.736, and 7.245 mg/kg-day for the 0, 25, 50, 100, 200, and 300 ppm exposure groups, respectively. For Parameter Set "2" (Table 41), human surrogate dose levels for VRG, styrene oxide (SO) are estimated to be: 0, 1.552, 2.794, 4.451, 6.520, and 7.866 mg/kg-day for the 0, 25, 50, 100, 200, and 300 ppm exposure groups, respectively.

From the female rat data, potency values for the three human doses estimated above are calculated for the maximum likelihood estimate q_1 (MLE) and 95 percent upper bound q_1* (Table 48). For the administered dose range (six degrees of freedom for fit, p = 0.39), q_1 (MLE) = 0.04304, and q_1* = 0.07526 $(mg/kg\text{-}d)^{-1}$. Using Parameter Set "1" for the styrene oxide AUC VRG surrogate dose levels (p = 0.63), q_1(MLE) = 0.1317 and q_1* = 0.2143 $(mg/kg\text{-}d)^{-1}$. Using Parameter Set "2" SO AUC VRG surrogate dose levels (p = 0.60), q_1(MLE) = 0.1170 and q_1* = 0.1910 $(mg/kg\text{-}d)^{-1}$. Body weight for a female rat is assumed to be 0.35 kg and for a human is 70 kg, the correction factor to apply to the body weight ¾ scaled potency estimates for 2/3 scaling is $(70/0.35)^{(3/4-2/3)}$ = 1.56.

Table 48. Human Cancer Potency Values Derived from Female Rat Benign and Malignant Mammary Tumors (Conti *et al*., 1988)*

Dose Metric	Goodness of Fit, P	q_1 (MLE) $(mg/kg\text{-}d)^{-1}$	q_1* 3/4 scaled $(mg/kg\text{-}d)^{-1}$	q_1* 2/3 scaled $(mg/kg\text{-}d)^{-1}$
Administered Dose	0.39	0.0430	0.075	0.12
PBPK SO VRG AUC				
Parameter set 1	0.63	0.1317	0.21	0.33
Parameter set 2	0.60	0.117	0.19	0.30

Note: q_1* values based on 2/3 scaling calculated from ¾ scaled values by multiplying by $(70/0.35)^{(3/4-2/3)}$ = 1.56.

Beliles *et al.* (1985) calculated administered doses of 0, 12, and 21 mg/kg-day to female rats based on the mean drinking water consumption rates of 118.3, 106.8, and 62.9 g/kg-day for control, low, and high dose groups, respectively. The mean body weight of 0.4 kg at 52 weeks (mean of mean values for three dose groups) is used to calculate the interspecies factors: interspecies factor for 2/3 scaling = $(70/0.4056)^{1/3}$ = 5.568 and interspecies factor for ¾ scaling = $(70/0.4056)^{1/4}$ = 3.624.

From the female rat data, values for the administered dose range (no degrees of freedom for fit) are q_1 (MLE) = 0.01734 and q_1* = 0.04550 $(mg/kg\text{-}d)^{-1}$. No significant change is observed using the PBPK Parameter Set "1" VRG surrogate dose levels (Table 42). However, when different scaling factors are applied, q_1* = 0.1656 (for 3/4 scaling) and q_1* = 0.2533 (for 2/3 scaling) are calculated.

For the third study, using data from Cruzan *et al.* (2001), both male and female mice demonstrated increased incidence of lung adenoma and carcinoma following inhalation exposure to styrene. To calculate cancer potencies, the intermittent administered doses are first adjusted for continuous exposure as follows for 100 ppm styrene: 100 x 4.26 μg/L × 6/24 hours × 5/7 days = 76.07 μg/L. An additional time adjustment is needed for female mice since they were necropsied at week 97 instead of the standard 104 weeks.

Human equivalent doses are calculated assuming a breathing rate of 20,000 L/day and a 70 kg body weight for a human adult. Therefore, using the adjusted dose of 076.07 μg/L (see above) × 20,000 L/day ÷ 70 kg = 21733 μg/kg-day, or 21.7 mg/kg-day. For the administered concentrations of 0, 20, 40, 80, and 160 ppm (in air), human equivalent doses are 0, 4.56, 9.13, 18.04, and 34.99 mg/kg-day, respectively.

For female lung adenoma and carcinoma combined, the full data set is 8/66, 22/67, 22/69, 14/70, and 30/67. The summary of potency estimates is given in Table 49. The full data set was not adequately fit by the LMS model. Excluding the 80 ppm outlier group resulted in an acceptable fit and this adjusted data set was used for the PBPK dose surrogates. For the administered dose, the potency values were 0.022 and 0.041 $(mg/kg\text{-}d)^{-1}$ for ¾ and 2/3 interspecies scaling, respectively. For the PBPK dose metrics with two parameter sets, potency values ranged from 0.026 to 0.041 $(mg/kg\text{-}d)^{-1}$ for ¾ scaling and from 0.049 to 0.077 $(mg/kg\text{-}d)^{-1}$ for 2/3 scaling.

For male lung adenoma and carcinoma combined the potency values are summarized in Table 50. The administered dose gave potency values of 0.021 and 0.039 $(mg/kg\text{-}d)^{-1}$ for ¾ and 2/3 scaling, respectively. The PBPK dose metrics with two parameter sets gave values ranging from 0.026 to 0.040 $(mg/kg\text{-}d)^{-1}$ for ¾ scaling and 0.048 to 0.074 $(mg/kg\text{-}d)^{-1}$ for 2/3 scaling.

Table 49. Human Cancer Potency Values Derived from Female Mouse Lung Adenomas and Carcinomas Combined (Cruzan *et al*., 2001)[a]

Dose Metric, Data Set	Goodness of Fit, P	q_1 (MLE) $(mg/kg\text{-}d)^{-1}$	q_1* 3/4 scaled $(mg/kg\text{-}d)^{-1}$	q_1* 2/3 scaled $(mg/kg\text{-}d)^{-1}$
Administered Dose				
Full data set	0.003 (poor fit)	0.0447	0.016	0.030
Exclude top dose group	0.004 (poor fit)	0.00377	0.015	0.028
Exclude top two dose groups	0.12	0.0409	0.059	0.111
Exclude 80 ppm outlier dose group	0.054	0.0154	0.022	0.041
PBPK Lung all metabolites				
Full data set, Parameter set 1	0.0035 (poor fit)	0.0156	0.028	0.053
Exclude 80 ppm group, parameter set 1	0.20	0.0308	0.041	0.077
Exclude 80 ppm group, parameter set 2	0.088	0.0212	0.029	0.054
PBPK Lung SO AUC				
Exclude 80 ppm group, parameter set 1	0.17	0.029	0.039	0.073
Exclude 80 ppm group, parameter set 2	0.080	0.01987	0.026	0.049

[a] Note: Values include correction for less than lifetime study $(104/97)^3 = 1.232$; q_1* values based on 2/3 scaling calculated from ¾ scaled values by multiplying by $(70/0.035)^{(3/4-2/3)} = 1.88$.

Using the data from NCI (1979) for male mouse tumors, the reported dose levels were 0, 150, and 300 mg/kg for five days/week for 78 weeks, followed by a 13 week observation period. Administered continuous doses of 0, 91.83, and 183.66 mg/kg-day were calculated by multiplying the oral (gavage) doses by 5/7 days and by 78/91 weeks to adjust to lifetime exposure. The PBPK dose metric "Lung SO AUC" gave adjusted doses of 0, 91.83, and 195.38 mg/kg-d. The PBPK metric "Lung all metabolites" gave the same values as the administered dose. The quantal tumor data were 0/20, 6/44, and 9/43, respectively. Correction for less than lifetime study duration was $(104/91)^3 = 1.4927$. It was assumed that male mice weighed 35 grams, a reasonable approximation of the mean weight from Figure 4 in NCI (1979). The interspecies 2/3 scaling factor was $(70/0.035)^{1/3} = 12.60$. The interspecies ¾ scaling factor was $(70/0.035)^{1/4} = 6.687$.

Table 50. Human Cancer Potency Values Derived from Male Mouse Lung Adenomas and Carcinomas Combined (Cruzan *et al.*, 2001)[a]

Dose Metric, Data Set	Goodness of Fit, P	q_1 (MLE) $(mg/kg\text{-}d)^{-1}$	q_1* ¾ scaled $(mg/kg\text{-}d)^{-1}$	q_1* 2/3 scaled $(mg/kg\text{-}d)^{-1}$
Administered Dose	0.078	0.014	0.021	0.039
PBPK LUNG ALL METABOLITES				
Parameter set 1	0.067	0.0314	0.040	0.074
Parameter set 2	0.13	0.019	0.027	0.050
PBPK Lung SO AUC				
Parameter set 1	0.24	0.02647	0.036	0.067
Parameter set 2	0.12	0.018	0.026	0.048

[a]Note: q_1* values based on 2/3 scaling calculated from ¾ scaled values by multiplying by $(70/0.043)^{(3/4-2/3)} = 1.85$.

The potency values are summarized in Table 51. For the administered dose the ¾ scaled potency was 0.031 $(mg/kg\text{-}d)^{-1}$ whereas the potency based on the styrene oxide lung AUC dose metric was 0.030 $(mg/kg\text{-}d)^{-1}$. The 2/3 scaled potencies were 0.058 and 0.056 $(mg/kg\text{-}d)^{-1}$, respectively.

Table 51. Human Cancer Potency Values Derived from Male Mouse Lung Tumors (NCI, 1979)[a]

Dose Metric, Data Set	Goodness of Fit, P	q_1 (MLE) $(mg/kg\text{-}d)^{-1}$	q_1* ¾ scaled $(mg/kg\text{-}d)^{-1}$	q_1* 2/3 scaled $(mg/kg\text{-}d)^{-1}$
Administered Dose	0.92	0.0207	0.031	0.058
PBPK SO Lung AUC				
Parameter set 1	0.29	0.0199	0.0296	0.056

[a]Note: Values include correction for less than lifetime study $(104/91)^3 = 1.49$; q_1* values based on 2/3 scaling calculated from ¾ scaled values by multiplying by $(70/0.035)^{(3/4-2/3)} = 1.88$.

All the relevant potencies from the above analyses are summarized in Table 52. Dose surrogates in Table 52 address differences in high and low dose pharmacokinetics in the rodent, but not cross-species differences between rodents and humans. The potency calculation assumes that the standard interspecies scaling applied is sufficient to take into account cross-species differences in pharmacokinetics. Set 1 parameters are from papers of Csanady *et al.* (1994), Hynes *et al.* (1999), and Mendrala *et al.* (1993). Set 2 parameters are from Cohen *et al.* (2002). All tumorigenicity was assumed to be explained by surrogate dose. Pharmacokinetically adjusted doses were the result of scaling of administered doses relative to the surrogate dose.

Table 52. Summary of Selected Cancer Potency Estimates by the LMS Method

Study and Reference	Tumor Type	Dose Surrogate	Potency [(mg/kg-d)$^{-1}$] [a]	
			3/4 Scaling[1]	2/3 Scaling[2]
Conti *et al.* (1988) Female Rat Inhalation	Benign and Malignant Mammary	Vessel Rich Group AUC Set "1" parameters Set "2" parameters	 0.21 0.19	 0.33 0.30
		Administered Dose	0.075	0.12
Beliles *et al.* (1985) Female Rat Drinking Water	Mammary	Administered Dose[3]	0.16	0.25
NCI (1979) Male Mouse Gavage	Lung	Lung SO AUC	0.030	0.056
		Administered dose or All lung metabolites	0.031	0.058
Cruzan *et al.* (2001) Female Mouse Inhalation	Lung	Lung Styrene Oxide AUC Set 1 parameters Set 2 parameters	 0.039* 0.026*	 0.073* 0.049*
		Lung All Metabolites Set 1 parameters Set 2 parameters	 0.041* 0.029*	 0.077* 0.054*
		Administered dose	0.022*	0.043*
Cruzan *et al.* (2001) Male Mouse Inhalation	Lung	Lung Styrene Oxide AUC Set 1 parameters Set 2 parameters	 0.036 0.026	 0.067 0.048
		Lung All Metabolites Set 1 parameters Set 2 parameters	 0.040 0.027	 0.074 0.050
		Administered dose	0.021	0.039

[a] Unadjusted for rodent/human differences in SO.
[1] For oral studies, assumes that human mg/kg-d dose is equivalent to rodent mg/kg-d dose multiplied by $(bw_{rodent}/bw_{human})^{1/4}$; for inhalation that ppm in air give rise to equivalent risk, regardless of species.
[2] Assumes that, regardless of route, human mg/kg-d dose is equivalent to rodent mg/kg-d dose multiplied by $(bw_{rodent}/bw_{human})^{1/3}$
[3] No significant difference with pharmacokinetic adjustment.
* Outlier data point (80 ppm) removed.

Benchmark Response Method (BMR)

U.S. EPA (2003) and others (*e.g.*, Gaylor *et al.*, 1994) have more recently advocated a benchmark dose method (BMR, benchmark response) for estimating cancer risk. This involves fitting a mathematical equation to the dose-response data. A linear or multistage procedure is often used. A point of departure (POD) on the fitted curve is defined; for animal carcinogenesis bioassays, this is usually the lower 95 percent confidence bound on the dose predicted to cause a 10 percent increase in tumor incidence (LED_{10}). Linear

extrapolation from the point of departure to zero dose is used to estimate risk at low doses, either when mutagenicity or other data imply that this is appropriate, or in the default case where no data on mechanism are available. The slope factor thus determined from the experimental data is corrected for experimental duration in the same way as the q_1^* adjustments described for the linearized multistage (LMS) procedure.

Cancer potency values based on BMR methodology and the tumor data sets from Conti *et al.* (1988) are presented in Table 53. The first column gives the tumor type, either malignant mammary tumors (MT) or benign and malignant (BMT), and the dose metric used for the dose response relation. The second column gives the model fit to the data and the fit statistics by the Chi-squared goodness of fit test. A fit criterion of $P \geq 0.1$ is considered acceptable. The 10 percent effective dose (ED_{10}) and its 95 percent lower bound (LED_{10}) follow in the next two columns. The animal potency is simply $0.1/LED_{10}$ and this value is scaled to give a human equivalent potency in the last two columns. Applied doses are scaled using the factor $(W_h/W_a)^{1/4}$. PBPK metrics are scaled using $(W_h/W_a)^{1/8}$ assuming that sufficient pharmacokinetic adjustment has already been made with the use of the PBPK model. This approach assumes equal contributions of pharmacokinetic and pharmacodynamic differences to the overall animal to human extrapolation.

In Table 53 the BMR analysis of the Conti *et al.* (1988) female rat mammary tumor data is summarized. All the dose metrics evaluated gave adequate model fits. Dose metrics based on internal dosimetry of the styrene oxide metabolite, i.e., the area under the concentration x time curve (AUCso) for either blood or vessel rich tissue group, with either metabolic parameter set, gave superior fits to the mammary tumor data with potency values in a close range, 0.13-0.14 $(mg/kg\text{-}d)^{-1}$.

Table 53. Styrene Cancer Potency Estimates (Conti *et al*., 1988 Female Rat Mammary Tumors) by BMR

Tumor	Dose*	Dose response model, X^2, P	ED_{10}	LED_{10}	Animal Potency $0.1/LED_{10}$ $(mg/kg\text{-}d)^{-1}$	Human Potency $(mg/kg\text{-}d)^{-1}$
Malignant tumors only MT applied dose	0, 4.25, 8.50, 17.0, 34.0, 51.0 mg/kg-d	QL 3.66 0.454	13.3	8.57	0.012	0.046
BMT applied dose		QL 4.10 0.393	5.74	3.28	0.03	0.12
BMT PBPK AUCso vrg set #1	0, 5.30, 9.0, 14.0, 20.0, 26.0 μM hr/d	QL 2.62 0.623	2.84	1.747 (= 1.40 mg/kg-d by interpolation)	0.071	0.14
BMT PBPK AUCso vrg set #2	0, 15.0, 27.0, 43.0, 63.0, 76.0 μM hr/d	QL 2.75 0.60	8.702	5.329 (= 1.51 mg/kg-d by interpolation)	0.0662	0.13
BMT PBPK AUCso blood Set #1	0, 4.0, 6.7, 11.0, 16.0, 19.0 μM hr/d	QL 2.64 0.62	2.17	1.33 (= 1.41 mg/kg-d external)	0.071	0.14
BMT PBPK AUCso blood Set #2	0, 11.0, 20.0, 32.0, 47.0, 57.0 μM hr/d	QL 2.78 0.594	6.52	3.99 (= 1.54 mg/kg-d external)	0.065	0.128
BMT AMETst total Set #1	0, 48.0, 94.0, 190, 370, 540 μmol/kg-d	QL 3.99 0.407	61.00	35.0 (= 3.098 mg/kg-d external)	0.032	0.063
BMT AMETst total Set #2	0, 47.0, 95.0, 190, 380, 560 μmol/kg-d	QL 4.08 0.396	63.45	36.33 (= 3.285 mg/kg-d external)	0.030	0.060

*Note: Lifetime average dose adjustment = 4/24 hr x 5/7 days x 52/104 weeks; MT = malignant tumors; BMT = benign and malignant tumors; VRG = vessel rich group; QL = quantal linear dose response model; M = moles/L; ST = styrene; SO = styrene oxide; ED_{10} = dose giving a 10 percent increase in tumors over control; LED_{10} = 95 percent lower bound on the ED_{10}; $(W_h/W_a)^{1/4}$ = interspecies scaling for pharmacokinetic and pharmacodynamic differences; $(W_h/W_a)^{1/8}$ = interspecies scaling for pharmacodynamic differences only. AUC and AMET values converted to external mg/kg-d equivalents.

Tables 54 and 55 provide a similar summary of the analysis of the Cruzan *et al*. (2001) female mouse lung tumor data with parameter sets one and two, respectively. In Table 54 only three metrics provided adequate fits by the Chi-squared goodness of fit test: AUCso in lung and vessel rich group and the amount of styrene metabolized by the lung (AMETst lung). Of these the AUCso vrg gave a marginally better fit, albeit all three metrics gave very similar human potency values of 0.025-0.028 $(mg/kg\text{-}d)^{-1}$. In Table 51 with parameter set 2, only two metrics gave adequate fits: AUCso blood and AUCso vrg with a (rounded) potency value of 0.020 $(mg/kg\text{-}d)^{-1}$.

Table 54. Styrene Animal and Human Cancer Potency and Unit Risk Estimates (Cruzan *et al*., 2001 Female Mouse Lung Tumors) by BMR, Parameter Set 1

Dose metrics*	**Dose response model, X^2, P**	**ED_{10}**	**LED_{10}**	**Pa $0.1/LED_{10}$ $(mg/kg\text{-}d)^{-1}$**	**Ph $(mg/kg\text{-}d)^{-1}$ $(70/0.035)^{1/8}$**	**Uh $(\mu g/m^3)^{-1}$**
Applied mg/m^3	QL 5.83 0.054 n.s.	29.34	18.81			
AUCso Blood µMhr/d	QL 7.75 0.021 n.s.	165.1	105.3			
AUCso Lung µMhr/d	QL 3.51 0.172	30.43	20.79 (= 10.17 mg/m^3)	0.00982	0.0254	7.26E-6
AUCso Vrg µMhr/d	QL 3.00 0.22	15.64	10.82 (= 9.37 mg/m^3)	0.01066	0.0276	7.88E-6
AMETst Lung µmol/kg-d	QL 3.26 0.196	32.42	22.29 (= 9.86 mg/m^3)	0.01013	0.0262	7.49E-6
AMETst Liver µmol/kg-d	QL 6.64 0.036 n.s.	331.2	208.0			
AMETst Total µmol/kg-d	QL 6.21 0.045 n.s.	356.7	226.4			

*Note: Lifetime average dose adjustment = 6/24 hr x 5/7 days. Female data set analyzed without outlier and with $(104/97)^3$ adjustment for less than lifetime observation; QL = quantal linear dose response model; n.s. = unacceptable fit; M = moles/L; ST = styrene; SO = styrene oxide; ED_{10} = dose giving a 10 percent increase in tumors over control; LED_{10} = 95percent lower bound on the ED_{10}; Pa = animal potency; Ph = human potency; Uh = human unit risk; $(W_h/W_a)^{1/4}$ = interspecies scaling for pharmacokinetic and pharmacodynamic differences; $(W_h/W_a)^{1/8}$ = interspecies scaling for pharmacodynamic differences only. AUC and AMET values were converted to external mg/m^3 equivalents.

Table 55. Styrene Animal and Human Cancer Potency and Unit Risk Estimates (Cruzan *et al*., 2001 Female Mouse Lung Tumors) by BMR, Parameter Set 2

Dose metrics*	Dose response model, X^2, P	ED_{10}	LED_{10}	Pa $0.1/LED_{10}$ $(mg/kg\text{-}d)^{-1}$	Ph $(mg/kg\text{-}d)^{-1}$ $(70/0.035)^{1/8}$	Uh $(\mu g/m^3)^{-1}$
Applied mg/m^3	QL 5.83 0.054 n.s.	29.34	18.81			
AUCso Blood µMhr/d	QL 4.59 0.101	4.71	3.12 (= 12.95 mg/m^3)	0.00772	0.01995	5.70E-6
AUCso Lung µMhr/d	QL 5.23 0.073 n.s.	9.95	6.49			
AUCSO Vrg µMhr/d	QL 4.54 0.103	6.26	4.15 (= 13.18 mg/m^3)	0.00758	0.0196	5.60E-6
AMETst Lung µmol/kg-d	QL 5.04 0.080 n.s.	109.98	72.07			
AMETst Liver µmol/kg-d	QL 6.98 0.030 n.s.	180.99	112.58			
AMETst Total µmol/kg-d	QL 6.24 0.044 n.s.	295.78	187.50			

*Note: Lifetime average dose adjustment = 6/24 hr x 5/7 days. QL = quantal linear dose response model; n.s. = unacceptable fit; M = moles/L; ST = styrene; SO = styrene oxide; ED_{10} = dose giving 10 percent increase in tumors over control; LED_{10} = 95 percent lower bound on the ED_{10}; Pa = animal potency; Ph = human potency; Uh = human unit risk; $(W_h/W_a)^{1/4}$ = interspecies scaling for pharmacokinetic and pharmacodynamic differences; $(W_h/W_a)^{1/8}$ = interspecies scaling for pharmacodynamic differences only. AUC values were converted to external mg/m^3 equivalents.

Tables 56 and 57 summarize the male mouse lung tumor data with parameter sets one and two, respectively. All of the internal metrics based on styrene oxide AUC in blood, lung, and vessel rich tissues gave adequate model fits to the tumor data with parameter set "1". Of these the AUCso blood gave a marginally superior fit with a potency value of 0.027 $(mg/kg\text{-}d)^{-1}$. The metabolized dose metric of AMETst lung also gave a superior fit and a potency value of 0.028 $(mg/kg\text{-}d)^{-1}$. With parameter set two, three metrics gave adequate fits: AUCSO blood and AUCSO VRG with potency values of 0.018 and 0.014 $(mg/kg\text{-}d)^{-1}$, respectively, and AMETst lung with a potency value of 0.017 $(mg/kg\text{-}d)^{-1}$. As with the female data the parameter set 1 derived metrics gave better fits than those derived from parameter set 2. The best single value from the Cruzan *et al.* (2001) data is

0.028 $(mg/kg\text{-}d)^{-1}$ representing both the female AUCso vrg based and male AMETst lung-based potencies.

Table 56. Styrene Animal and Human Cancer Potency and Unit Risk Estimates (Cruzan *et al*., 2001 Male Mouse Lung Tumors) by BMR, Parameter Set 1

Dose metrics*	**Dose response model, X^2, P**	**ED_{10}**	**LED_{10}**	**Pa $0.1/LED_{10}$ $(mg/kg\text{-}d)^{-1}$**	**Ph $(mg/kg\text{-}d)^{-1}$ $(70/0.043)^{1/8}$**	**Uh $(\mu g/m^3)^{-1}$**
Applied mg/m^3	QL 5.83 0.054 n.s.	29.34	18.81			
AUCso Blood µMhr/d	QL 3.86 0.277	10.33	6.89 (= 8.01 mg/m^3)	0.0109	0.0266	7.84E-6
AUCso Lung µMhr/d	QL 4.38 0.223	27.18	17.91 (= 8.97 mg/m^3)	0.00975	0.0237	7.00E-6
AUCso Vrg µMhr/d	QL 5.08 0.166	15.47	10.68 (= 12.10 mg/m^3)	0.00723	0.0176	5.19E-6
AMETst Lung µmol/kg-d	QL 3.73 0.293	32.65	21.87 (= 7.69 mg/m^3)	0.01138	0.0277	8.17E-6
AMETst Liver µmol/kg-d	QL 7.27 0.064 n.s.	288.31	174.63			
AMETst Total µmol/kg-d	QL 6.76 0.080 n.s.	314.55	193.69			

*Note: Lifetime average dose adjustment = 6/24 hr x 5/7 days. QL = quantal linear dose response model; n.s. = unacceptable fit; M = moles/L; ST = styrene; SO = styrene oxide; ED_{10} = dose giving a 10% increase in tumors over control; LED_{10} = 95% lower bound on the ED_{10}; Pa = animal potency; Ph = human potency; Uh = human unit risk; $(W_h/W_a)^{1/4}$ = interspecies scaling for pharmacokinetic and pharmacodynamic differences; $(W_h/W_a)^{1/8}$ = interspecies scaling for pharmacodynamic differences only. AUC values were converted to external mg/m^3 equivalents.

Table 57. Styrene Animal and Human Cancer Potency and Unit Risk Estimates (Cruzan *et al*., 2001 Male Mouse Lung Tumors) by BMR, Parameter Set 2

Dose metrics*	Dose response model, X^2, P	ED_{10}	LED_{10}	Pa $0.1/LED_{10}$ $(mg/kg\text{-}d)^{-1}$	Ph $(mg/kg\text{-}d)^{-1}$ $(70/0.043)^{1/8}$	Uh $(\mu g/m^3)^{-1}$
Applied mg/m^3	QL 5.83 0.054 n.s.	29.34	18.81			
AUCso Blood μMhr/d	QL 5.53 0.137	4.31	2.75 (= 11.70 mg/m^3)	0.00748	0.0182	5.37E-6
AUCso Lung μMhr/d	QL 6.33 0.0967	8.78	5.48			
AUCso vrg μMhr/d	QL 6.17 0.104	6.42	4.05 (= 15.07 mg/m^3)	0.00580	0.0142	4.17E-6
AMETst Lung μmol/kg-d	QL 5.69 0.128	106.5	67.67 (= 12.49 mg/m^3)	0.0070	0.0171	5.03E-6
AMETst Liver μmol/kg-d	QL 8.06 0.045 n.s.	173.1	101.9			
AMETst Total μmol/kg-d	QL 7.06 0.070 n.s.	274.9	167.5			

*Note: Lifetime average dose adjustment = 6/24 hr x 5/7 days. QL = quantal linear dose response model; n.s. = unacceptable fit; M = moles/L; ST = styrene; SO = styrene oxide; ED_{10} = dose giving a 10 percent increase in tumors over control; LED_{10} = 95 percent lower bound on the ED_{10}; Pa = animal potency; Ph = human potency; Uh = human unit risk; $(W_h/W_a)^{1/4}$ = interspecies scaling for pharmacokinetic and pharmacodynamic differences; $(W_h/W_a)^{1/8}$ = interspecies scaling for pharmacodynamic differences only. AUC values converted to external mg/m^3 equivalents.

The results of the analysis of the NCI (1979) male mouse lung tumor data are given in Table 58. While all the metrics evaluated gave adequate fits to the quantal tumor data, the applied dose (lifetime average) appeared to give the best fit with a potency value of 0.015 $(mg/kg\text{-}d)^{-1}$.

The analytical summary of the mammary tumor data from the drinking water study of Beliles *et al.* (1985) is given in Table 59. Here also the applied dose appeared to give the best fit based on the study authors' estimated doses, with a potency value of 0.054 $(mg/kg\text{-}d)^{-1}$. A simulated PBPK dose based on average water intake values from the study gave a better model fit but nearly the same potency of 0.048 $(mg/kg\text{-}d)^{-1}$. The best value from this study is probably that based on the applied dose.

Table 58. Styrene Animal and Human Cancer Potency and Unit Risk Estimates (NCI, 1979 Male Mouse Lung Tumors) by BMR, Parameter Set 1

Dose metrics*	Dose response model, X^2, P	ED_{10}	LED_{10}	Pa $0.1/LED_{10}$ $(mg/kg\text{-}d)^{-1}$	Ph $(mg/kg\text{-}d)^{-1}$
Applied Dose mg/kg-d	QL 0.18 0.915	75.82	50.95	0.00224	0.015(a)
AUCso Lung mg/kg-d converted from μM-hr/d	QL 1.99 0.369	91.24	61.30	0.00186	0.0048(b)
AMETst Lung mg/kg-d	QL 0.39 0.822	8.07	5.42	0.0211	0.054(b)
AMETst Liver mg/kg-d	QL 0.29 0.87	122.72	82.4	0.00139	0.0036(b)

*Note: Lifetime average dose adjustment = 5/7 days x 78/91 weeks. Short study adjustment of $(104/91)^3$ applied to potencies. QL = quantal linear dose response model; M = moles/L; ST = styrene; SO = styrene oxide; ED_{10} = dose giving a 10 percent increase in tumors over control; LED_{10} = 95 percent lower bound on the ED_{10}; Pa = animal potency; Ph = human potency; a, $(W_h/W_a)^{1/4}$ = interspecies scaling for pharmacokinetic and pharmacodynamic differences; b, $(W_h/W_a)^{1/8}$ = interspecies scaling for pharmacodynamic differences only.

Table 59. Styrene Animal and Human Cancer Potency and Unit Risk Estimates (Beliles *et al*., 1985 Female Rat Mammary Tumors) by BMR, Parameter Set 1

Dose metrics*	Dose response model, X^2, P	ED_{10}	LED_{10}	Pa $0.1/LED_{10}$ $(mg/kg\text{-}d)^{-1}$	Ph $(mg/kg\text{-}d)^{-1}$
Applied Dose mg/kg-d	QQ 0.11 0.746	9.04	6.77	0.0148	0.054(a)
AMET mg/kg-d	QQ 0.16 0.68	7.43	5.62	0.0178	0.034(b)
PBPK Simulated Applied Dose mg/kg-d	QL 0 0.984	6.99	3.98	0.0251	0.048(b)

*Note: Doses calculated by study authors 0, 12, 21 mg/kg-d, mean body weight at 52 weeks = 405.6 g. QL = quantal linear dose response model; QQ = quantal quadratic dose response model; M = moles/L; ST = styrene; SO = styrene oxide; ED_{10} = dose giving a 10 percent increase in tumors over control; LED_{10} = 95 percent lower bound on the ED_{10}; Pa = animal potency; Ph = human potency; a, $(W_h/W_a)^{1/4}$ = interspecies scaling for pharmacokinetic and pharmacodynamic differences; b, $(W_h/W_a)^{1/8}$ = interspecies scaling for pharmacodynamic differences only.

In Table 60, health protective concentrations of styrene in drinking water are calculated based on the selected or best potency values from the key studies, assuming a 70 kg average human body weight. Values are shown for ingestion consumption only and for multiroute exposure to contaminated water (including bathing, showering, flushing toilets, and consumption of produce watered with or growing in contaminated groundwater) derived using the CalTOX[3] multiroute environmental distribution and exposure model (see output in attached Appendix). The model estimates inhalation, ingestion and dermal exposures derived from low-level contaminated ground water (Appendix p. 9). Summing these exposures and calculating their proportions to each other yields a combined exposure estimate, in which inhalation represents 43.5 percent of the total, ingestion 38.1 percent, and dermal 18.3 percent. The combined (multiroute) exposure value is derived from the ratio of total dose (from groundwater) compared to the dose from ingestion, or 2 L/day x 2.78/1.06 = 5.25 Leq/day.

Table 60. Health Protective Concentrations (C) for Styrene Calculated from Selected Cancer Potency Values Based on BMR Methodology.

Study, Species, Route, Site	Cancer Potency $(mg/kg\text{-}d)^{-1}$	C calculated for ingestion only mg/L* (ppb)	C calculated for multiroute exposure mg/L (ppb)
Conti *et al*. (1998) Female Rat Inhalation Mammary Tumors	0.14 (0.112)[#]	2.5E-4 (0.25)	9.51E-5 (0.095)
Cruzan *et al*. (2001) Male and Female Mice Inhalation, Lung tumors	0.026 (male 0.027, female 0.026)	1.35E-3 (1.35)	5.1E-4 (0.51)
Beliles *et al*. (1985) Female Rat Drinking Water Mammary Tumors	0.054 (0.0535)	6.48E-4 (0.65)	2.46E-4 (0.25)
NCI (1979) Male Mice Gavage Lung Tumors	0.015 (0.0194)	2.33E-3 (2.3)	8.87E-4 (0.89)
Geometric Mean (Arithmetic Grand Mean)	0.042 (0.046)	8.33E-4	3.15E-4
Geometric mean in ppb		0.83	0.32
Kogevinas *et al*. (1994) Human Inhalation Malignant Lymphoma	0.017	2.1E-3 (2.1)	7.8E-4 (0.78)

* Note C = 70 kg x $1x10^{-6}$ risk/(potency x L/d); ingestion only = 2 L/d; multiroute = 5.26 Leq/d.
[#] Values in parentheses (column 1) are arithmetic means of all adequately fitting dose metrics.

[3] CalTOX is a spreadsheet model prepared by the California Dept. of Toxic Substances Control to assist in health-risk assessments that address contaminated soils and adjacent air, surface water, sediments, and ground water. The spreadsheet includes a dynamic multimedia transport and transformation model, multiple pathway exposure scenario models, and strategies to quantify and reduce uncertainty in multimedia, multiple-pathway exposure models.

For ingestion only, C values range from 0.25 to 2.3 ppb (μg/L). For multiroute exposures the values are lower, 0.10 to 0.90 ppb. The geometric mean potency derived from the four studies is 0.042 $(mg/kg\text{-}d)^{-1}$ and concentrations derived from this value are 0.83 and 0.32 ppb (μg/L), respectively. An alternative arithmetic grand mean potency derived from the study means of all adequately fit dose metrics was 0.046 $(mg/kg\text{-}d)^{-1}$.

CALCULATION OF PHG

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks for carcinogens or noncarcinogens must take into account the toxicity of the chemical itself, as well as the potential exposure of individuals using the water. Tap water is used directly as drinking water and for preparing foods and beverages. It is also used for bathing or showering, and in washing, flushing toilets, and other household uses, resulting in potential dermal and inhalation exposures.

Noncarcinogenic Effects

From the toxicological data review and the dose-response analysis, there were three different endpoints from which to select a NOAEL or LOAEL to develop a PHG based on noncarcinogenic toxicity. In general, uncertainty and adjustment factors are used to derive levels considered safe for the general and variable exposed population. This includes considering uncertainty and additional protection for population subgroups that may be more sensitive or susceptible than the average population due to life stage (e.g., infants, fetuses, the elderly), genetic makeup, and health or nutritional status.

Calculation of a public health-protective concentration (C) for styrene in drinking water for noncarcinogenic endpoints follows the general equation:

$$C = \frac{NOAEL/LOAEL \times BW \times RSC}{UF \times WC}$$

where,

NOAEL/LOAEL	=	no-observed-adverse-effect-level or lowest-observed-adverse-effect-level;
BW	=	adult body weight (a default of 70 kg for male, 60 kg for female, or 10 kg for a child);
RSC	=	relative source contribution (a default of 0.2 to 0.8, or 20 to 80 percent of the exposure from water);
UF	=	uncertainty factors (typical defaults of 10 for subchronic to chronic extrapolation, 10 for interspecies extrapolation, and 10 for human variability, but may use separate allowances for toxicokinetic and toxicodynamic components (e.g., Renwick, 2000) or other values based on identified sensitive subpopulations;
WC	=	daily water consumption rate: default values of 2 L/d for 60-70 kg adult; 1 L/d for 10 kg child; higher values of L equivalents (Leq/d)

are used for volatile organic compounds to account for inhalation and dermal exposure through other household uses of tap water.

Alternatively, a Benchmark Dose approach may be used to estimate a critical dose or concentration of a chemical. A mathematical model is fit to the dose-response data and the concentration corresponding to a specific low percentage response (usually 5 percent) is determined. The lower 95 percent confidence limit on that specified response is considered the benchmark dose (BMDL). The BMDL is substituted for the NOAEL/LOAEL in the above equation.

Currently few sources of drinking water are contaminated with styrene and people are more likely to be exposed to styrene in air from industrial emissions and cigarette smoke (see Section on Environmental Occurrence and Human Exposure). When the predominant exposures are estimated to be from non-drinking water sources, with inadequate data to more precisely calculate a relative source contribution, OEHHA has customarily used the default value of 0.2, as has the U.S. EPA (U.S. EPA, 2000). Thus the default RSC of 0.2 was used for styrene.

A multi-route exposure assessment was performed using the CalTOX multicompartment exposure model, available through the California Department of Toxic Substances Control (DTSC, 1994). This Excel spreadsheet multi-compartment model utilizes the physicochemical properties of a substance to estimate the proportion of a chemical that will distribute into household air and other exposure sources from normal uses of tap water, and the resulting human exposures to the chemical via the inhalation and dermal routes. Input of the physicochemical parameters specific to styrene resulted in an estimate of 38 percent of total exposure from ingestion, 44 percent from inhalation, and 18 percent from dermal uptake. Based on an ingestion volume of 2 L/day, this can be summarized as an equivalent exposure to the styrene contained in 5.25 L/day of water. The CalTOX program output is shown in Appendix 1.

Neurotoxicity (humans):

Mutti *et al*., 1984b (inhalation route to oral route)

The Benchmark Concentration (BMC_{05}) for workers of 1.7 ppm styrene in air was calculated above to be equivalent to a continuous oral dose of 0.48 mg/kg-day. An uncertainty factor of 10 to account for variations in human sensitivity to styrene was judged to be applicable. Calculation of a public health-protective concentration (C) for styrene in drinking water for noncarcinogenic endpoints by ingestion follows:

$$C = \frac{BMDL \times BW \times RSC}{UF \times WC}$$

$$C = \frac{0.48 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{10 \times 5.25 \text{ Leq/day}} = 0.114 \text{ mg/L} = 100 \text{ ppb}$$

Respiratory (animals):

Cruzan *et al*., 2001 (mice)

Decreased eosinophilic staining of epithelial cells in terminal bronchioles, bronchiolar epithelial hyperplasia, bronchiolar epithelial hyperplasia extending into alveolar ducts, and bronchioloalveolar hyperplasia of male mice were the most sensitive adverse responses. The LOAEL for these lung effects was 20 ppm. Adjusting for duration and frequency of exposure, 20 ppm is equivalent to 3.57 ppm (3.57 ppm = 20 × 6 hours/24 hours × 5 days/7 days). Assuming that this is a systemic-dose effect, this can be converted to a corresponding daily oral dose (mg/kg-day) as follows: (0.65 × 3.57 ppm × 4.26 mg/m^3-ppm × 20 m^3/day) ÷ 70 kg = 2.82 mg/kg-day. The factor of 0.65 adjusts for an absorption intake of 65 percent styrene via inhalation, and assumes 100 percent absorption via the oral route.

A Benchmark Dose approach was also applied to the data on decreased eosinophilic staining of terminal bronchioles in male mice. Fitting the probit model to the log dose and response data using the U.S. EPA BMDS software gave an MLE_{05} of 3.8 ppm and a BMC_{05} of 1.1 ppm. Using a route-to-route calculation as in the previous paragraph, 1.1 ppm is equivalent to an oral dose of 0.155 mg/kg-day.

The uncertainty and adjustment factors applied to obtain a dose of styrene in water at which respiratory toxicity is not expected to occur in humans include 10 to adjust from a LOAEL to NOAEL (UF_L) (unless BMD is used), and 10 to account for (intraspecies) variability within the human population (UF_H). In this case, the interspecies (animal to human) factor (UF_A) of 10 has been subdivided into toxicokinetic and toxicodynamic components. A default factor of 3 (rounded from 3.16, the square root of 10) is applied for interspecies differences in toxicodynamics. For toxicokinetic differences the U.S. EPA (1994) has derived a Regional Gas Disposition Ratio (RGDR) which uses animal and human minute volumes and surface areas in various regions of the lung (extrathoracic, tracheobronchial, and pulmonary) to address differences in toxicokinetics. OEHHA has used this approach in deriving some chronic Reference Exposure Levels (OEHHA, 2000). Using values of 500 cm^2 and 540,000 cm^2 for mouse and human pulmonary surface areas (SA), respectively, and 0.041 L/min and 13.9 L/min for mouse and human minute volumes (MV), respectively, RGDR was derived as follows:

$$RGDR = (MVa/MVh)/(SAa/SAh) = (0.041/13.9)/(500/540{,}000) = 3.19$$

In this instance the calculated RGDR of 3.19 is equivalent to the default value.

Using the LOAEL approach, the calculation of a health-protective concentration for ingestion is:

$$C = \frac{LOAEL \times BW \times RSC}{UF \times WC}$$

$$C = \frac{2.82 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{(10 \times 3.19 \times 3 \times 10) \times 5.25 \text{ Leq/day}} = 0.00786 \text{ mg/L} = 8 \text{ ppb}$$

Using the BMD approach, the equivalent calculation for ingestion is:

$$C = \frac{0.155 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{(1 \times 3.19 \times 3 \times 10) \times 5.25 \text{ Leq/day}} = 0.0043 \text{ mg/L} = 4 \text{ ppb}$$

Cruzan *et al*., 1998 (rats)

Exposure to 50 ppm styrene vapors for two years resulted in atrophy and/or degeneration of the olfactory epithelium and "prominent" Bowman's glands in the olfactory epithelium. Among the female rats, significant atrophy and/or degeneration of the olfactory epithelium was detected. The LOAEL is 50 ppm and the males appear to be more sensitive.

Uncertainty factors to be applied to the LOAEL include 10 to adjust from a LOAEL to a NOAEL (UF_L), 10 to adjust from rodent to human (interspecies variability) (UF_A), and 10 to account for human intraspecies variability (UF_H).

The concentration of 50 ppm is first converted from a discontinuous to continuous daily exposure to yield a concentration of 8.92 ppm (50 ppm x 6/24 hours x 5/7 days = 8.92 ppm). Assuming that these effects result from systemic absorption of the chemical rather than a direct effect the units of ppm can be converted to mg/kg-day to yield a dose of 7.02 mg/kg-day (8.92 ppm x 4.26 mg/m^3 per ppm x 20 m^3/day x 0.65 retention factor) / 70 kg = 7.02 mg/kg-day) for risk assessment purposes. Using the LOAEL approach, the calculation of a health-protective concentration for ingestion is:

$$C = \frac{\text{LOAEL} \times \text{BW} \times \text{RSC}}{\text{UF} \times \text{WC}}$$

$$C = \frac{7.02 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 5.25 \text{ Leq/day}} = 0.0187 \text{ mg/L} = 20 \text{ ppb}$$

Hepatotoxicity (animals):

Quast *et al*., 1979 (dogs)

The study of Quast *et al*. (1979) was used as the basis of the U.S. EPA Reference Dose (RfD) of 0.2 mg/kg-day for styrene. Due to the small group sizes of only four dogs each, OEHHA decided not to use this study in calculation of a health-protective concentration.

Srivastava *et al*., 1982 (rats)

In the Dose Response Assessment a LOAEL of 171 mg/kg-day was derived for elevated enzyme levels in rat liver. The uncertainty and adjustment factors to be applied to estimate a health-protective concentration are 10 to adjust from a LOAEL to a NOAEL (UF_L), 3 to adjust from 100 days to lifetime (subchronic to chronic) (UF_S), 10 to adjust from a rodent to a human dose (interspecies variability) (UF_A), and 10 to account for variability within the human population (UF_H).

The concentration in water at which the hepatotoxic effects of styrene are not expected to occur in humans by ingestion is:

$$C = \frac{LOAEL \times BW \times RSC}{UF \times WC}$$

$$C = \frac{171 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{3{,}000 \times 5.25 \text{ Leq/day}} = 0.152 \text{ mg/L (ppm)} = 200 \text{ ppb}$$

For noncarcinogenic effects the range of health-protective concentrations obtained for multiroute exposures was 4 to 200 ppb. The most health protective value was 4 ppb using the benchmark approach with the data on bronchiolar effects in male mice (Cruzan *et al*., 2001). The drinking water concentration estimated to be fully protective against non-cancer effects of styrene for a lifetime of exposure, is therefore proposed to be 4 ppb.

Carcinogenic Effects

For calculation of carcinogenic potency of styrene exposure, data were available from both human and animal studies. Assessment of dose-response was done on both data sets, as described below. From the Kogevinas *et al.* (1994) study of occupational styrene exposure, cancer potency values were estimated using a modeling approach for pancreatic cancer and malignant lymphomas in units of excess relative risk per ppm-year. These values were used to derive an estimated excess lifetime risk for continuous exposure in California, with resulting potency values of 0.0089 $(\text{mg/kg-day})^{-1}$ (pancreatic cancer) and 0.017 $(\text{mg/kg-day})^{-1}$ (malignant lymphomas). However, neither of these data sets yielded particularly good fits and confidence bounds (see Figures 3 and 4).

Animal studies provided potency values ranging from 0.016 to 0.33 $(\text{mg/kg-d})^{-1}$ for LMS methodology and 0.015 to 0.14 $(\text{mg/kg-d})^{-1}$ for the BMR approach. Different interspecies scaling assumptions with PBPK dosimetry were used in these two approaches. The estimated cancer potency derived from the human data was similar to that derived from the animal studies, but the animal studies were judged to provide more reliable data (better-characterized exposures, better dose-response, and tighter confidence bounds on the potencies). Therefore the animal studies were judged to be superior for extrapolation in this case.

For carcinogens, the following general equation can be used to calculate the public health-protective concentration (C) for styrene in drinking water (in mg/L):

$$C = \frac{R \times BW}{CSF \times WC} = \text{mg/L (ppm)}$$

where,

R = *de minimis* lifetime excess individual cancer risk (a default of 10^{-6});

BW = adult body weight (a default of 70 kg);

CSF = cancer slope factor, a potency derived from the lower 95% confidence limit on the 10% tumor dose (LED_{10}). q_1* is the upper 95%

confidence limit on the cancer potency slope calculated by the LMS model, CSF = $0.1/LED_{10}$. Both potency estimates are converted to human equivalent (in $[mg/kg\text{-}day]^{-1}$) using $BW^{3/4}$ scaling, i.e. human slope factor = animal slope factor x $(BW_{human}/BW_{animal})^{1/4}$ or x $(BW_{human}/BW_{animal})^{1/8}$ for PBPK adjusted dosimetry;

WC = volume of water consumed daily (L/d), which may include a multiroute exposure estimate for volatile organic compounds (i.e., Leq/d).

Using values from Tables 52 and 60, the following calculations of health protective concentrations of styrene in drinking water can be made.

1. Cruzan female mouse administered dose and LMS method

   C = $(10^{-6}$ x 70 kg)/(0.022 x 2 L/d) = 0.00159 mg/L = 1.6 ppb

2. Cruzan female and male mouse lung tumors, PBPK dose metric, multiroute exposure, and BMR method

   C = $(10^{-6}$ x 70 kg)/(0.026 x 5.25 Leq/d) = 0.00051 mg/L = 0.51 ppb

3. NCI male mouse gavage and LMS method

   C = $(10^{-6}$ x 70 kg)/(0.030 x 2 L/d) = 0.00117 mg/L = 1.2 ppb

4. NCI male mouse gavage and BMR method

   C = $(10^{-6}$ x 70 kg)/(0.015 x 5.25 Leq/d) = 0.000887 mg/L = 0.89 ppb

5. Conti female rat mammary PS 2 (highest potency) and LMS

   C = $(10^{-6}$ x 70 kg)/(0.21 x 2 L/d) = 0.000166 mg/L = 0.17 ppb

6. Conti female rat mammary tumors and BMR method

   C = $(10^{-6}$ x 70 kg)/(0.14 x 5.25 Leq/d) = 9.51×10^{-5} mg/L = 0.095 ppb

7. Beliles female rat mammary tumors and BMR method (only drinking water study)

C = (10^{-6} x 70 kg)/(0.054 x 5.25 Leq/d) = 0.000246 mg/L = 0.25 ppb

Summary

Carcinogenic effects yielded more health protective (lower) values than noncarcinogenic effects. For carcinogenic effects, the best value based on a single study is that from the Cruzan *et al.* (2001) mouse study. The study was conducted recently, was a lifetime study, and used an adequate number of animals and of concentrations of styrene. With the exception of the female mice exposed to 80 ppm, it gave a convincing dose response. Health-protective *de minimis* risk values of 1.4 ppb by ingestion only and of 0.5 ppb (calculation 2 above rounded from 0.51) for multiroute exposure to styrene in drinking water (BMR method) were estimated from this study. The PHG value for styrene, based on a one in one million theoretical lifetime risk of cancer, is therefore proposed to be 0.0005 mg/L or 0.5 ppb.

The proposed PHG value is considered to be fully protective against effects in potentially sensitive subpopulations, including infants and children, pregnant women, and the elderly, based on the following rationale: (1) It is based on cancer in mice, a species which appears to be more sensitive than humans to the carcinogenic effects of styrene. (2) The proposed PHG results in a dose to humans that is approximately eight orders of magnitude below the animal developmental NOAEL of 300 mg/kg day and the animal reproductive NOAEL of 200 mg/kg-day. (3) The cytochrome P450 isozymes, which activate styrene to the reactive metabolite styrene-7,8-oxide, are not elevated in these sensitive groups, and epoxide hydrase and glutathione transferases, which detoxify styrene-7,8-oxide, are not decreased in these sensitive subgroups.

RISK CHARACTERIZATION

The primary sources of uncertainty in the development of the PHG for styrene in drinking water are also the general issues of uncertainty in any risk assessment, particularly mode of action, extrapolation of risk versus dose, and extrapolation across species. Inter- and intra-species extrapolation and RSC are also relevant for estimation of a non-cancer health-protective value.

The proposed PHG of 0.5 ppb was calculated based on the carcinogenic potency of styrene. In calculating the proposed PHG, a *de minimis* theoretical excess individual cancer risk level of 10^{-6} was assumed based on a linear risk extrapolation. The corresponding drinking water concentrations for cancer risk levels of 10^{-5} and 10^{-4} are 5 and 50 ppb, respectively.

For PHGs, our use of the RSC has, with a few exceptions, followed U.S. EPA drinking water risk assessment methodology. Our current thinking for development of RSCs for noncarcinogens is based on the guidance in the U.S. EPA Ambient Water Quality Criteria (U.S. EPA, 2000). U.S. EPA has treated carcinogens differently from noncarcinogens with respect to the use of RSCs. For noncarcinogens, RfDs (in mg/kg-day), drinking water equivalent levels (DWELs, in mg/L) and MCLGs (in mg/L) are calculated using

uncertainty factors (UFs), body weights and water consumption rates (L/day), and the RSC, respectively. The RSC defaults are 20, 40, and 80 percent (0.2, 04, and 0.8); other values may be used depending on the scientific evidence (U.S. EPA, 2000).

For approaches that use low-dose extrapolation based on quantitative risk assessment, U.S. EPA does not factor in an RSC. The use of low-dose extrapolation is considered by U.S. EPA to be adequately health-protective without the additional source contributions. In developing PHGs, we have adopted the assumption that RSCs should not be factored in for carcinogens for which we have calculated a cancer potency based on low-dose extrapolation. This is an area of uncertainty and scientific debate; it is not clear how this assumption impacts the overall health risk assessment, nor how these procedures may change under the newer U.S. EPA cancer guidelines (U.S. EPA, 2005a).

For the non-cancer extrapolation, it may be questioned whether effects observed in the respiratory and olfactory system in an inhalation study are applicable to the ingestion route. For purposes of this risk assessment, we have assumed that they can be, if the effect is not an irritant or site-of-contact effect. For example, bleomycin can cause pulmonary fibrosis in animals both by injection (McCullough *et al*., 1978) and by intratracheal instillation (Starcher *et al*., 1978). Thus bleomycin can induce lung fibrosis both systemically and by direct administration to the lung.

Inhalation of benzo(a)pyrene results in carcinogenicity of the lung (Thyssen *et al*., 1981), while ingestion of benzo(a)pyrene causes mainly stomach cancer in animals (Rigdon and Neal, 1966), and dermal application leads to skin cancer (Wynder *et al.*, 1957). Thus, benzo(a)pyrene mainly causes cancer at the site of contact. In animals, styrene has caused bronchiolar-alveolar adenomas and carcinomas both by ingestion and by inhalation (Table 13). Thus it is also plausible that the multiple effects on respiratory tissue (in the study of Cruzan *et al.*, 2001) associated with styrene inhalation may also occur with styrene ingestion.

We have concluded that moderate and higher styrene exposures cause reproductive and developmental toxicity in animal studies. A recent review of styrene by the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) has concluded that there is no reproductive toxicity in rats, while the NOAEL for developmental effects was 150 ppm by inhalation (NTP, 2005, 2006). While we disagree in part with the interpretations of the applicable studies and the conclusions of the CERHR report, this has no effect on the development of the non-cancer PHG, which is derived from other, more-sensitive endpoints.

Many styrene studies have been conducted since the U.S. EPA reviewed styrene toxicity in the late 1980s for the oral reference dose (RfD) which led to the current federal MCLG and MCL, or the early 1990s for the inhalation reference concentration (RfC). The new epidemiology reports, animal studies, and *in vivo* and *in vitro* genotoxicity and mutagenicity data have provided much new data related to potential carcinogenicity of styrene. However, U.S. EPA has not yet completed a cancer risk assessment for styrene (U.S. EPA, 2009). The International Agency for Research on Cancer (IARC) in 2002 found “limited” evidence of carcinogenicity for styrene in humans and animals. In IARC parlance this means a positive association between styrene exposure and cancer is found for which a causal interpretation is considered to be credible, but IARC could not rule out

chance, bias, or confounding with reasonable confidence to identify the chemical as a known human carcinogen. OEHHA similarly finds suggestive but not definitive direct evidence of cancer in the human cancer data. The excess malignancies observed most frequently are of the lymphatic and hematopoietic systems.

An NTP expert panel (NTP, 2008a,b) recently recommended that styrene should be listed in the NTP Report on Carcinogens as "reasonably anticipated to be a human carcinogen based on limited evidence of carcinogenicity in humans and sufficient evidence in animals." The vote for this identification was eight to two, and the two members of the panel who voted against the motion are reported to have done so because, in their opinion, styrene should be listed as "known to be a human carcinogen" (NTP, 2008b).

Indirect evidence of human carcinogenicity comes from animal cancer bioassays and studies of genotoxicity and pharmacokinetics of styrene. Several animal cancer bioassays for styrene indicate its carcinogenic potential for humans. Lung tumors were observed in female and male mice exposed by inhalation for their lifetime and in male mice exposed by gavage. Lung tumors developed in the progeny of mice exposed to styrene *in utero* and then after birth by gavage. Female rats developed mammary gland tumors when exposed by inhalation or drinking water ingestion. Styrene has given positive results in several genotoxicity tests including reverse mutation in *Salmonella typhimurium* (Ames test) and induction of DNA strand breaks and chromosomal aberrations in human and animal cells. Styrene is metabolized *in vivo* to styrene-7,8-oxide, a mutagen and carcinogen. Other metabolites of styrene can also be toxic.

Overall OEHHA concludes that there is sufficient evidence that styrene causes cancer in animals. While several epidemiological studies of styrene and cancer in workers exposed in reinforced plastics and other industries have been published, the human data do not show proof of carcinogenicity. Although we believe it is prudent to assume carcinogenicity for the purposes of risk assessment, this represents an area of controversy and uncertainty. If the PHG were based on non-cancer effects, the health-protective level could be significantly higher. However, whether to incorporate an extra uncertainty factor for possible carcinogenicity would have to be addressed in such a calculation.

Populations potentially sensitive to styrene effects might include persons with liver diseases, because of the hepatotoxic effects and the rapid hepatic metabolism of this chemical in healthy individuals. Higher exposures are expected in infants who consume tap water, because of the greater water consumption on a body weight basis in this group. Early-in-life exposures to carcinogens can also result in heightened tumor risk because of the greater lifespan remaining for tumor development. U.S. EPA's recently released Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (U.S. EPA, 2005b) recommends that cancer risk assessments accommodate early-life exposures by multiplying cancer risk potencies by factors intended to account for higher exposures and potential susceptibility in infants and children. For exposures before 2 years of age, a 10-fold adjustment to cancer potency is recommended; for exposures between 2 and <16 years of age, a 3-fold adjustment is recommended. Together, these adjustments correspond to a time-weighted factor of 1.66. It is recommended that a cancer potency factor be multiplied by this value, if that factor was determined in a study which did not include exposures during infancy. OEHHA has also established guidelines for the inclusion of children's higher exposure and potential

susceptibility, which incorporate similar considerations and correction factors (OEHHA, 2009). We are not suggesting an adjustment to the cancer potency for styrene based on these principles because this risk assessment was prepared, externally reviewed, and posted for public comment prior to formal adoption of this guideline. Comments are invited on this issue.

OTHER GUIDANCE VALUES AND REGULATORY STANDARDS

A Maximum Contaminant Level (MCL) of 0.1 mg/L (100 μg/L, 100 ppb) was established by the California Department of Public Health (Title 22 CCR, Division 4, Chapter 15, Article 4, Section 64431) in 1994. The current detection limit for the purposes of reporting, or DLR, is 0.5 ppb. U.S. EPA set a Maximum Contaminant Level Goal (MCLG) and Maximum Contaminant Level (MCL) of 0.1 mg/L for styrene in 1991 (U.S. EPA, 1993). The federal MCL is based on potential liver, kidney, or circulatory system problems above this level (U.S. EPA, 2002).

Under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65), styrene has not been considered for listing as a chemical known to the State of California to cause cancer or reproductive harm. The International Agency for Research on Cancer (IARC, 1994) classified styrene as 2B, possibly carcinogenic to humans and reviewed and confirmed this classification in 2002 (IARC, 2002). The U.S. EPA Integrated Risk Information System (IRIS) states that styrene carcinogenicity is being reassessed (as of 01/02/1998) and that the carcinogenicity assessment is "Not available at this time" (U.S. EPA, 2009). However, a consumer fact sheet on styrene which is part of the National Primary Drinking Water Regulations publication (U.S. EPA, 2002) notes that "Styrene has the potential to cause the following effects from a lifetime exposure at levels above the MCL: liver and nerve tissue damage; cancer."

Styrene is listed as a hazardous air pollutant (HAP), generally known or suspected to cause serious health problems. The Clean Air Act, as amended in 1990, directs U.S. EPA to set standards requiring major sources to sharply reduce routine emissions of toxic pollutants. Discharges of styrene into water are regulated under section 311(b)(2)(A) of the Federal Water Pollution Control Act, which designated styrene a hazardous substance; styrene discharges are further regulated by the Clean Water Act Amendments of 1977 and 1978 (HSDB, 2007). State drinking water standards or guidelines have been established in at least four states, ranging from a high in Arizona of 140 μg/L to a low in Maine of 5 μg/L (U.S. EPA, 1995).

The U.S. Food and Drug Administration (FDA) permits styrene (U.S. FDA, 1999; CFR, 2001) as a food additive permitted for direct addition to food for human consumption as a synthetic flavoring substance and adjuvant used in the minimum quantity required to produce its intended effect, and otherwise in accordance with good manufacturing practice. FDA also permits styrene as an indirect food additive for use only as a component of adhesives. U.S. EPA has exempted styrene from a requirement for a food tolerance when used as an inert ingredient in pesticide formulations applied to growing crops, raw agricultural commodities after harvest, and animals (U.S. EPA, 1998).

The National Research Council (NRC, 1977) reported an **Acceptable Daily Intake** (ADI) for styrene of 0.133 mg/kg-day. This value was calculated on the basis of a no-adverse-effect level from a six-month gastric intubation study in rats (Wolf *et al*., 1956, as reported by NRC, 1977). Dosage-related effects at higher doses in this study were limited to increased liver and kidney weights. Based on this ADI, a "suggested no-adverse-effect level in drinking water" of 0.9 mg/L was calculated (NRC, 1977).

The U.S. Occupational Safety and Health Administration (OSHA) reported that a voluntary compliance program had been adopted in 1996 by industries using styrene (OSHA, 2002). This voluntary program reduces styrene exposures to a 50 ppm time-weighted average (TWA) with a 100 ppm (15 minute) ceiling (OSHA, 2002). OSHA originally established these levels in a 1989 rule that was later invalidated by the courts because of challenges to levels established for other substances (OSHA, 1996). Styrene levels were based on avoidance of narcotic effects on workers (OSHA, 1996).

U.S. EPA (2009) has a Reference Dose (RfD) of 0.2 mg/kg-day for styrene (last updated 9/01/90) based on red blood cell and liver effects in beagle dogs (Quast *et al*., 1979). The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily (usually oral) exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

U.S. EPA (2009) also has a Reference Concentration (RfC) of 1,000 $\mu g/m^3$ (last updated 7/01/93) based on central nervous system effects in workers (Mutti *et al*., 1984b). The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

Styrene is listed as a chemical subject to California's Air Toxics Hot Spots Emissions and Assessment Act of 1987 (California Health and Safety Code Section 44300 *et seq*.). Under the Act OEHHA is mandated to develop health risk assessment guidelines. OEHHA (1999) developed an acute Reference Exposure Level (REL) of 21,000 $\mu g/m^3$ based on eye and throat irritation in three human volunteers (Stewart *et al*., 1968). An acute REL is the concentration at or below which no adverse health effects are anticipated for an exposure duration of one hour.

OEHHA (2000) developed a chronic inhalation REL of 900 $\mu g/m^3$, also based on central nervous system effects in workers (Mutti *et al*., 1984b). A chronic REL is a concentration at or below which no adverse health effects are anticipated for an extended duration of exposure to a chemical.

REFERENCES

ACGIH (1997). Styrene, Monomer. Documentation of the Threshold Limit Values and Biological Exposure Indices. Supplements to the 6th edition (1991). American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

Aikawa H, Kinoue T, Takahiko Y, Kayama F, Sakabe K (2001). Behavioural change in the pups produced by perinatal styrene exposure during their brain development stage. Jpn J Clin Ecol 10(1):33-41.

Albano JD, Ward E, Jemal A, Anderson R, Cokkinides VE, Murray T, Henley J, Liff J, Thun MJ (2007). Cancer mortality in the United States by education level and race. J Natl Cancer Inst 99(18):1384-94.

Amoore JE, Hautala E (1983). Odor as an aid to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J Appl Toxicol 3(6):272-90.

Andersen ME, Gargas ML, Ramsey JC (1984). Inhalation pharmacokinetics: evaluating systemic extraction, total *in vivo* metabolism, and the time course of enzyme induction for inhaled styrene in rats based on arterial blood: inhaled air concentration ratios. Toxicol Appl Pharmacol 73(1):176-87

Anderson EL, Carcinogen Assessment Group of the U.S. Environmental Protection Agency (1983). Quantitative approaches in use to assess cancer risk. Risk Anal 3(4):277-95.

Anderson D, Francis AJ, Godbert P, Jenkinson PC, Butterworth KR (1993). Variability in chromosome aberrations, sister chromatid exchanges, and mitogen-induced blastogenesis in peripheral lymphocytes from control individuals. Environ Health Perspect 101 Suppl 3:83-8.

Andersson HC, Tranberg EA, Uggla AH, Zetterberg G (1980). Chromosomal aberrations and sister-chromatid exchanges in lymphocytes of men occupationally exposed to styrene in a plastic-boat factory. Mutat Res 73(2):387-401.

Andjelkovic D, Taulbee J, Symons M (1976). Mortality experience of a cohort of rubber workers, 1964-1973. J Occup Med 18(6):387-94.

Andjelkovic D, Taulbee J, Symons M, Williams T (1977). Mortality of rubber workers with reference to work experience. J Occup Med 19(6):397-405.

Anttila A, Pukkala E, Riala R, Sallmen M, Hemminki K (1998). Cancer incidence among Finnish workers exposed to aromatic hydrocarbons. Int Arch Occup Environ Health 71:187-93.

Anwar WA, Shamy MY (1995). Chromosomal aberrations and micronuclei in reinforced plastics workers exposed to styrene. Mutat Res 327(1-2):41-7.

Arfini G, Mutti A, Vescovi P, Ferroni C, Ferrari M *et al.* (1987). Impaired dopaminergic modulation of pituitary secretion in workers occupationally exposed to styrene: further evidence from PRL response to TRH stimulation. J Occup Med 29:826-30.

Arlien-Soborg P (1992). Styrene. In: Solvent Neurotoxicity. Arlien-Soborg P, ed. CRC Press, Boca Raton, FL, pp. 129-53.

Arnedo-Pena A, Bellido-Blasco J, Villamarin-Vazquez JL, Aranda-Mares JL, Font-Cardona N, Gobba F, Kogevinas M (2003). Acute health effects after accidental exposure to styrene from drinking water in Spain. Environ Health 2(1):6.

Arrighi HM, Hertz-Picciotto I (1996). Controlling the healthy worker survivor effect: an example using arsenic exposure and respiratory cancer. Occup Environ Med 53:455-62.

Arrighi HM, Hertz-Picciotto I (1993). Re: Definitions, sources, magnitude, effect modifiers, and strategies of reduction of the healthy worker effect (Letter). J Occup Med 35:890-91.

Arrighi HM, Hertz-Picciotto I (1994). The evolving concept of the healthy worker survivor effect. Epidemiology 5:189-96.

Artuso M, Angotzi G, Bonassi S, Bonatti S, De Ferrari M, Gargano D *et al.* (1995). Cytogenetic biomonitoring of styrene-exposed plastic boat builders. Arch Environ Contam Toxicol 29(2):270-74.

Astrand I, Kilbom A, Ovrum P, Wahlberg I, Vesterberg O (1974). Exposure to styrene. I. Concentration in alveolar air and blood at rest and during exercise and metabolism. Work Environ Health 11:69-85.

ATSDR (1992). Toxicological Profile for Styrene. Agency for Toxic Substances and Disease Registry, U.S. Public Health Service, Atlanta, GA.

Bakhtizina GZ, Koval'skii GB, Popuchiyev VV (1983). [Morphofunctional investigation of the ovaries upon long-term exposure to styrene.] Gig Sanit (3):77-9. [In Russian]

Bakke OM, Scheline RR (1970). Hydroxylation of aromatic hydrocarbons in the rat. Toxicol Appl Pharmacol 16(3):691-700.

Baquet CR, Commiskey P (2000). Socioeconomic factors and breast carcinoma in multicultural women. Cancer 88(5 Suppl):1256-64.

Barale R (1991). The genetic toxicology of styrene and styrene oxide. Mutat Res 257(2):107-26.

Barcellona PS, Barale R, Campana A, Zucconi D, Rossi V, Caranti S (1987). Correlations between embryotoxic and genotoxic effects of phenytoin in mice. Teratog Carcinog Mutagen 7(2):159-68.

Bardodej A, Bardodejova E (1970). Biotransformation of ethyl benzene, styrene and alpha-methylstyrene in man. Am Ind Hyg Assoc J 31(2):206-9.

Baron JM, Zwadlo-Klarwasser G, Jugert F, Hamann W, Rubben A, Mukhtar H *et al.* (1998). Cytochrome P450 1B1: A major P450 isoenzyme in human blood monocytes and macrophage subsets. Biochem Pharmacol 56:1105-10.

Bastlová T, Podlutsky A (1996). Molecular analysis of styrene oxide-induced hprt mutation in human T-lymphocytes. Mutagenesis 11(6):581-91.

Bastlova T, Vodicka P, Peterkova K, Hemminki K, Lambert B (1995). Styrene oxide-induced HPRT mutations, DNA adducts and DNA strand breaks in cultured human

lymphocytes. Carcinogenesis 16(10):2357-62.

Bauer C, Leporini C, Bronzetti G, Corsi C, Nieri R *et al.* (1980). The problem of negative results for styrene in the *in vitro* mutagenesis test with metabolic activation (microsomal assay): explanation by gas chromatographic analysis. Boll Soc Ital Biol Sper 56(3):203-7.

Behari M, Choudhary C, Roy S, Maheshwari MC (1986). Styrene-induced peripheral neuropathy. A case report. Eur Neurol 25:424-7.

Beije B, Jenssen D(1982). Investigation of styrene in the liver perfusion/cell culture system. No indication of styrene-7,8-oxide as the principal mutagenic metabolite produced by the intact rat liver. Chem Biol Interact 39(1):57-76.

Beliles RP, Butala JH, Stack CR, Makris S (1985). Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. Fundam Appl Toxicol 5(5):855-68.

Belvedere G, Blezza D, Cantoni L (1982). Ferrodoxin reductase catalyzes styrene oxidation to styrene oxide. Experientia 38(3):306-7.

Belvedere G, Tursi F (1981). Styrene oxidation to styrene oxide in human blood erythrocytes and lymphocytes. Res Commun Chem Pathol Pharmacol 33(2):273-82.

Belvedere G, Tursi F, Vainio H (1983). Non-microsomal activation of styrene to styrene oxide. In: Extrahepatic Drug Metabolism and Chemical Carcinogenesis. Rydstrom J, Montelius J, Bengtsson M, eds. Elsevier Publ, Netherlands, pp. 193-200.

Benignus VA, Geller AM, Boyes WK, Bushnell PJ (2005). Human neurobehavioral effects of long-term exposure to styrene: a meta-analysis. Environ Health Perspect 113(5):532-8.

Bergamaschi E, Smargiassi A, Mutti A, Cavazzini S, Vettori MV *et al.* (1997). Peripheral markers of catecholaminergic dysfunction and symptoms of neurotoxicity among styrene-exposed workers. Int Arch Occup Environ Health 69(3):209-14.

Bergamaschi E, Mutti A, Cavazzini S, Vettori MV, Renzulli FS, Franchini I (1996). Peripheral markers of neurochemical effects among styrene-exposed workers. Neurotoxicology 17(3-4):753-9.

Bergamaschi E, Smargiassi A, Mutti A, Franchini I, Lucchini R (1995). Immunological changes among workers occupationally exposed to styrene. Int Arch Occup Environ Health 67:165-71.

Bergman K (1979). Whole-body autoradiography and allied tracer techniques in distribution and elimination studies of some organic solvents: benzene, toluene, xylene, styrene, methylene chloride, chloroform, carbon tetrachloride and trichloroethylene. Scand J Work Environ Health 5(Suppl 1):1-263.

Bergman K (1983). Application and results of whole-body autoradiography in distribution studies of organic solvents. Crit Rev Toxicol 12(1):59-118.

Bernardini S, Hirvonen A, Jarventaus H, Norppa H (2002). Influence of GSTM1 and GSTT1 genotypes on sister chromatid exchange induction by styrene in cultured human lymphocytes. Carcinogenesis 23(5):893-7.

Bernauer U, Vieth B, Ellrich R, Heinrich-Hersch B, Janig G-R, Gundert-Remy U (2000). CYP2E1 expression in bone marrow and its intra- and interspecies variability: approaches for a more reliable extrapolation from one species to another in the risk assessment of chemicals. Arch Toxicol 73:618-24.

Berode M, Droz PO, Boillat MA, Guillemin M (1986). Effect of alcohol on the kinetics of styrene and its metabolites in volunteers and in workers. Appl Ind Hyg 1(1):25-8.

Berode M, Droz PO, Guillemin M (1985). Human exposure to styrene. VI. Percutaneous absorption in human volunteers. Int Arch Occup Environ Health 55(4): 331-6.

Bhamre S, Anandatheerathavarada HK, Shankar SK, Boyd MR, Ravindranath V (1993). Purification of multiple forms of cytochrome P450 from a human brain and reconstitution of catalytic activities. Arch Biochem Biophys 301:251-5.

Bigbee WL, Grant SG, Langlois RG, Jensen RH, Anttila A, Pfaffli P *et al.* (1996). Glycophorin A somatic cell mutation frequencies in Finnish reinforced plastics workers exposed to styrene. Cancer Epidemiol Biomarkers Prev 5(10):801-10.

Biro A, Pallinger E, Major J, Jakab MG, Klupp T, Falus A, Tompa A (2002). Lymphocyte phenotype analysis and chromosome aberration frequency of workers occupationally exposed to styrene, benzene, polycyclic aromatic hydrocarbons or mixed solvents. Immunol Lett 81(2):133-40.

Bjørge C, Brunborg G, Wiger R, Holme JA, Scholz T, Dybing E, Søderlund EJ (1996). A comparative study of chemically induced DNA damage in isolated human and rat testicular cells. Reprod Toxicol 10(6):509-19.

Blake MJ, Castro L, Leeder JS, Kearns GL (2005). Ontogeny of drug metabolizing enzymes in the neonate. Semin Fetal Neonatal Med 10(2):123-38.

Boffetta P, Sali D, Kolstad H, Coggon D, Olsen J, Andersen A *et al*. (1998). Mortality of short-term workers in two international cohorts. J Occup Environ Med 40(12):1120-6.

Boffetta P, Adami HO, Cole P, Trichopoulos D, Mandel JS (2009). Epidemiologic studies of styrene and cancer: A review of the literature. J Occup Environ Med 51:xx-xx. Published online 22 October 2009, doi: 10.1097/JOM.0b013e3181ad49b2.

Bonassi S, Montanaro F, Ceppi M, Abbondandolo A (1996). Is human exposure to styrene a cause of cytogenetic damage? A re-analysis of the available evidence. Biomarkers 1:217-25.

Bond GG, Bodner KM, Olsen, GW, Cook RR (1992). Mortality among workers engaged in the development or manufacture of styrene-based products – an update. Scand J Work Environ Health 18:145-4.

Bond JA (1989). Review of the toxicology of styrene. Crit Rev Toxicol 19(3):227-49.

Bond JA, Csanady GA, Gargas ML, Guengerich FP, Leavens T *et al.* (1994). 1,3-Butadiene: linking metabolism, dosimetry, and mutation induction. Environ Health Perspect 102(Suppl 9):87-94.

Boogaard PJ, de Kloe KP, Sumner SCJ, van Elburg PA, Wong BA (2000a). Disposition of [ring-U-^{14}C] styrene in rats and mice exposed by recirculating nose-only inhalation.

Toxicol Sci 58:161-72.

Boogaard PJ, de Kloe KP, Wong BA, Sumner SCJ, Watson WP, van Sittert NJ (2000b). Quantitation of DNA adducts formed in liver, lungs and isolated lung cells of rats and mice exposed to ^{14}C-styrene by nose-only inhalation. Toxicol Sci 57:203-16.

Brams A, Buchet JP, Crutzen-Fayt MC, De Meester C, Lauwerys R, Leonard A (1987). A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure). Toxicol Lett 38(1-2):123-33.

Brenner DD, Jeffrey AM, Latriano L, Wazneh L, Warburton D *et al.* (1991). Biomarkers in styrene-exposed boatbuilders. Mutat Res 261(3):225-36.

Brodkin CA, Moon JD, Camp J, Echeverria D, Redlich CA, Willson RA, Checkoway H (2001). Serum hepatic biochemical activity in two populations of workers exposed to styrene. Occup Environ Med 58(2):95-102.

Brown WR (1999). SYI/7: Styrene: 104-Week Repeat Dose Inhalation Combined Toxicity/carcinogenicity Study in Mice. Immunohistochemistry of Primary Lung Tumors. (unpublished report). Styrene Information and Research Center, Arlington, VA.

Brown NA (1991). Reproductive and developmental toxicity of styrene. Reprod Toxicol 5:3-29.

Brown NA, Lamb JC, Brown SM, Neal BH (2000). A review of the developmental and reproductive toxicity of styrene. Regul Toxicol Pharmacol 32:228-47.

Brunnemann KD, Rivenson A, Cheng SC, Saa V, Hoffmann D (1992). A study of tobacco carcinogenesis. XLVII. Bioassays of vinylpyridines for genotoxicity and for tumorigenicity in A/J mice. Cancer Lett 65(2):107-13.

Burns CJ, Cruzan G, Tsai SP (2006). Styrene and breast cancer incidence in Texas: a comment on an ecological association. Breast Cancer Res Treat 97(3):339-40.

Busk L (1977). Mutagenicity testing of styrene and styrene epoxide in Salmonella typhimurium. Bull Environ Contam Toxicol 17(6):739-42.

CARB (2000). Annual Statewide Toxics Summary. Styrene. California Air Resources Board. Accessed 10/02/01 at: http://www.arb.ca.gov/. Last updated June 26, 2001.

CARB (2001). California Emissions Inventory Development and Reporting System (CEIDARS). Information collected under the Hot Spots Program. California Air Resources Board. Run date, October 18, 2001.

Campagna D, Mergler D, Huel G, Belanger S, Truchon G *et al.* (1995). Visual dysfunction among styrene-exposed workers. Scand J Work Environ Health 21:382-90.

Camurri L, Codeluppi S, Pedroni C, Scarduelli L (1983). Chromosomal aberrations and sister-chromatid exchanges in workers exposed to styrene. Mutat Res 119(3):361-9.

Camurri L, Codeluppi S, Scarduelli L, Candela S (1984). Sister chromatid exchanges in workers exposed to low doses of styrene. Basic Life Sci 29 Pt B:957-63.

Cantoni L, Slamona M, Facchinetti T, Pantarotto C, Belvedere G (1978). Hepatic and extrahepatic formation and hydration of styrene oxide *in vitro* in animals of different species. Toxicol Lett 2:79-186.

Cantor KP, Stewart PA, Brinton LA, Dosemeci M (1995). Occupational exposures and female breast cancer mortality in the United States. J Occup Environ Med 37 (3):336-48.

Cantoreggi S, Lutz WK (1993). Covalent binding of styrene to DNA in rat and mouse. Carcinogenesis 14(3):355-60.

Carlson GP (1997). Effects of inducers and inhibitors on the microsomal metabolism of styrene to styrene oxide in mice. J Toxicol Environ Health 51(5):477-88.

Carlson GP (1998). Metabolism of styrene oxide to styrene glycol by mouse liver and lung. J Toxicol Environ Health Part A 53(1):19-27.

Carlson GP, Hynes DE, Mantick NA (1998). Effects of inhibitors of CYP1A and CYP2B on styrene metabolism in mouse liver and lung microsomes. Toxicol Lett 98(3):131-7.

Carlson GP, Mantick NA, Powley MW (2000). Metabolism of styrene by human liver and lung. J Toxicol Environ Health, Part A 59:591-5.

Carlson GP, Rivera AAP, Mantick NA (2001). Metabolism of the styrene metabolite 4-vinylphenol by rat and mouse liver and lung. J Toxicol Environ Health Part A 63:541-51.

Carlson GP, Ullman M, Mantick NA, Snyder PW (2002). 4-Vinylphenol-induced pneumotoxicity and hepatotoxicity in mice. Toxicol Pathol 30(5):565-9.

Carlson GP (2003). In vitro metabolism of styrene to styrene oxide in liver and lung of CYP2E1 knockout mice. J Toxicol Environ Health A 66(9):861-9.

Carlsson A (1981). Distribution and elimination of ^{14}C-styrene in rat. Scand J Work Environ Health 7:45-50.

Carpenter CP, Shaffer CB, Weil CS, Smyth HF Jr (1944). Studies on the inhalation of 1,3 butadiene; with a comparison of its narcotic effect with benzol, toluol, and styrene, and a note on the elimination of styrene by the human. J Ind Hyg Toxicol 26:69-77.

Cascorbi I, Henning S, Brockmoller J, Gephart J, Meisel C, Muller MM *et al.* (2000). Substantially reduced risk of cancer of the aerodigestive tract in subjects with variant-463A of the myeloperoxidase gene. Cancer Res 60:644-9.

Castranova V, Rabovsky J, Tucker JH, Miles PR (1988). The alveolar Type II epithelial cell: a multifunctional pneumocyte. Toxicol Appl Pharmacol 93:472-83.

CDHS (1985). Guidelines for Chemical Carcinogen Risk Assessments and their Scientific Rationale. California Department of Health Services, Health and Welfare Agency, Sacramento, CA.

CFR (2001). Synthetic flavoring substances and adjuvants. 21 CFR § 172.515 (4/1/01). Accessed at: http://a257.g.akamaitech.net/7/257/2422/04nov20031500/edocket.access.gpo.gov /cfr_2001/aprqtr/pdf/21cfr172.515.pdf.

Chakrabarti S, Brodeur J (1981). Effects of microsomal induction and inhibition on styrene-induced acute hepatotoxicity in rats. J Toxicol Environ Health 8(4):599-607.

Chakrabarti S, Duhr MA, Senecal-Quevillon M, Richer CL (1993). Dose-dependent genotoxic effects of styrene on human blood lymphocytes and the relationship to its oxidative and metabolic effects. Environ Mol Mutagen 22(2):85-92.

Chakrabarti S, Malick MA (1991). *In vivo* nephrotoxic action of an isomeric mixture of S-(1-phenyl-2-hydroxyethyl)glutathione and S-(2-phenyl-2-hydroxyethyl)glutathione in Fischer-344 rats. Toxicology 67(1):15-28.

Chakrabarti SK (1999). Styrene and styrene oxide affect the transport of dopamine in purified rat striatal synaptic vesicles. Biochem Biophys Res Commun 255(1):70-4.

Chakrabarti SK (2000). Altered regulation of dopaminergic activity and impairment in motor function in rats after subchronic exposure to styrene. Pharmacol Biochem Behav 66(3):523-32.

Chakrabarti SK, Tuchweber B (1987). Effects of various pretreatments on the acute nephrotoxic potential of styrene in Fischer-344 rats. Toxicology 46(3):343-56.

Chandra M, Riley MG, Johnson DE (1992). Spontaneous neoplasms in aged Sprague-Dawley rats. Arch Toxicol 66:496-592.

Checkoway H, Echeverria D, Moon J-D, Heyer N, Costa LG (1994). Platelet monoamine oxidase B activity in workers exposed to styrene. Int Arch Occup Health 66:359-62.

Checkoway H, Williams TM (1982). A hematology survey of workers at a styrene-butadiene synthetic rubber manufacturing plant. Am Ind Hyg Assoc J 43(3):164-9.

Chen BQ (1989). [The effects of inhalation of styrene on the neurobehavior in the offsprings] [Chinese]. Chen Zhonghua Yu Fang Yi Xue Za Zhi 23(6):342-5.

Chen G-D, Chi L-H, Kostyniak PJ, Henderson D (2007). Styrene induced alterations in biomarkers of exposure and effects in the cochlea: mechanisms of hearing loss. Toxicol Sci 98(1):167–77.

Chernoff N, Setzer RW, Miller DB, Rosen MB, Rogers JM (1990). Effects of chemically induced maternal toxicity on prenatal development in the rat. Teratology 42(6):651-8.

Cherry N, Gautrin D (1990). Neurotoxic effects of styrene: further evidence. Br J Ind Med 47:29-37.

Chia S-E, Jeyaratnam J, Ong C-N, Ng T-P, Lee H-S (1994). Impairment of color vision among workers exposed to low concentrations of styrene. Am J Ind Med 26:481-8.

Chiang CL (1984). The life table and its applications. Robert E Krieger Publ Co., Malabar, FL.

Chmielewski J, Dolmierski R, Renke W, Kwiatkowski SR (1977). [Long-term effects of styrene on workers at the working place] (German) Z Gesamte Hyg 23(9):639-43.

Cho SI, Damokosh AI, Ryan LM, Chen D, Hu YA, Smith TJ, Christiani DC, Xu X (2001). Effects of exposure to organic solvents on menstrual cycle length. J Occup Environ Med 43:567-75.

Chovanec M, Näslund M, Spivak I, Dusinská M, Cedervall B, Kolman A (1998). Rejoining of DNA strand breaks induced by propylene oxide and epichlorohydrin in human diploid fibroblasts. Environ Mol Mutagen 32(3):223-8.

Christakopoulos A, Bergmark E, Zorcec V, Norppa H, Maki-Paakkanen J, Osterman-Golkar S (1993). Monitoring occupational exposure to styrene from hemoglobin adducts and metabolites in blood. Scand J Work Environ Health 19(4):255-63.

Coccini T, Fenoglio C, Nano R, De Piceis Polver P, Moscato G, Manzo L (1997). Styrene-induced alterations in the respiratory tract of rats treated by inhalation or intraperitoneally. J Toxicol Environ Health 52(1):63-77.

Coccini T, Randine G, Li B, Manzo L, Costa LG (1999). Effect of styrene on monoamine oxidase B activity in rat brain. J Toxicol Environ Health A 56(1):59-68.

Coggon D, Osmond C, Pannett B, Simmonds S, Winter PD, Acheson ED (1987). Mortality of workers exposed to styrene in the manufacture of glass-reinforced plastics. Scand J Work Environ Health 13:94-9.

Cohen JT, Carlson G, Charnley G, Coggon D, Delzell E, Graham JD *et al*. (2002). A comprehensive evaluation of the potential health risks associated with occupational and environmental exposure to styrene. J Toxicol Environ Health B Crit Rev 5(1-2):1-265.

Collins JF, Alexeeff GV, Lewis DC, Dodge DE, Marty MA, *et al.* (2004). Development of acute inhalation reference exposure levels (RELs) to protect the public from predictable excursions of airborne toxicants. J Appl Toxicol 24(2):155-66.

Compton-Quintana PJ, Jensen RH, Bigbee WL, Grant SG, Langlois RG, Smith MT, Rappaport SM (1993). Use of the glycophorin A human mutation assay to study workers exposed to styrene. Environ Health Perspect 99:297-301.

Conner MK, Alarie Y, Dombroske RL (1979). Sister chromatid exchange in regenerating liver and bone marrow cells of mice exposed to styrene. Toxicol Appl Pharmacol 50(2):365-7.

Conner MK, Alarie Y, Dombroske RL (1980). Sister chromatid exchange in murine alveolar macrophages, bone marrow, and regenerating liver cells induced by styrene inhalation. Toxicol Appl Pharmacol 55(1):37-42.

Conti B, Maltoni C, Perino G, Ciliberti A (1988). Long-term carcinogenicity bioassays on styrene administered by inhalation, ingestion and injection and styrene oxide administered by ingestion in Sprague-Dawley rats, and para-methylstyrene administered by ingestion in Sprague-Dawley rats and Swiss mice. Ann N Y Acad Sci 534:203-34.

Corsini E, Luster MI, Morgan DL, Craig WA, Rosenthal GJ (1994). Styrene inhalation and immune function in mice. Inhal Toxicol 6:647-54.

Cowles SR, Tsai SP, Snyder PJ, Ross CE *et al.* (1994). Mortality, morbidity, and haematologic results from a cohort of long-term workers involved in 1,3-butadiene monomer production. Occup Environ Med 51:323-29.

Coyle YM, Hynan LS, Euhus DM, Minhajuddin ATM (2005). An ecological study of the association of environmental chemicals on breast cancer incidence in Texas. Breast Cancer Res Treat 92(2):107-14.

Crump KS (1981). An improved procedure for low-dose carcinogenic risk assessment from animal data. J Environ Path Toxicol 5:675-84.

Crump KS, Guess HA, Deal KL (1977). Confidence intervals and test of hypotheses concerning dose response relations inferred from animal carcinogenicity data. Biometrics 33(3):437-51.

Cruzan G, Bus J, Banton M, Gingell R, Carlson G (2009). Mouse specific lung tumors from CYP2F2-mediated cytotoxic metabolism: An endpoint/toxic response where data from multiple chemicals converge to support a mode of action. Regul Toxicol Pharmacol 55(2):205:18.

Cruzan G, Carlson GP, Johnson KA, Andrews LS, Banton MI, Bevan C, Cushman JR (2002). Styrene respiratory tract toxicity and mouse lung tumors are mediated by CYP2F-generated metabolites. Regul Toxicol Pharmacol 35(3):308-19.

Cruzan G, Carlson GP, Turner M, Mellert W (2005c). Ring-oxidized metabolites of styrene contribute to styrene-induced Clara-cell toxicity in mice. J Toxicol Environ Health (A) 68:229-37.

Cruzan G, Cushman JR, Andrews LS, Granville GC, Miller RR, Hardy CJ *et al.* (1997). Subchronic inhalation studies of styrene in CD rats and CD-1 mice. Fundam Appl Toxicol 35(2):152-65.

Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Hardy CJ, Coombs DW, Mullins PA, Brown WR (1998). Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks. Toxicol Sci 46(2):266-81.

Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA *et al*. (2001). Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. J Appl Toxicol 21(3):185-98.

Cruzan G, Faber WD, Johnson KA, Roberts LS, Hellwig J, Carney E, Yarrington JT, Stump DG (2005a) Two generation reproduction study of styrene by inhalation in Crl-CD rats. Birth Defects Res B Dev Reprod Toxicol 74(3):211-20.

Cruzan G, Faber WD, Johnson KA, Roberts LS, Hellwig J, Maurissen J, Beck MJ, Radovsky A, Stump DG (2005b). Developmental neurotoxicity study of styrene by inhalation in Crl-CD rats. Birth Defects Res B Dev Reprod Toxicol 74(3):221-32.

Csanády GA, Filser JG (1994). Improvement of inhalation toxicokinetics: linkage between a data based two compartment model and physiological models. Naunyn-Schmiedeberg's Arch Pharmacol 349(Suppl):R128.

Csanády GA, Mendrala AL, Nolan RJ, Filser JG (1994). A physiologic pharmacokinetic model for styrene and styrene-7,8-oxide in mouse, rat and man. Arch Toxicol 68:143-57.

Csanady GA, Kessler W, Hoffmann HD, Filser JG (2003). A toxicokinetic model for styrene and its metabolite styrene-7,8-oxide in mouse, rat and human with special emphasis on the lung. Toxicol Lett 138(1-2):75-102.

Daré E, Tofighi R, Vettori MV, Momoi T, Poli D, Saido TC, Mutti A, Ceccatelli S (2002). Styrene 7,8-oxide induces caspase activation and regular DNA fragmentation in neuronal cells. Brain Res 933(1):12-22.

Daston GP, Overmann GJ, Taubeneck MW, Lehman-McKeeman LD, Rogers JM, Keen CL (1991a). The role of metallothionein induction and altered zinc status in maternally mediated developmental toxicity: comparison of the effects of urethane and styrene in rats. Toxicol Appl Pharmacol 110(3):450-63.

Daston GP, Baines D, Yonker JE (1991b). Chick embryo neural retina cell culture as a

screen for developmental toxicity. Toxicol Appl Pharmacol 109(2):352-66.

Date K, Ohno K, Azuma Y, Hirano S, Kobayashi K *et al*. (2002). Endocrine-disrupting effects of styrene oligomers that migrated from polystyrene containers into food. Food Chem Toxicol 40(1):65-75.

De Flora S (1979). Metabolic activation and deactivation of mutagens and carcinogens. Ital J Biochem 28(2):81-103.

De Meester C, Poncelet F, Roberfroid M, Rondelet J, Mercier M (1977). Mutagenic activity of styrene and styrene oxide. A preliminary study [proceedings] Arch Int Physiol Biochim 85(2):398-9.

De Meester C, Duverger-van Bogaert M, Lambotte-Vandepaer M, Mercier M, Poncelet F (1981). Mutagenicity of styrene in the Salmonella typhimurium test system. Mutat Res 90(4):443-50.

De Palma G, Manini P, Mozzoni P, Andreoli E, Bergamaschi E, Cavazzini S *et al.* (2001). Polymorphism of xenobiotic-metabolizing enzymes and excretion of styrene-specific mercapturic acids. Chem Res Toxicol 14:1393-400.

De Raat WK (1978). Induction of sister chromatid exchanges by styrene and its presumed metabolite styrene oxide in the presence of rat liver homogenate. Chem Biol Interact 20(2):163-70.

De Rosa E, Cellini M, Sessa G, Scapellato ML, Marcuzzo G, Bartolucci GB (1996). The importance of sampling time and coexposure to acetone in the biological monitoring of styrene-exposed workers. Appl Occup Environ Hyg 11(5):471-5.

Del Carratore R, Bronzetti G, Bauer C, Corsi C, Nieri R, Paolini M, Giagoni P (1983). Cytochrome P-450 factors determining synthesis in strain D7 Saccharomyces cerevisiae. An alternative system to microsomal assay. Mutat Res 121(2):117-23.

Delzell E, Macaluso M, Sathiakumar N, Matthews R (2001). Leukemia and exposure to 1,3-butadiene, styrene and dimethyldithiocarbamate among workers in the synthetic rubber industry. Chem Biol Interact 135-136:515-34.

Delzell E, Sathiakumar N, Graff J, Macaluso M, Maldonado G, Matthews R (2006). An updated study of mortality among North American synthetic rubber industry workers. Research Report 132, Health Effects Institute, Boston MA. Available at: http://pubs.healtheffects.org/types.php?type=1; posted final December 2006.

Delzell E, Sathiakumar N, Hovinga M, Macaluso M, Julian J, Larson R, Cole P, Muir DC (1996). A follow-up study of synthetic rubber workers. Toxicology 113(1-3):182-9.

Devereux TR, Hook GER, Fouts JR (1979). Foreign compound metabolism by isolated cells from rabbit lung. Drug Metab Dispos 7:70-5.

Diez U, Rehwagen M, Rolle-Kampczyk U, Wetzig H, Schulz R, Richter M, Lehmann I, Borte M, Herbarth O (2003). Redecoration of apartments promotes obstructive bronchitis in atopy risk infants--results of the LARS Study. Int J Hyg Environ Health 206(3):173-9.

Divine BJ (1990). An update on mortality among workers at a 1,3-butadiene facility - preliminary results. Environ Health Perspect 86:119-28.

Divine BJ, Hartman CM (1996). Mortality update of butadiene production workers. Toxicology 113:169-81.

Divine BJ, Wendt JK, Hartman CM (1993). Cancer mortality among workers at a butadiene production facility. In: Butadiene and Styrene: Assessment of Health Hazards. M Sorsa, K Peltonen, H Vainio, K Hemminki, eds. IARC Scientific Publ No. 127. Lyon, France, pp. 345-62.

Dogra RK, Khanna S, Srivastava SN, Shukla LJ, Shanker R (1989). Styrene-induced immunomodulation in mice. Int J Immunopharmacol 11(5):577-86.

Donner M, Sorsa M, Vainio H (1979). Recessive lethals induced by styrene and styrene oxide in Drosophila melanogaster. Mutat Res 67(4):373-6.

Dorne JL, Walton K, Renwick AG (2005). Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. Food Chem Toxicol 43(2):203-16.

Downs TD, Crane MM, Kim KW (1987). Mortality among workers at a butadiene facility. Am J Ind Med 12:311-29.

Dowty BJ, Laseter JL, Storer J (1976). The transplacental migration and accumulation in blood of volatile organic constituents. Pediatr Res 10:696-701.

Drummond L, Caldwell J, Wilson HK (1989). The metabolism of ethylbenzene and styrene to mandelic acid: stereochemical considerations. Xenobiotica 19(2):199-208.

DTSC (1994). CalTOX™, A Multimedia Total Exposure Model for Hazardous Waste Sites, Ver 1.5. Prepared by Univ. of California, Davis, in cooperation with Lawrence Livermore National Laboratory for Dept. of Toxic Substances Control, California Environmental Protection Agency, Sacramento, California. Accessed at: http://www.dtsc.ca.gov/AssessingRisk/ctox_dwn.cfm.

Dumas S, Parent M-E, Siemiatycki J, Brisson J (2000). Rectal cancer and occupational risk factors: a hypothesis-generating, exposure-based case-control study. Int J Cancer 87:874-9.

Edling C, Ekberg K (1985). No acute behavioral effects of exposure to styrene: a safe level of exposure? Br J Ind Med 42:301-4.

Elovaara E, Engstrom K, Nakajima T, Park SS, Gelboin HV, Vainio H (1991). Metabolism of inhaled styrene in acetone-, phenobarbital- and 3-methylcholanthrene-pretreated rats: stimulation and stereochemical effects by induction of cytochromes P450IIE1, P450IIB and P450IA. Xenobiotica 21(5):651-61.

Engelhardt G, Gamer A, Vodicka P, Barta I, Hoffmann HD, Veenstra G (2003). A re-assessment of styrene-induced clastogenicity in mice in a subacute inhalation study. Arch Toxicol 77(1):56-61.

Engstrom J, Bjurstrom R, Astrand I, Ovrum P (1978). Uptake, distribution and elimination of styrene in man. Scand J Work Environ Health 4:315-23.

Engstrom K, Harkonen H, Kalliokoski P, Rantanen J (1976). Urinary mandelic acid concentration after occupational exposure to styrene and its use as a biological exposure

test. Scand J Work Environ Health 2(1):21-6.

Erixon K, Ahnström G (1979). Single-strand breaks in DNA during repair of UV-induced damage in normal human and xeroderma pigmentosum cells as determined by alkaline DNA unwinding and hydroxylapatite chromatography: effects of hydroxyurea, 5-fluorodeoxyuridine and 1-beta-D-arabinofuranosylcytosine on the kinetics of repair. Mutat Res 59(2):257-71.

Evelo CT, Oostendorp JG, ten Berge WF, Borm PJ (1993). Physiologically based toxicokinetic modeling of 1,3-butadiene lung metabolism in mice becomes more important at low doses. Environ Health Perspect 101(6):496-502.

Fallas C, Fallas J, Maslard P, Dally S (1992). Subclinical impairment of colour vision among workers exposed to styrene. Br J Ind Med 49:679-82.

Filser JG, Johanson G, Kessler W, Kreuzer PE, Stei P *et al*. (1993). A pharmacokinetic model to describe toxicokinetic interactions between 1,3-butadiene and styrene in rats: predictions for human exposure. In: Butadiene and Styrene: Assessment of Health Hazards. M Sorsa, K Peltonen, H Vainio, K Hemminki, eds. IARC Scientific Publ No. 127. Lyon, France, pp. 65-78.

Fishbein L (1992). Exposure from occupational versus other sources. Scand J Work Environ Health 18 (Suppl 1):5-16.

Fleig I, Thiess AM (1978). Mutagenicity study of workers employed in the styrene and polystyrene processing and manufacturing industry. Scand J Work Environ Health 4 Suppl 2:254-8.

Flodin U, Ekberg K, Andersson L (1989). Neuropsychiatric effects of low exposure to styrene. Br J Ind Med 46:805-8.

Flodin U, Fredriksson M, Axelson O, Persson B, Hardell L (1986). Background radiation, electrical work, and some other exposures associated with acute myeloid leukemia in a case-referent study. Arch Environ Health 41 (2):77-84.

Florin I, Rutberg L, Curvall M, Enzell CR (1980). Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology 15(3):219-32.

Forni A, Goggi E, Ortisi E, Cechetti R, Cortona G, Sesana G, Alessio L (1988). Cytogenetic findings in styrene workers in relation to exposure. In: Environmental hygiene. Seemayer NH, Hadnagy W, eds. Berlin, Springer, pp. 159-62.

Foster JR (1999). The role of cytochrome P450 in styrene-induced nasal lesions in the mouse. Rep No. CTL/R/1418. Styrene Information and Research Center, Arlington, VA.

Foureman GL, Harris C, Guengerich FP, Bend JR (1989). Stereoselectivity of styrene oxidation in microsomes and in purified cytochrome P-450 enzymes from rat liver. J Pharmacol Exp Ther 248(2):492-7.

Fowles JR, Alexeeff GV, Dodge D (1999). The use of benchmark dose methodology with acute inhalation lethality data. Regul Toxicol Pharmacol 29(3):262-78.

Franchini I, Cavatora A, Falzoi M, Lucertini S, Mutti A (1983). Early indicators of renal damage in workers exposed to organic solvents. Int Arch Occup Environ Health 52:1-9.

Frentzel-Beyme R, Thiess AM, Wieland R (1978). Survey of mortality among employees engaged in the manufacture of styrene and polystyrene at the BASF Ludwigshafen works. Scand J Work Environ Health 4(Suppl. 2):231-9.

Gagnaire F, Chalansonnet M, Carabin N, Micillino JC (2006). Effects of subchronic exposure to styrene on the extracellular and tissue levels of dopamine, serotonin and their metabolites in rat brain. Arch Toxicol 80(10):703-12.

Gamberale F, Hultengren M (1974). Exposure to styrene. II. Psychological functions. Work Environ Health 11(2):86-93.

Gamberale F, Lisper HO, Anshelm-Olson B (1975). Effect of styrene gases on reaction time among workers in plastic boat industry. Arbete och Halsa 8:23 (in Swedish; cited in World Health Organization, 1983).

Gamer AO, Leibold E, Deckardt K, Kittel B, Kaufmann W, Tennekes HA, van Ravenzwaay B (2004). The effects of styrene on lung cells in female mice and rats. Food Chem Toxicol 42(10):1655-67.

Gargas ML, Burgess RJ, Voisard DE, Cason GH, Andersen ME (1989). Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol Appl Pharmacol 98:87-99.

Gaylor DW, Kodell RL, Chen JJ, Springer JA, Lorentzen RJ, Scheuplein RJ (1994). Point estimates of cancer risk at low doses. Risk Anal 14(5):843-50.

Geigert J, Lee TD, Dalietos DJ, Hirano DS, Neidleman SL (1986). Epoxidation of alkenes by chloroperoxidase catalysis. Biochem Biophys Res Commun 136(2):778-82.

Gerin M, Siemiatycki J, Desy M, Krewski D (1998). Associations between several sites of cancer and occupational exposure to benzene, toluene, xylene, and styrene: results of a case-control study in Montreal. Am J Ind Med 34:144-56.

Ghantous H, Dencker L, Gabrielsson J, Danielsson BRG, Bergmann K (1990). Accumulation and turnover of metabolites of toluene and xylene in nasal mucosa and olfactory bulb in the mouse. Pharmacol Toxicol 66(2):87-92.

Ghittori S, Maestri L, Imbriani M, Capodaglio E, Cavalleri A (1997). Urinary excretion of specific mercapturic acids in workers exposed to styrene. Am J Ind Med 31(5):636-44.

Girre C, Lucas D, Hispard E, Menez C, Dally S, Menez J-F (1994). Assessment of cytochrome P4502E1 induction in alcoholic patients by chlorzoxazone pharmacokinetics. Biochem Pharmacol 47(9):1503-8.

Glosnicka R, Dziadziuszko H (1986). Mutagenic action of styrene and its metabolites. II. Genotoxic activity of styrene, styrene oxide, styrene glycol and benzoic acid tested with the SOS chromotest. Bull Inst Marit Trop Med Gdynia 37(3-4):295-302.

Gobba F, Cavelleri A (1993). Kinetics of urinary excretion and effects on colour vision after exposure to styrene. IARC Scientific Publ 127:79-88.

Godderis L, De Boeck M, Haufroid V, Emmery M, Mateuca R, Gardinal S, Kirsch-Volders M, Veulemans H, Lison D (2004). Influence of genetic polymorphisms on

biomarkers of exposure and genotoxic effects in styrene-exposed workers. Environ Mol Mutagen 44(4):293-303.

Gotell P, Axelson O, Lindelof B (1972). Field studies on human styrene exposure. Work Environ Health 9(2):76-83 (in Swedish; cited in World Health Organization, 1983).

Governa M, Valentino M, Visona I (1994). Chemotactic activity of human polymorphonuclear leukocytes and industrial xenobiotics: a brief review. Toxicology 91:165-77.

Graff JJ, Sathiakumar N, Macaluso M, Maldonado G, Matthews R, Delzell E (2005). Chemical exposures in the synthetic rubber industry and lymphohematopoietic cancer mortality. J Occup Environ Med 47(9):916-32.

Grant SG, Bigbee WL (1993). In vivo somatic mutation and segregation at the human glycophorin A (GPA) locus: phenotypic variation encompassing both gene-specific and chromosomal mechanisms. Mutat Res 288(1):163-72.

Green T, Lee R, Toghill A, Meadowcroft S, Lund V, Foster J (2001). The toxicity of styrene to the nasal epithelium of mice and rats: studies on the mode of action and relevance to humans. Chem-Biol Interact 137(2):185-202.

Guengerich FP (1990). Enzymatic oxidation of xenobiotic chemicals. Fasman GD, ed. Crit Rev Biochem Molec Biol CRC Press, Boca Raton, FL, pp. 97-153.

Guengerich FP, Wang P, Mason PS, Mitchell MB (1979a). Rat and human microsomal epoxide hydratase. Immunological characterization of various forms of the enzyme. J Biol Chem 254(23):12255-9.

Guengerich FP, Wang P, Mitchell MB, Mason PS (1979b). Rat and human liver microsomal epoxide hydratase. Purification and evidence for the existence of multiple forms. J Biol Chem 254(23):12248-4.

Guillemin M, Bauer D, Martin B, Marazzi A (1982). Human exposure to styrene. IV. Industrial hygiene investigations and biological monitoring in the polyester industry. Int Arch Occup Environ Health 51:139-50.

Guillemin MP, Bauer D (1979). Human exposure to styrene. III. Elimination kinetics of urinary mandelic and phenylglyoxylic acids after single experimental exposure. Int Arch Occup Environ Health 44:249-63.

Gunning WT, Castonguay A, Goldblatt PJ, Stoner GD (1991). Strain A/J mouse lung adenoma growth patterns vary when induced by different carcinogens. Toxicol Pathol 19:168-75.

Hagmar L, Hogstedt B, Welinder H, Karlsson A, Rassner F (1989). Cytogenetic and hematological effects in plastics workers exposed to styrene. Scand J Work Environ Health 15(2):136-41.

Hallier E, Goergens HW, Hallier K, Bolt HM (1994). Intervention study on the influence of reduction of occupational exposure to styrene on sister chromatid exchanges in lymphocytes. Int Arch Occup Environ Health 66(3):167-72.

Hallier E, Goergens HW, Karels H, Golka K (1995). A note on individual differences

inthe urinary excretion of optical enantiomers of styrene metabolites and of styrene-derived mercapturic acids in humans. Arch Toxicol 69(5):300-5.

Hansteen IL, Jelmert O, Torgrimsen T, Forsund B (1984). Low human exposure to styrene in relation to chromosome breaks, gaps and sister chromatid exchanges. Hereditas 100(1):87-91.

Harkonen H, Lindstrom K, Seppalainen AM, Sisko A Hernberg S (1978). Exposure-response relationship between styrene exposure and central nervous functions. Scand J Work Environ Health 4:53-9.

Harkonen H, Holmberg P (1982). Obstetric histories of women occupationally exposed to styrene. Scand J Work Environ Health 8:74-7.

Harkonen H, Tola S, Korkala M, Hernberg S (1984a). Congenital malformations, mortality and styrene exposure. Ann Acad Med (Singapore) 13:404-7.

Harkonen H, Lehtniemi A, Aitio A (1984b). Styrene exposure and the liver. Scand J Work Environ Health 10(1):59-61.

Harvilchuck JA, Carlson GP (2009). Effect of multiple doses of styrene and R-styrene oxide on CC10, bax, and bcl-2 expression in isolated Clara cells of CD-1 mice. Toxicology 259(3):149-52.

Harvilchuck JA, Pu X, Klaunig JE, Carlson GP (2009). Indicators of oxidative stress and apoptosis in mouse whole lung and Clara cells following exposure to styrene and its metabolites. Toxicology 264(3):171-8.

Harvilchuck JA, Zurbrugg RJ, Carlson GP (2008). CC10 mRNA and protein expression in Clara cells of CD-1 mice following exposure to styrene or its metabolites styrene oxide or 4-vinylphenol. Toxicol Lett 183(1-3):28-35.

Haseman JK, Johnson FM (1996). Analysis of National Toxicology Program rodent bioassay data for anticarcinogenic effects. Mutat Res 350(1):131-41.

Haseman JK, Rao GN (1992). Effects of corn oil, time-related changes, and inter-laboratory variability on tumor occurrence in control Fischer 344 (F344/N) rats. Toxicol Pathol 20(1):52-60.

Hassett C, Aicher L, Sidhu JS, Omiecinski CJ (1994). Human microsomal epoxide hydrolase: genetic polymorphism and functional expression *in vitro* of amino acid variants. Hum Molec Genetics 3(3):421-8.

Hassett C, Lin J, Carty CL, Laurenzana EM, Omiencinski CJ (1997). Human hepatic microsomal epoxide hydrolase comparative analysis of polymorphic expression. Arch Biochem Biophys 337(2):275-83.

Hayes JP, Lambourn L, Hopkirk JA, Durham SR, Taylor AJ (1991). Occupational asthma due to styrene. Thorax 46(5):396-7.

Heikes DL, Jensen SR, Fleming-Jones ME (1995). Purge and trap extraction with GC-MS determination of volatile organic compounds in table-ready foods. J Agric Food Chem 43:2869-75.

Hellman B, Vaghef H, Boström B (1995). The concepts of tail moment and tail inertia in

the single cell gel electrophoresis assay. Mutat Res 336(2):123-31.

Hemminki K, Franssila E, Vainio H (1980). Spontaneous abortions among female chemical workers in Finland. Intl Arch Occup Environ Health 45:123-6.

Hemminki K, Lindbohm ML, Hemminki T, Vainio H (1984). Reproductive hazards and the plastics industry. In: Industrial Hazards of Plastics and Synthetic Elastomers. J Jarvisalo, P Pfaffli, H Vainio, eds. Alan R. Liss, Inc., New York, NY, pp. 79-89.

Henderson LM, Speit G (2005). Review of the genotoxicity of styrene in humans. Mutat Res 589(3):158-91.

Hertz-Picciotto I, Arrighi HM, Hu S-W (2000). Does arsenic exposure increase the risk for circulatory disease? Am J Epidemiol 151:174-81.

Hicks SM, Vassallo JD, Dieter MZ, Lewis CL, Whiteley LO, Fix AS, Lehman-McKeeman LD (2003). Immunohistochemical analysis of Clara cell secretory protein expression in a transgenic model of mouse lung carcinogenesis. Toxicology 187(2-3):217-28.

Hodges VM, Molloy GY, Wickramasinghe SN (2000). Demonstration of mRNA for five species of cytochrome P450 in human bone marrow, bone marrow-derived macrophages and human haemopoietic cell lines. Br J Haemotol 108:151-6.

Hodgson AT, Daisey JM, Mahanama KRR, Ten Brinke J, Alevantis LE (1996). Use of volative tracers to determine the contribution of environmental tobacco smoke to concentrations of volatile organic compounds in smoking environments. Environ Internat 22(3):295-307.

Hodgson JT, Jones RD (1985). Mortality of styrene production, polymerization and processing workers at a site in northwest England. Scand J Work Environ Health 11:347-52.

Hoffmann C, Putz C, Semder B, Faller TH, Csanady GA, Filser JG (2006). Styrene-7,8-oxide burden in ventilated, perfused lungs of mice and rats exposed to vaporous styrene. Toxicol Sci 90(1):39-48.

Hogstedt B, Hedner K, Mark-Vendel E, Mitelman F, Schutz A, Skerfving S (1979). Increased frequency of chromosome aberrations in workers exposed to styrene. Scand J Work Environ Health 5(4):333-5.

Hogstedt B, Akesson B, Axell K, Gullberg B, Mitelman F *et al*. (1983). Increased frequency of lymphocyte micronuclei in workers producing reinforced polyester resin with low exposure to styrene. Scand J Work Environ Health 9(3):241-6.

Holmberg P (1979). Central nervous system defects in children born to mothers exposed to organic solvents during pregnancy. Lancet 177-9.

Holmberg P, Hernberg S, Kurppa K, Rantala K (1982). Oral clefts and organic solvent exposure during pregnancy. Int Arch Occup Environ Health 50:371-6.

Holmberg P, Kurppa K, Riala R, Rantala K, Kuosma E (1986). Solvent exposure and birth defects: an epidemiologic survey. In: Safety and Health Aspects of Organic Solvents: Proc Internatl Course on Safety and Health Aspects of Organic Solvents,

Espoo, Finland, April 22-26, 1985. V Riihimaki, U Ulfvarson, eds. Alan R. Liss, New York, pp. 179-85.

Holmberg PC, Nurminen M (1980). Congenital defects to the central nervous system and occupational factors during pregnancy; a case-referent study. Am J Ind Med 1:167-76.

Holz O, Scherer G, Brodtmeier S, Koops F, Warncke K, *et al*. (1995). Determination of low level exposure to volatile aromatic hydrocarbons and genotoxic effects in workers at a styrene plant. Occup Environ Med 52(6):420-8.

Hook GER, Gilmore LB, Gupta RP, Patton SE, Jetten AM, Nettesheim P (1990). The functions of pulmonary Clara Cells. In: Biology, Toxicology, and Carcinogenesis of Respiratory Epithelium. Proc. of a meeting hosted by the Lovelace Inhalation Toxicology Research Institute, Nov 14-16, 1988, Thomassen DG, Netteshem P, eds. Hemisphere Publishing Corp., Albuquerque, NM.

Horvath E, Pongracz K, Rappaport S, Bodell WJ (1994). 32P-post-labeling detection of DNA adducts in mononuclear cells of workers occupationally exposed to styrene. Carcinogenesis 15(7):1309-15.

Hotz P, Guillemin MP, Lob M (1980). Study of some hepatic effects (induction and toxicity) caused by occupational exposure to styrene in the polyester industry. Scand J Work Environ Health 6(3):206-15.

Hruban Z (1976). Pulmonary changes induced by amphophilic drugs. Environ Health Perspect 16:111-8.

HSDB (2007). Styrene. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD. Accessed at http://toxnet.nlm.nih.gov/.

Huff J, Cirvello J, Haseman J, Bucher J (1991). Chemicals associated with site-specific neoplasia in 1394 long-term carcinogenesis experiments in laboratory rodents. Environ Health Perspect 93:247-70.

Hukkanen J, Hakkola J, Antilla S, Piipare R, Karjalainen A *et al.* (1997). Detection of mRNA encoding xenobiotic-metabolizing cytochrome P450s in human bronchoalveolar macrophages and peripheral blood lymphocytes. Mol Carcinog 20:224-30.

Hynes DE, DeNicola DB, Carlson GP (1999). Metabolism of styrene by mouse and rat isolated lung cells. Toxicol Sci 51:195-201.

IARC (1979). Styrene, polystyrene and styrene-butadiene copolymers. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some monomers, plastics and synthetic elastomers, and acrolein. 19:231-74. International Agency for Research on Cancer, Lyon, France.

IARC (1982a). IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. The Rubber Industry. 28:1-454. IARC, Lyon, France.

IARC (1982b). Benzene. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some industrial chemicals and dyestuffs. 29:93-148. IARC, Lyon, France.

IARC (1994a). Styrene. IARC Monographs on the Evaluation of Carcinogenic Risks to

Humans. Some Industrial Chemicals. 60:233-320. IARC, Lyon, France.

IARC (1994b). Styrene-7,8-oxide. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Industrial Chemicals. 60:321-46. IARC, Lyon, France.

IARC (1999). Short-term tests for defining mutagenic carcinogens. Waters MD, Stack HF, Jackson MA. 146:499-536. IARC Scientific Publications, Lyon, France.

IARC (2002). Styrene. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. 82:437-550. IARC, Lyon, France.

IARC (2006). IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. List of all agents evaluated to date (by group). Accessed Nov. 21, 2006 at: http://monographs.iarc.fr/ENG/Classification/crthall.php.

Ikeda M, Imamura T, Hayaskh M, Tabuchi T, Hara I (1974). Evaluation of hippuric, phenylglyoxylic and mandelic acids in urine as indices of styrene exposure. Int Arch Arbeitsmed 32(1):93-101.

Irons RD, Pyatt DW (1998). Commentary: Dithiocarbamates as potential confounders in butadiene carcinogenesis. Carcinogenesis 19(4):539-42.

Ishidate M Jr, Yoshikawa K (1980). Chromosome aberration tests with Chinese hamster cells in vitro with metabolic activation – a comparative study on mutagens and carcinogens. Arch Toxicol Suppl 4:41-44.

Jablonicka A, Karelova J, Polakova H, Vargova M (1988). Analysis of chromosomes in peripheral blood lymphocytes of styrene-exposed workers. Mutat Res 206(2):167-9.

Jang JY, Droz PO, Berode M (1997). Ethnic differences in biological monitoring of several organic solvents. I. Human exposure experiment. Int Arch Occup Environ Health 69(5):343-9.

Jantunen K, Maki-Paakkanen J, Norppa H (1986). Induction of chromosome aberrations by styrene and vinylacetate in cultured human lymphocytes: dependence on erythrocytes. Mutat Res 159(1-2):109-16.

Jelnes JE (1988). Semen quality in workers producing reinforced plastics. Reprod Toxicol 2:209-12.

Jersey GC, Balmer MF, Quast JF, Park CN, Schuetz SJ, Beyer JE *et al.* (1978). Two-year chronic inhalation toxicity and carcinogenicity study on monomeric styrene in rats. Final Report. Toxicology Research Laboratory. Health and Environmental Research. Dow Chemical U.S.A. Midland, MI. MCA No. STY 1.1-TOX-INH (2 YR).

Johanson G, Ernstgard L, Gullstrand E, Lof A, Osterman-Golkar S, Williams CC *et al.* (2000). Styrene oxide in blood, hemoglobin adducts, and urinary metabolites in human volunteers exposed to $^{13}C_8$-styrene vapors. Toxicol Appl Pharmacol 168:36-49.

Johanson G, Filser JG (1993). A physiologically based pharmacokinetic model for butadiene and its metabolite butadiene monoxide in rat and mouse and its significance for risk extrapolation. Arch Toxicol 67(3):151-63.

Kankaanpää JT, Elovaara E, Hemminki K, Vainio H (1980). The effect of maternally inhaled styrene on embryonal and foetal development in mice and Chinese hamsters. Acta Pharmacol Toxicol (Copenh) 47(2):127-9.

Kapitulnik J, Levin W, Morecki R, Dansette PM, Jerina DM, Conney AH (1977). Hydration of arene and alkene oxides by epoxide hydrase in human liver microsomes. Clin Pharmacol Ther 21(2):158-65.

Karakaya AE, Karahalil B, Yilmazer M, Aygun N, Sardas S, Burgaz S (1997). Evaluation of genotoxic potential of styrene in furniture workers using unsaturated polyester resins. Mutat Res 392(3):261-8.

Katakura Y, Kishi R, Ikeda T, Miyake H (1999). Effects of prenatal exposure to styrene on neurochemical levels in rat brain. Toxicol Lett 105:239-49.

Katakura Y, Kishi R, Ikeda T, Miyake H (2001). Effects of prenatal styrene exposure on postnatal development and brain serotonin and catecholamine levels in rats. Environ Res 85(1):41-7.

Kato S, Bowman ED, Harrington AM, Blomeke B, Shields PG (1995). Human lung carcinogen-DNA adduct levels mediated by genetic polymorphisms in vivo. J Natl Cancer Inst 87(12):902-7.

Kauffman SL (1981). Histogenesis of the papillary Clara cell adenoma. Am J Pathol 103:174-80.

Kaufmann W, Mellert W, van Ravenzwaay B, Landsiedel R, Poole A (2005). Effects of styrene and its metabolites on different lung compartments of the mouse - cell proliferation and histomorphology. Regul Toxicol Pharmacol 42(1):24-36.

Keenan KP, Ballam GC, Soper KA, Laroque P, Coleman JB, Dixit R (1999). Diet, caloric restriction, and the rodent bioassay. Toxicol Sci 52(Suppl 1):24-34.

Keenan KP, Soper KA, Smith PF, Ballam GC, Clark RL (1995). Diet, overfeeding, and moderate dietary restriction in control Sprague-Dawley rats: I. Effects on spontaneous neoplasms. Toxicol Pathol 23(3):269-86.

Kelsey KT, Smith TJ, Hammond SK, Letz R, Little JB (1990). Sister-chromatid exchanges in lymphocytes from styrene-exposed boat builders. Mutat Res 241(2):215-21.

Khanna VK, Husain R, Hanig JP, Seth PK (1991). Increased neurobehavioral toxicity of styrene in protein-malnourished rats. Neurotoxicol Teratol 13(2):153-9.

Khanna VK, Husain R, Seth PK (1994). Effect of protein malnutrition on the neurobehavioral toxicity of styrene in young rats. J Appl Toxicol 14:351-6.

Kim H, Wang RS, Elovaara E, Raunio H, Pelkonen O, Aoyama T *et al.* (1997). Cytochrome P450 isozymes responsible for the metabolism of toluene and styrene in human liver microsomes. Xenobiotica 27(7):657-65.

Kim RB, Yamazake H, Chiba K, O'Shea D, Imiura M, Guengerich FP *et al.* (1996). In vivo and in vitro characterization of CYP2E1 activity in Japanese and Caucasians. J Pharmacol Exp Ther 279(1):4-11.

Kishi R, Chen BQ, Katadura Y, Ikeda T, Miyake H (1995). Effect of prenatal exposure to styrene on the neurobehavioral development, activity, motor coordination, and learning behavior of rats. Neurotoxicol Teratol 17:121-30.

Kishi R, Katakura Y, Okui T, Ogawa H, Ikeda T, Miyake H (1989). Placental transfer and tissue distribution of 14C-styrene: an autoradiographic study in mice. Br J Ind Med 46(6):376-83.

Kishi R, Katakura Y, Ikeda T, Chen BQ, Miyake H (1992). Neurochemical effects in rats following gestational exposure to styrene. Toxicol Lett 63(2):141-6.

Kligerman AD, Allen JW, Bryant MF, Campbell JA, Collins BW *et al*. (1992). Cytogenetic studies of mice exposed to styrene by inhalation. Mutat Res 280(1):35-43.

Kligerman AD, Allen JW, Erexson GL, Morgan DL (1993). Cytogenetic studies of rodents exposed to styrene by inhalation. IARC Sci Publ (127):217-24.

Kogevinas M, Boffetta P (1991). Correspondence on: A cohort mortality study and a case-control study of workers potentially exposed to styrene in the reinforced plastics and composites industry. Brit J Ind Med 48:575.

Kogevinas M, Ferro G, Andersen A, Bellander T, Biocca M *et al.* (1994). Cancer mortality in a historical cohort study of workers exposed to styrene. Scand J Work Environ Health 20(4):251-61.

Kolstad HA, Juel K, Olsen J, Lynge E (1995). Exposure to styrene and chronic health effects: mortality and incidence of solid cancers in the Danish reinforced plastics industry. Occup Environ Med 52:320-7.

Kolstad HA, Lynge E, Olsen J, Breum N (1994). Incidence of lymphohematopoietic malignancies among styrene-exposed workers of the reinforced plastics industry. Scand J Work Environ Health 20:272-8.

Kolstad HA, Olsen J (1999). Why do short-term workers have high mortality? Am J Epid 149(4):347-52.

Kolstad HA, Pedersen B, Olsen J, Lynge E, Jensen G, Lisse I *et al.* (1996). Clonal chromosome aberrations in myeloid leukemia after styrene exposure. Scand J Work Environ Health 22(1):58-61.

Kolstad HA, Bisanti L, Roeleveld N, Baldi R, Bonde JP, Joffe M (2000). Time to pregnancy among male workers of the reinforced plastics industry in Denmark, Italy and The Netherlands. ASCLEPIOS. Scand J Work Environ Health 26:353-8.

Kolstad HA, Bisanti L, Roeleveld N, Bonde J, Joffe M (1999a). Time to pregnancy for men occupationally exposed to styrene in several European reinforced plastics companies. ASCLEPIOS. Scand J Work Environ Health 25(Suppl 1):66-9; discussion 76-8.

Kolstad HA, Bonde JP, Spano M, Giwercman A, Zschiesche W *et al*. (1999b). Change in semen quality and sperm chromatin structure following occupational styrene exposure. ASCLEPIOS. Int Arch Occup Environ Health 72:135-41.

Korn M, Gfroerer W, Filser JG, Kessler W (1994). Styrene-7,8-oxide in blood of workers

exposed to styrene. Arch Toxicol 68(8):524-7.

Korn M, Wodarz R, Schoknecht W, Weichardt H, Bayer E (1984). Styrene metabolism in man: gas chromatographic separation of mandelic acid enantiomers in the urine of exposed persons. Arch Toxicol 55:59-63.

Koskinen M, Plna K (2000). Specific DNA adducts induced by some mono-substituted epoxides in vitro and in vivo. Chem-Biol Interact 129:209-29.

Koskinen M, Vodicka P, Hemminki K (2001). Identification of 1-adenine DNA adducts in workers occupationally exposed to styrene. J Occup Environ Med 43:694-700.

Krovat BC, Tracy JH, Omiecinski CJ (2000). Fingerprinting of cytochrome P450 and microsomal epoxide hydrolase gene expression in human blood cells. Toxicol Sci 55:352-60.

Kuwada M, Kawashima R, Nakamura K, Kojima H, Hasumi H, Maki J *et al*. (2002). Neonatal exposure to endocrine disruptors suppresses juvenile testis weight and steroidogenesis but spermatogenesis is considerably restored during puberty. Biochem Biophys Res Commun 295:193-7.

Kwong SL, Allen M, Wright WE (2005). Cancer in California: 1988-2002. Sacramento, CA: California Department of Health Services, Cancer Surveillance Section, December 2005.

Ladefoged O, Lam HR, Ostergaard G, Hansen EV, Hass U, Lund SP *et al.* (1998). Toxicity of the styrene metabolite, phenylglyoxylic acid, in rats after three months' oral dosing. Neurotoxicology 19(4-5):721-37.

Laffon B, Pasaro E, Mendez J (2001). Genotoxic effects of styrene-7,8-oxide in human white blood cells: comet assay in relation to the induction of sister-chromatid exchanges and micronuclei. Mutat Res 491:163-72.

Laffon B, Pasaro E, Mendez J (2002a). DNA damage and repair in human leukocytes exposed to styrene-7,8-oxide measured by the comet assay. Toxicol Lett 126:61-8.

Laffon B, Pasaro E, Mendez J (2002b). Evaluation of genotoxic effects in a group of workers exposed to low levels of styrene. Toxicology 171:175-86.

Laib RJ, Tucholski M, Filser JG, Csanady GA (1992). Pharmacokinetic interaction between 1,3-butadiene and styrene in Sprague-Dawley rats. Arch Toxicol 66(5):310-4.

Landrigan PJ (1993). Critical assessment of epidemiological studies on the carcinogenicity of 1,3-butadiene and styrene. In: Butadiene and Styrene: Assessment of Health Hazards. M Sorsa, K Peltonen, H Vainio, K Hemminki, eds. IARC Scientific Publ No. 127. Lyon, France, pp. 375-88.

Lang P (1995). Spontaneous Neoplastic Lesions in the Crl:CD-1 ®BR Mouse. Report prepared for Charles River Laboratories. March 1995. Accessed 08/13/01 at http://www.criver.com/techdocs/95mar_sn/index.html.

Lang PL, White WJ (1996). Growth, development, life span, and select lesion incidence in the aging CD-1® mouse. In: Pathobiology of the Aging Mouse. Mohr U, Dungworth DL, Capen CC, Carlton WW, Sundberg JP, Ward JM, eds. International Life Sciences

Institute, Washington, D.C, pp. 27-44.

Laws GM, Skopek TR, Reddy MV, Storer RD, Glaab WE (2001). Detection of DNA adducts using a quantitative long PCR technique and the fluorogenic 5' nuclease assay (TaqMan®). Mutat Res 484:3-18.

Lazutka JR, Lekevicius R, Dedonyte V, Maciuleviciute-Gervers L *et al*. (1999). Chromosomal aberrations and sister-chromatid exchanges in Lithuanian populations: effects of occupational and environmental exposures. Mutat Res 445:225-39.

Leavens TL, Moss OR, Turner MJ, Janszen DB, Bond JA (1996). Metabolic interactions of 1,3-butadiene and styrene in male B6C3F1 mice. Toxicol Appl Pharmacol 141:628-36.

Lee SH, Norppa H (1995). Effects of indomethacin and arachidonic acid on sister chromatid exchange induction by styrene and styrene-7,8-oxide. Mutat Res 348:175-81.

Leibman KC (1975). Metabolism and toxicity of styrene. Environ Health Perspect 11:115-9.

Lemasters GK, Carson A, Samuels SJ (1985a). Occupational styrene exposure for twelve product categories in the reinforced-plastics industry. Am Ind Hyg Assoc J 46:434-41.

Lemasters GK, Hagen A, Samuels SJ (1985b). Reproductive outcomes in women exposed to solvents in 36 reinforced plastics companies. I. Menstrual dysfunction. J Occup Med 27:490-4.

Lemasters GK, Samuels SJ, Morrison JA, Brooks SM (1989). Reproductive outcomes of pregnant workers employed at 36 reinforced plastics companies. II. Lowered birth weight. J Occup Med 31:115-20.

Lewis R (1999). Overview of the rubber industry and tire manufacturing. Occupational Medicine: State of the Art Reviews 14 (4):707-18. October-December.

Limasset JC, Simon P, Poirot P, Subra I, Grzebyk M (1999). Estimation of thepercutaneous absorption of styrene in an industrial situation. Int Arch Occup Environ Health 72:46-51.

Lindbohm ML, Hemminki K, Bonhomme MG, Anttila A *et al*. (1991). Effects of paternal occupational exposure on spontaneous abortions. Am J Publ Health 81:1029-33.

Lindbohm ML, Taskinen H, Sallmen M, Hemminki K (1990). Spontaneous abortions among women exposed to organic solvents. Am J Ind Med 17:449-63.

Lindstrom K, Harkonen H, Hernberg S (1976). Disturbances in psychological functions of workers occupationally exposed to styrene. Scand J Work Environ Health 3:129-39.

Linhart I, Gut I, Smejkal J, Novak J (2000). Biotransformation of styrene in mice. Stereochemical aspects. Chem Res Toxicol 13:36-43.

Linhart I, Smejkal J, Mladkova I (1998). Stereochemical aspects of styrene biotransformation. Toxicol Lett 94(2):127-35.

Linnainmaa K, Meretoja T, Sorsa M, Vainio H (1978a). Cytogenetic effects of styrene and styrene oxide. Mutat Res 58(2-3):277-86

Linnainmaa K, Meretoja T, Sorsa M, Vainio H (1978b). Cytogenetic effects of styrene

and styrene oxide on human lymphocytes and Allium cepa. Scand J Work Environ Health. 4 Suppl 2:156-62.

Linnoila RI, Szabo E, DeMayo F, Witschi H, Sabourin C, Malkinson A (2000). The role of CC10 in pulmonary carcinogenesis: from a marker to tumor suppression. Ann N Y Acad Sci 923:249-67.

Liu S-F, Fang Q-M, Jin Z-L, Rappaport MS (2001). Investigation of protein-styrene oxide adducts as a molecular biomarker of human exposed to styrene. J Environ Sci 13(4):391-7.

Lof A, Gullstrand E, Lundgren E, Nordqvist MB (1984). Occurrence of styrene-7,8-oxide and styrene glycol in mouse after the administration of styrene. Scand J Work Environ Health 10:179-87.

Lof A, Johanson G (1993). Dose-dependent kinetics of inhaled styrene in man. IARC Sci Publ 127:89-99.

Lof A, Lundgren E, Nordqvist B (1986). Kinetics of styrene in workers from a plastics industry after controlled exposure: a comparison with subjects not previously exposed. Br J Ind Med 43(8):537-43.

London SJ, Lehman TA, Taylor JA (1997). Myeloperoxidase genetic polymorphism and lung cancer risk. Cancer Res 57:5001-3.

Loprieno N, Abbondandolo A, Barale R, Baroncelli S, Bonatti S *et al.* (1976). Mutagenicity of industrial compounds: styrene and its possible metabolite styrene oxide. Mutat Res 40(4):317-24.

Lorimer WV, Lilis R, Nicholson WJ, Anderson H, Fischbein A, Daum S *et al.* (1976). Clinical studies of styrene workers: initial findings. Environ Health Perspect 17:171-81.

Lorimer WV, Lilis R, Fischbein A, Daum S, Anderson H, Wolff MS, Selikoff IJ (1978). Health status of styrene-polystyrene polymerization workers. Scand J Work Environ Health 4 Suppl 2:220-6.

Loughlin JE, Rothman KJ, Dreyer NA (1999). Lymphatic and haematopoietic cancer mortality in a population attending school adjacent to styrene-butadiene facilities, 1963-1993. J Epidemiol Community Health 53:283-7.

Luderer U, Tornero-Velez R, Shay T, Rappaport S, Heyer N, Echeverria D (2004). Temporal association between serum prolactin concentration and exposure to styrene. Occup Environ Med 61(4):325-33.

Lynge E, Anttila A, Hemminki K (1997). Organic solvents and cancer. Cancer Causes Contr 8:406-19.

Macaluso M, Larson R, Delzell E, Sathiakumar N, Hovinga M *et al*. (1996). Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. Toxicology 113(1-3):190-202

Macaluso M, Larson R, Lynch J, Lipton S, Delzell E (2004). Historical estimation of exposure to 1,3-butadiene, styrene, and dimethyldithiocarbamate among synthetic rubber workers. J Occup Environ Hyg 1(6):371-90.

Mackay CJ, Kelman GR (1986). Choice reaction time in workers exposed to styrene vapour. Human Toxicol 5:85-9.

Maestri L, Imbriani M, Ghittori S, Capodaglio E, Gobba F, Cavalleri A (1997). Excretion of N-acetyl-S-(1-phenyl-2-hydroxyethyl)-cysteine and N-acetyl-S-(2-phenyl-2-hydroxyethyl)-cysteine in workers exposed to styrene. Sci Total Environ 199(1-2):13-22.

Maki-Paakkanen J (1987). Chromosome aberrations, micronuclei and sister-chromatid exchanges in blood lymphocytes after occupational exposure to low levels of styrene. Mutat Res 189(4):399-406.

Maki-Paakkanen J, Walles S, Osterman-Golkar S, Norppa H (1991). Single-strand breaks, chromosome aberrations, sister-chromatid exchanges, and micronuclei in blood lymphocytes of workers exposed to styrene during the production of reinforced plastics. Environ Mol Mutagen 17(1):27-31.

Makitie A, Pirvola U, Pyykko I, Sakakibara H, Riihimaki V, Ylikoski J (2002). Functional and morphological effects of styrene on the auditory system of the rat. Arch Toxicol 76(1):40-7.

Male R, Lillehaug JR, Djurhuus R, Pryme IF (1985). In vitro transformation and tumor promotion studies of styrene and styrene oxide. Carcinogenesis 6(9):1367-70.

Malkinson AM (1992). Primary lung tumors in mice: An experimentally manipulable model of human adenocarcinoma. Cancer Res 52(Suppl):2670s-6s.

Maltoni C, Ciliberti A, Carretti D (1982). Experimental contributions in identifying brain potential carcinogens in the petrochemical industry. Ann N Y Acad Sci 381:216-49.

Maltoni C, Cotti G, Perino G (1988a). Long-term carcinogenicity bioassays on methylene chloride administered by ingestion to Sprague-Dawley rats and Swiss mice and by inhalation to Sprague-Dawley rats. Ann N Y Acad Sci 534:352-66.

Maltoni C, Lefemine G, Tovoli D, Perino G (1988b). Long-term carcinogenicity bioassays on three chlorofluorocarbons (trichlorofluoromethane, FC11; dichlorodifluoromethane, FC12; chlorodifluoromethane, FC22) administered by inhalation to Sprague-Dawley rats and Swiss mice. Ann N Y Acad Sci 534:261-82.

Maltoni C (1978). [Letter to International Cooperative Study Group on the Long-term Effects of Styrene]. 2 October 1978.

Mancuso TF, Ciocco A, El-Attar AA (1968). An epidemiological approach to the rubber industry. J Occup Med 10:213-32.

Manini P, Buzio L, Andreoli R, Goldoni M, Bergamaschi E, Jakubowski M, Vodicka P, Hirvonen A, Mutti A (2003). Assessment of biotransformation of the arene moiety of styrene in volunteers and occupationally exposed workers. Toxicol Appl Pharmacol 189(3):160-9.

Marczynski B, Rozynek PEHJ, Korn M, Baur X (1997). Detection of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, in white blood cells of workers occupationally exposed to styrene. Arch Toxicol 71(8):496-500.

Marniemi J, Suolinna E-M, Kaartinen N, Vainio H (1977). Covalent binding of styrene

oxide to rat liver macromolecules in vivo and in vitro. Ullich V *et al.*, eds. Microsomes and Drug Oxidations. Pergamon Press, New York, pp. 698-702.

Maron DM, Ames BN (1983). Revised methods for the Salmonella mutagenicity test. Mutat Res 113(3-4):173-215.

Mason RJ, Kalina M, Nielsen LD, Malkinson AM, Shannon JM (2000). Surfactant protein C expression in urethane-induced murine pulmonary tumors. Am J Pathol 156:175-82.

Matanoski G, Elliott E, Tao X, Francis M, Correa-Villasenor A, Santos-Burgoa C (1997). Lymphohematopoietic cancers and butadiene and styrene exposure in synthetic rubber manufacture. Ann N Y Acad Sci 837:157-69

Matanoski G, Francis M, Correa-Villasenor A, Elliott E, Santos-Burgoa C *et al*. (1993). Cancer epidemiology among styrene-butadiene rubber workers. In: Butadiene and Styrene: Assessment of Health Hazards. M Sorsa, K Peltonen, H Vainio, K Hemminki, eds. IARC Scientific Publ No. 127. International Agency for Research on Cancer, Lyon, France, pp. 363-74.

Matanoski GM, Santos-Burgoa C, Schwartz L (1990). Mortality of a cohort of workers in the styrene-butadiene polymer manufacturing industry (1943-1982). Environ Health Perspect 86:107-17.

Matanoski GM, Tao X (2002). Case-cohort study of styrene exposure and ischemic heart disease. Res Rep Health Eff Inst 108:1-29; discussion 31-7.

Matanoski GM, Tao XG (2003). Styrene exposure and ischemic heart disease: a case-cohort study. Am J Epidemiol 158(10):988-95.

Mathew J, Cattan AR, Hall AG, Hines JE, Nelson R, Eastham E, Burt AD (1992). Glutathione S-transferases in neonatal liver disease. J Clin Pathol 45(8):679-83.

Matiella JE, Hsieh T C-Y (1991). Volatile compounds in scrambled eggs. J Food Sci 56(2):387-91.

Matikainen E, Forsman-Gronholm L, Pfaffli P, Juntunen J (1993). Neurotoxicity in workers exposed to styrene. IARC Scientific Publ 127:153-61.

Matsuoka A, Hayashi M, Ishidate M Jr (1979). Chromosomal aberration tests on 29 chemicals combined with S9 mix in vitro. Mutat Res 66(3):277-90.

McCullough B, Collins JF, Johanson WG Jr, Grover FL (1978). Bleomycin-induced diffuse interstitial pulmonary fibrosis in baboons. J Clin Invest 61(1):79-88.

McDougal JN, Jepson GW, Clewell HJI, Gargas ML, Andersen ME (1990). Dermal absorption of organic chemical vapors in rats and humans. Fund Appl Toxicol 14:299-308.

McGee J, Fitzpatrick F (1985). Enzymatic hydration of leukotriene A4: purification and characterization of a novel epoxide hydrolase from human erythrocytes. J Biol Chem 260(23):12832-7.

McGregor D, Anderson D (1999). DNA damage and repair in mammalian cells in vitro and in vivo as indicators of exposure to carcinogens. IARC Sci Publ 146:309-54.

McMartin DN, Sahota PS, Gunson DE, Hsu HH, Spaet RH (1992). Neoplasms and related proliferative lesions in control Sprague-Dawley rats from carcinogenicity studies. Historical data and diagnostic considerations. Toxicol Pathol 20(2):212-25.

McMichael AJ, Spirtas R, Gamble JF, Tousey PM (1976). Mortality among rubber workers: relationship to specific jobs. J Occup Med 18(3):178-85.

McMichael AJ, Spirtas R, Kupper LL (1974). An epidemiologic study of mortality within a cohort of rubber workers, 1964-72. J Occup Med 16:458-64.

McMichael AJ, Spirtas R, Kupper LL, Gamble JF (1975). Solvent exposure and leukemia among rubber workers: an epidemiologic study. J Occup Med 17:234-9.

Meinhardt TJ, Lemen RA, Crandall MS, Young RJ (1982). Environmental epidemiologic investigation of the styrene-butadiene rubber industry: Mortality patterns with discussion of the hematopoietic and lymphatic malignancies. Scand J Work Environ Health 8:250-9.

Meites J (1980). Relation of the neuroendocrine system to the development and growth of experimental mammary tumors. J Neural Transmission 48:25-42.

Melnick RL (2002). Carcinogenicity and mechanistic insights on the behavior of epoxides and epoxides-forming chemical. Ann N Y Acad Sci 982:177-89.

Melnick RL, Huff JE (1993). 1,3-Butadiene induces cancer in experimental animals at all concentrations from 6.25 to 8000 parts per million. In: Butadiene and Styrene: Assessment of Health Hazards. M Sorsa, K Peltonen, H Vainio, K Hemminki, eds. IARC Scientific Publications No. 127. Lyon, France. pp. 309-22.

Mendrala AL, Langvardt PW, Nitschke KD, Quast JF, Nolan RJ (1993). In vitro kinetics of styrene and styrene oxide metabolism in rat, mouse, and human. Arch Toxicol 67:18-27.

Mera N, Ohmori S, Itahashi K, Kiuchi M, Igarashi T, Rikihisa T, Kitada M (1994). Immunochemical evidence for the occurrence of Mu class glutathione S-transferase in human fetal livers. J Biochem (Tokyo) 116(2):315-20.

Meretoja T, Jarventaus H, Sorsa M, Vainio H (1978a). Chromosome aberrations in lymphocytes of workers exposed to styrene. Scand J Work Environ Health 4 Suppl 2:259-64.

Meretoja T, Vainio H, Jarventaus H (1978b). Clastogenic effects of styrene exposure on bone marrow cells of rats. Toxicol Lett 1:315-318.

Miller VP, DePillis GD, Ferrer JC, Mauk AG, deMontellano PRO (1992). Monooxygenase activity of cytochrome C peroxidase. J Biol Chem 267:8936-42.

Minchin RF, Boyd MR (1983). Localization of metabolic activation and deactivation systems in the lung: significance to the pulmonary toxicity of xenobiotics. Ann Rev Pharmacol 23:217-38.

Moller C, Odkvist L, Larsby B, Tham R, Ledin T, Bergholtz L (1990). Otoneurological findings in workers exposed to styrene. Scand J Work Environ Health 16(3):189-94.

Monson RR, Nakano KK (1976). Mortality among rubber workers: I. White male union employees in Akron, Ohio. Am J Epidem 103(3):284-96.

Morata TC, Johnson A-C, Nylen P, Svensson EB, Cheng J, Krieg EF *et al.* (2002). Audiometric findings in workers exposed to low levels of styrene and noise. J Occup Environ Med 44:806-14.

Morel G, Cossec B, Lambert AM, Binet S (1999). Evaluation of rat hepatic 2E1 activity in function of age, sex and inducers: choice of an experimental model capable of testing the hepatotoxicity of low molecular weight compounds. Toxicol Lett 106:171-80.

Morgan DL, Cooper SW, Carlock DL, Sykora JJ, Sutton B, Mattie DR *et al.* (1991). Dermal absorption of neat and aqueous volatile organic chemicals in the Fischer 344 rat. Environ Res 55(1):51-63.

Morgan DL, Mahler JF, Dill JA, Price HC Jr, O'Conner RW, Adkins B Jr (1993). Styrene inhalation toxicity studies in mice. II. Sex differences in susceptibility of B6C3F1 mice. Fundam Appl Toxicol 21:317-25.

Morgenstern R, Depierre JW (1988). Membrane-bound glutathione transferases. In: Glutathione Conjugation. Mechanisms and Biological Significance. Sies H, Ketterer B, eds. Academic Press, Ltd, London, pp. 157-74.

Morioka I, Kuroda M, Miyashita K, Takeda S (1999). Evaluation of organic solvent ototoxicity by the upper limit of hearing. Arch Environ Health 54(5):341-6.

Morisset Y, P'an S, Jegier A (1979). Effect of styrene and fiber glass on small airways of mice. J Toxicol Environ Health 5:943-56.

Morris JB (2000). Uptake of styrene in the upper respiratory tract of the CD mouse and Sprague-Dawley rat. Toxicol Sci 54:222-8.

Moscato G, Biscaldi G, Cottica D, Pugliese F, Candura S, Candura F (1987). Occupational asthma due to styrene: two case reports. J Occup Med 29(12):957-60.

Murata K, Araki S, Yokoyama K (1991). Assessment of the peripheral, central, and autonomic nervous system function in styrene workers. Am J Ind Med 20:775-84.

Murray FJ, John JA, Balmer MF, Schwetz BA (1978). Teratologic evaluation of styrene given to rats and rabbits by inhalation or by gavage. Toxicology 11(4):335-43.

Mutti A (1988). Styrene exposure and serum prolactin. J Occup Med 30:481-2.

Mutti A (1993). Mechanisms and biomarkers of solvent-induced behavioral and neuroendocrine effects. In: Use of Biomarkers in Assessing Health and Environmental Impacts of Chemical Pollutants. Travis CC, ed.. Plenum Press, New York, pp 183-99.

Mutti A, Buzio C, Perazzoli F, Bergamaschi E, Bocchi MC, Selis L, Mineo F, Franchini I (1992). [Lymphocyte subpopulations in workers exposed occupationally to styrene] [Italian]. Med Lav 83(2):167-77.

Mutti A, Coccini T, Alinovi R, Toubeau G, Broeckaert F *et al.* (1999). Exposure to hydrocarbons and renal disease: an experimental animal model. Ren Fail 21(3-4):369-85.

Mutti A, Falzoi M, Romanelli A, Franchini I (1984a). Regional alterations of brain catecholamines by styrene exposure in rabbits. Arch Toxicol 55(3):173-7.

Mutti A, Falzoi M, Romanelli MC, Bocchi MC, Ferroni C, Frandhini I (1988). Brain dopamine as a target for solvent toxicity: effects of some monocyclic aromatic hydrocarbons. Toxicology 49:77-82.

Mutti A, Mazzucchi A, Rustichelli P, Frigeri G, Arfini G, Franchini I (1984b). Exposure-effect and exposure-response relationships between occupational exposure to styrene and neuropsychological functions. Am J Ind Med 5:275-86.

Mutti A, Vescovi PP, Falzoi M, Arfini G, Valenti G, Franchini I (1984c). Neuroendocrine effects of styrene on occupationally exposed workers. Scand J Work Environ Health 10:225-8.

Nakajima T, Elovaara E, Gonzalez FJ, Gelboin HV, Raunio H *et al*. (1994a). Styrene metabolism by cDNA-expressed human hepatic and pulmonary cytochromes P450. Chem Res Toxicol 7(6):891-6.

Nakajima T, Wang RS, Elovaara E, Gonzalez FJ, Gelboin HV *et al.* (1994b). CYP2C11 and CYP2B1 are major cytochrome P450 forms involved in styrene oxidation in liver and lung microsomes from untreated rats, respectively. Biochem Pharmacol 48(4):637-42.

NCI (1979). Bioassay of styrene for possible carcinogenicity. Technical Report Series No. 185. National Cancer Institute, National Institutes of Health, Bethesda, MD.

Nestmann ER, Lynch BS, Ratpan F (2005). Perspectives on the genotoxic risk of styrene. J Toxicol Environ Health B Crit Rev 8(2):95-107.

Nicholson WJ, Selikoff IJ, Seidman H (1978). Mortality experience of styrene-polystyrene polymerization workers: initial findings. Scand J Work Environ Health 4 (Suppl. 2):247-52.

NIOSH (1983). Criteria for a recommended standard: occupational exposure to styrene. National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services, Cincinnati, OH. NIOSH Publication No. 83-119. September.

NIOSH (1976). Proceedings of NIOSH Styrene-Butadiene Briefing, Covington, Kentucky, April 30, 1976. L Ede, ed. National Institute for Occupational Safety and Health, U.S. Department of Health Education and Welfare, Cincinnati, OH. DHEW/NIOSH Publication No. 77-129.

Nordenson I, Beckman L (1984). Chromosomal aberrations in lymphocytes of workers exposed to low levels of styrene. Hum Hered 34(3):178-82.

Nohynek GJ, Longeart L, Geffray B, Provost JP, Lodola A (1993). Fat, frail and dying young: survival, body weight and pathology of the Charles River Sprague-Dawley-derived rat prior to and since the introduction of the VAFR variant in 1988. Hum Exp Toxicol 12(2):87-98.

Nordqvist MB, Lof A, Osterman-Golkar S, Walles SA (1985). Covalent binding of styrene and styrene-7,8-oxide to plasma proteins, hemoglobin and DNA in the mouse. Chem Biol Interact 55(1-2):63-73.

Norppa H (1981). Styrene and vinyltoluene induce micronuclei in mouse bone marrow. Toxicol Lett 8(4-5):247-51.

Norppa H, Sorsa M, Pfaffli P, Vainio H (1980a). Styrene and styrene oxide induce SCEs and are metabolised in human lymphocyte cultures. Carcinogenesis 1(4):357-61.

Norppa H, Sorsa M, Vainio H (1980b). Chromosomal aberrations in bone marrow of Chinese hamsters exposed to styrene and ethanol. Toxicol Lett 5(3-4):241-4.

Norppa H, Tursi F (1984). Erythrocyte-mediated metabolic activation detected by SCE. In: Sister Chromatid Exchanges. Part B. Genetic Toxicology and Human Studies. Tice RR, Hollaender A, eds. Plenum Pres, New York, pp. 547-59.

Norppa H, Tursi F, Einisto P (1985). Erythrocytes as a metabolic activation system in mutagenicity tests. In: Mutagenesis and Genetic Toxicology. Theoretical and Practical Results. Janiaud P, Averbeck D, Moustacchi E, eds. INSERM Vol. 119. Paris, pp. 35-50.

Norppa H, Vainio H (1983). Genetic toxicity of styrene and some of its derivatives. Scand J Work Environ Health 9(2 Spec No):108-14.

Norppa H, Vainio H, Sorsa M (1983). Metabolic activation of styrene by erythrocytes detected as increased sister chromatid exchanges in cultured human lymphocytes. Cancer Res 43(8):3579-82.

Norstrom A, Lof A, Aringer L, Samuelsson R, Andersson B., Levin J-O *et al.* (1992). Determination of N-acetyl-S-(2-phenyl-2-hydroxyethyl)cysteine in human urine after experimental exposure. Chemosphere 24(11):1553-61.

NTP (1993). Toxicology and carcinogenesis studies of 1,3-butadiene (CAS no. 106-99-0) in B6C3F1 mice (inhalation studies) (TR No. 326), National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP (2001). Report on Carcinogens. What is under consideration for the 10th RoC? National Toxicology Program, Research Triangle Park, NC. Accessed 08/24/01 at: http://ntp-server.niehs.nih.gov/NewHomeRoC/10thConsideration.html.

NTP (2005). NTP-CERHR expert panel report on the potential human reproductive and developmental effects of styrene. Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, Research Triangle Park, NC. Accessed at: http://cerhr.niehs.nih.gov/chemicals/styrene/Styrene_final.pdf.

NTP (2006). NTP-CERHR monograph on the reproductive and developmental toxicity of styrene. Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, Research Triangle Park, NC. Accessed at: http://cerhr.niehs.nih.gov/chemicals/styrene/styrene-eval.html.

NTP (2008a). FINAL. Report on Carcinogens. Background Document for Styrene, September 29, 2008. National Toxicology Program, Research Triangle Park, NC. Accessed at: http://ntp.niehs.nih.gov/index.cfm?objectid=DFAFC5A1-F1F6-975E-766CD2956416305E.

NTP (2008b). Styrene Expert Panel Report. Part B – Recommendation for listing status for “styrene” in the Report on Carcinogens and scientific justification for the recommendation. National Toxicology Program, Research Triangle Park, NC. Accessed at: http://ntp.niehs.nih.gov/index.cfm?objectid=DFAFC5A1-F1F6-975E-766CD2956416305E.

Oberheitmann B, Frenzel-Beyme R, Hoffmann W (2001). An applicationof the challenge assay in boat builders exposed to low levels of styrene - a feasibility study of a possible biomarker for acquired susceptibility. Int J Hyg Environ Health 204:23-9.

OEHHA (1999). Air Toxics Hot Spots Program Risk Assessment Guidelines. Part I. The Determination of Acute Reference Exposure Levels for Airborne Toxicants. Office of Environmental Health Hazard Assessment. Available at www.oehha.ca.gov.

OEHHA (2000). Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Chronic Reference Exposure Levels. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. Available at: www.oehha.ca.gov.

OEHHA (2009). Technical Support Document for Cancer Potency Factors: Methodologies for derivation, listing of available values, and adjustments to allow for early life stage exposures. May 2009. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. Available at: http://www.oehha.ca.gov/air/hot_spots/tsd052909.html.

Oesch F (1972). Mammalian epoxide hydrases: inducible enzymes catalyzing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. Xenobiotica 3(5):305-40.

Oesch F, Glatt H, Schimassmann H (1977). The apparent ubiquity of epoxide hydratase in rat organs. Biochem Pharmacol 26(7):603-7.

Ohashi Y, Nakai Y, Ikeoka H, Koshimo H, Esaki Y, Horiguchi S *et al.* (1985). Electron microscopic study of the respiratory toxicity of styrene. Osaka City Med J 31(1):11-21.

Ohno K, Azuma Y, Nakano S, Kobayashi T, Hirano S, Nobuhara Y, Yamada T. (2001). Assessment of styrene oligomers eluted from polystyrene-made food containers for estrogenic effects in in vitro assays. Food Chem Toxicol 39(12):1233-41.

Okun AH, Beaumont JJ, Meinhardt TJ, Crandall MS (1985). Mortality patterns among styrene-exposed boatbuilders. Am J Ind Med 8:193-205.

Ollikainen T, Hirvonen A, Norppa H (1998). Influence of GSTT1 genotype on sister chromatid exchange induction by styrene-7,8-oxide in cultured human lymphocytes. Environ Molecular Mutagen 31(4):311-5.

Omiecinski CJ, Aicher L, Holubkov R, Checkoway H (1993). Human peripheral lymphocytes as indicators of microsomal epoxide hydrolase activity in liver and lung. Pharmacogenetics 3(3):150-8.

Omiecinski CJ, Remmel RP, Hosagrahara VP (1999). Concise review of the cytochrome P450s and their roles in toxicology. Toxicol Sci 48:151-6.

Ortiz de Montellano PR, Catalano CE (1985). Epoxidation of styrene by hemoglobin and myoglobin. Transfer of oxidizing equivalents to the protein surface. J Biol Chem 260(16):9265-71.

OSHA (1996). OSHA National news release: OSHA announces that styrene industry has adopted voluntary compliance program to improve worker protection. Dated March 1, 1996. Occupational Safety and Health Administration. Available at: www.osha.gov.

OSHA (2002). Safety and health topics: Styrene. Occupational Safety and Health Administration. Accessed October 17, 2002 at: www.osha.gov.

Osterman-Golkar S, Christakopoulos A, Zorcec V, Svensson K (1995). Dosimetry of styrene 7,8-oxide in styrene- and styrene oxide-exposed mice and rats by quantification of haemoglobin adducts. Chemico-Biol Interact 95(1-2):79-87.

Ott MG, Kolesar RC, Scharnweber HC, Schneider EJ, Venable JR (1980). A mortality survey of employees engaged in the development or manufacture of styrene-based products. J Occup Med 22(7):445-60.

Otteneder M, Lutz U, Lutz WK (2002). DNA adducts of styrene-7,8-oxide in target and non-target organs for tumor induction in rat and mouse after repeated inhalation exposure to styrene. Mutat Res 500:111-6.

Pacifici GM, Boobis AR, Brodie MJ, McManus ME, Davies DS (1981). Tissue and species differences in enzymes of epoxide metabolism. Xenobiotica 11(2):73-9.

Pacifici GM, Colizzi C, Giuliani L, Rane A (1983). Cytosolic epoxide hydrolase in fetal and adult human liver. Arch Toxicol 54:331-41.

Pacifici GM, Rane A (1982). Metabolism of styrene oxide in different human fetal tissues. Drug Metab Dispos 10(4):302-5.

Pacifici GM, Rane A (1983). Epoxide hydrolase in human fetal liver. Pharmacology 26(5):241-8.

Pacifici GM, Warholm M, Guthenberg C, Mannervik B, Rane A (1987). Detoxification of styrene oxide by human liver glutathione transferase. Hum Toxicol 6(6):483-9.

Pagano DA, Yagen B, Hernandez O, Bend JR, Zeiger E (1982). Mutagenicity of (R) and (S) styrene 7,8-oxide and the intermediary mercapturic acid metabolites formed from styrene 7,8-oxide. Environ Mutagen 4(5):575-84.

Pantarotto C, Fanelli R, Bidoli F, Morazzoni P, Salmona M, Szczawinska K (1978). Arene oxides in styrene metabolism, a new perspective in styrene toxicity? Scand J Work Environ Health 4(Suppl 2):67-77.

Parent M-E, Hua Y, Siemiatycki J (2000). Occupational risk factors for renal cell carcinoma in Montreal. Am J Ind Med 38:609-18.

Pauwels W, Vodiceka P, Severi M, Plna K, Veulemans H, Hemminki K (1996). Adduct formation on DNA and haemoglobin in mice intraperitoneally administered with styrene. Carcinogenesis 17(12):2673-80.

Pellizzari ED, Hartwell TD, Harris BSH III, Waddell RD, Whitaker DA, Erickson MD (1982). Purgeable organic compounds in mother's milk. Bull Environ Contam Toxicol 28:322-8.

Penttila M, Sorsa M, Vainio H (1980). Inability of styrene to induce nondisjunction in Drosophila or a positive micronucleus test in the Chinese hamster. Toxicol Lett 6:119-23.

Perbellini L, Mozzo P, Turri PV, Zedde A, Brugnone F (1988). Biological exposure index of styrene suggested by a physiologico-mathematical model. Int Arch Occup Environ Health 60:187-93.

Petruska JM, Mosebrook DR, Jakab GJ, Trush MA (1992). Myeloperoxidase-enhanced formation of (+/-)-trans,7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene-DNA adducts in lung tissue in vitro: a role of pulmonary inflammation in the bioactivation of a procarcinogen. Carcinogenesis 13(7):1075-81.

Pfaffli P, Hesso A, Vainio H, Hyvonen M (1981). 4-Vinylphenol excretion suggestive of arene oxide formation in workers occupationally exposed to styrene. Toxicol Appl Pharmacol 60(1):85-90.

Pierce CH, Dills RL, Silvey GW, Kalman DA (1996). Partition coefficients between human blood or adipose tissue and air for aromatic solvents. Scand J Work Environ Health 22(2):112-8.

Pohlova H, Rossner P, Sram RJ (1984). Cytogenetic analysis of human peripheral blood lymphocytes in culture exposed in vitro to styrene and styrene oxide. J Hyg Epidemiol Microbiol Immunol 29(3):269-74.

Pohlova H, Sram RJ (1985). Cytogenetic analysis of peripheral blood lymphocytes of workers occupationally exposed to styrene. J Hyg Epidemiol Microbiol Immunol 29(2):155-61.

Pokrowskij VA (1961). La toxicologie du styrene. Gig Tr Prof Zabol 5:3-7.

Poncelet F, de Meester C, Duverger-van Bogaert M, Lambotte-Vandepaer M, Roberfroid M *et al*. (1980). Influence of experimental factors on the mutagenicity of vinylic monomers. Arch Toxicol Suppl 4:63-6.

Ponomarkov V, Tomatis L (1978). Effects of long-term oral administration of styrene to mice and rats. Scand J Work Environ Health Suppl 2:127-35.

Prehn AW, West DW (1998). Evaluating local differences in breast cancer incidence rates: a census-based methodology (United States). Cancer Causes Control 9:511–7.

Prejean JD, Peckham JC, Casey AE, Griswold DP, Weisburger EK *et al*. (1973). Spontaneous tumors in Sprague-Dawley rats and Swiss mice. Cancer Res 33:2768-73.

Pryor GT, Rebert CS, Howd RA (1987). Hearing loss in rats caused by inhalation of mixed xylenes and styrene. J Appl Toxicol 7(1):55-61.

Quast JF, Humiston CG, Kalnins RV *et al*. (1979). Results of a toxicity study of monomeric styrene administered to beagle dogs by oral intubation for 19 months. Report to Manufacturing Chemists Association, Washington, DC by Health and Environmental Sciences, Dow Chemical USA, Midland, MI.

Purves D, Harvey C, Tweats D, Lumley CE (1995). Genotoxicity testing: current practices and strategies used by the pharmaceutical industry. Mutagenesis 10(4):297-312.

Rabovsky J, Fowles J, Hill MD, Lewis DC (2001). A health risk benchmark for the neurologic effects of styrene: comparison with NOAEL/LOAEL approach. Risk Anal 21(1):117-26.

Rabovsky J, Judy DJ, Sapola NA, Pailes WH, McPeek M, Castranova V (1989). Cytochrome P450-dependent alkoxyphenoxazone dealkylase activity in rat alveolar Type II cells: effect of pretreatment with beta-naphthaflavone. Cell Biochem Func 7(2):79-89.

Ragule N (1974). Embryotropic action of styrene. Gig Sanit 11:85-6. [Chem Abs 82:81357g, 1975. [cited in Brown NA et al., 2000]

Ramsey JC, Andersen ME (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol Appl Pharmacol 73:159-75.

Rao GN, Haseman JK, Grumbein S, Crawford DD, Eustis SL (1990). Growth, body weight, survival, and tumor trends in F344/N rats during an eleven-year period. Toxicol Pathol 18(1):(1 Pt 1):71-7.

Rappaport SM, Yeowell-O'Connell K, Bodell W, Yager JW, Symanski E (1996). An investigation of multiple biomarkers among workers exposed to styrene and styrene-7,8-oxide. Cancer Res 56(23):5410-16.

Rebert CS, Hall TA (1994). The neuroepidemiology of styrene: a critical review of representative literature. Crit Rev Toxicol 24(Suppl 1):S57-S106.

Reddy MV (1993). 32P-postlabelling analysis of small aromatic and of bulky non-aromatic DNA adducts. IARC Sci Publ 124:25-34.

Rehm S, Ward JM, Sass B (1994). Tumours of the lungs. IARC Sci Publ 111:325-55.

Renwick AG (2000). The use of safety or uncertainty factors in the setting of acute reference doses. Food Add Contam 17(7):627-35.

Rigdon RH, Neal J (1966). Gastric carcinomas and pulmonary adenomas in mice fed benzo (a) pyrene. Tex Rep Biol Med 24(2):195-207.

Riihimaki V, Pfaffli P (1978). Percutaneous absorption of solvent vapors in man. Scand J Work Environ Health 4:73-85.

Robbins AS, Brescianini S, Kelsey JL (1997). Regional differences in known risk factors and the higher incidence of breast cancer in San Francisco. J Natl Cancer Inst 89(13):960-5.

Röder-Stolinski C, Fischäder G, Oostingh GJ, Feltens R, Kohse F, von Bergen M, Mörbt N, Eder K, Duschl A, Lehmann I (2008). Styrene induces an inflammatory response in human lung epithelial cells via oxidative stress and NF-kappaB activation. Toxicol Appl Pharmacol 231(2):241-7.

Rolle-Kampczyk UE, Rehwagen M, Diez U, Richter M, Herbarth O, Borte M (2002). Passive smoking, excretion of metabolites, and health effects: results of the Leipzig's Allergy Risk Study (LARS). Arch Environ Health 57(4):326-31.

Rosen MB, JM Rogers, DB Miller, C Mattscheck, and N Chernoff (1988) Effects of chemical-induced maternal toxicity on the Sprague - Dawley rat. Teratology 37: 486

Rosengren LE, Haglid KG (1989). Long term neurotoxicity of styrene. A quantitative study of glial fibrillary acidic protein (GFA) and S-100. Br J Ind Med 46(5):316-20.

Ross D (1996). Metabolic basis of benzene toxicity. Eur J Haematol 57(Suppl):111-8.

Ruder AM, Ward EM, Dong M, Okun AH, Davis-King K (2004). Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: an update. Am J Ind Med 45(2):165-76.

Rueff J, Teixeira JP, Santos LS, Gaspar JF (2009). Genetic effects and biotoxicity monitoring of occupational styrene exposure. Clin Chim Acta 399:8–23.

Sallmen M, Lindbohm ML, Anttila A, Kyyronen P, Taskinen H *et al*. (1998). Time to pregnancy among the wives of men exposed to organic solvents. Occup Environ Med 55:24-30.

Santos-Burgoa C, Matanoski GM, Zeger S, Schwartz L (1992). Lymphohematopoietic cancer in styrene-butadiene polymerization workers. Am J Epid 136(7):843-54.

Sarangapani R, Teeguarden JG, Cruzan G, Clewell HJ, Andersen ME (2002). Physiologically based pharmacokinetic modeling of styrene and styrene oxide respiratory-tract dosimetry in rodents and humans. Inhal Toxicol 14(8):789-834.

Sathiakumar N, Brill I, Delzell E (2009). 1,3-Butadiene, styrene and lung cancer among synthetic rubber industry workers. J Occup Environ Med 51:xx-xx (posted online 22 October 2009, doi: 10.1097/JOM.0b013e3181c3c663.

Sathiakumar N, Delzell E (2007). A follow-up study of women in the synthetic rubber industry: Study methods. Chemico-Biological Interactions 166:25-8.

Sathiakumar N, Delzell E (2009). A follow-up study of mortality among women in the North American synthetic rubber industry. J Occup Environ Med 51:xx-xx (posted online 22 October 2009, doi: 10.1097/JOM.0b013e3181bd8972.

Sathiakumar N, Delzell E, Hovinga M, Macaluso M, Julian JA *et al*. (1998). Mortality from cancer and other causes of death among synthetic rubber workers. Occup Environ Med 55(4):230-5.

Savolainen H (1977). Some aspects of the mechanisms by which industrial solvents produce neurotoxic effects. Chem-Biol Interact 18:1-10.

Savolainen H, Vainio H (1977). Organ distribution and nervous system binding of styrene and styrene oxide. Toxicol 8(2):135-41.

Sbrana I, Lascialfari D, Rossi AM, Loprieno N, Bianchi M, Tortoreto M, Pantarotto C (1983). Bone marrow cell chromosomal aberrations and styrene biotransformation in mice given styrene on a repeated oral schedule. Chem Biol Interact 45(3):349-57.

Scelo G, Constantinescu V, Csiki I, Zaridze D, Szeszenia-Dabrowska N *et al.* (2004). Occupational exposure to vinyl chloride, acrylonitrile and styrene and lung cancer risk (Europe). Cancer Causes Control 15(5):445-52.

Schwegler U, Filser JG (1989). Inhalation kinetics of styrene in rat and mouse. Naunyn-Schmiedeberg's Arch Pharmacol 339(Suppl):R14.

Scorecard© (2000). Environmental Defense and GetActive Software. Used by permission. Scorecard is available at www.scorecard.org.

Scott D, Preston RJ (1994). A re-evaluation of the cytogenetic effects of styrene. Mutat Res 318(3):175-203.

Seeber A, Blaszkewicz M, Golka K, Hallier E, Kiesswetter E, Schaper M, Van Thriel C (2004). Neurobehavioral effects of experimental exposures to low levels of styrene. Toxicol Lett 151(1):183-92.

Seiler JP (1990). Chirality-dependent DNA reactivity as the possible cause of the differential mutagenicity of the two components in an enantiomeric pair of epoxides. Mutat Res 245(3):165-9.

Seilkop SK (1995). The effect of body weight on tumor incidence and carcinogenicity testing in B6C3F1 mice and F344 rats. Fundam Appl Toxicol 24:247-59.

Severi M, Pauwels W, Van Hummelen P, Roosels D, Kirsch-Volders M, Veulemans H (1994). Urinary mandelic acid and hemoglobin adducts in fiberglass-reinforced plastics workers exposed to styrene. Scand J Work Environ Health 20(6):451-8.

Sharief Y, Brown AM, Backer LC, Campbell JA, Westbrook-Collins B *et al*. (1986). Sister chromatid exchange and chromosome aberration analyses in mice after in vivo exposure to acrylonitrile, styrene, or butadiene monoxide. Environ Mutagen 8(3):439-48.

Sher SP, Jensen RD, Bokelman DL (1982). Spontaneous tumors in control F344 and charles river-CD rats and Charles River CD-1 and B6C3HF1 mice. Toxicol Lett 11(1-2):103-10.

Shield AJ, Sanderson BJ (2004). A recombinant model for assessing the role of GSTM1 in styrene-7,8-oxide toxicity and mutagenicity. Toxicology 195(1):61-8.

Shigeta S, Miyake K, Aikawa H, Misawa (1989). Effects of postnatal low-levels of exposure to styrene on behavior and development in rats. J Toxicol Sci 14(4):279-86.

Siegel IH (1975). Enzyme Kinetics. John Wiley & Sons, Inc. New York, NY.

Sielken RL Jr., Valdez-Flores C (2001). Dose-response implications of the University of Alabama study of lymphohematopoietic cancer among workers exposed to 1,3-butadiene and styrene in the synthetic rubber industry. Chem Biol Interact 135-136:637-51.

Sikov MR, Cannon WC, Carr DB, Miller RA, Niemeier RW, Hardin BD (1986). Reproductive toxicology of inhaled styrene oxide in rats and rabbits. J Appl Toxicol 6(3):155-64.

Simula AP, Priestly BG (1992). Species differences in the genotoxicity of cyclophosphamide and styrene in three in vivo assays. Mutat Res 271(1):49-58.

Sinha AK, Jersey GC, Linscombe VA, Adams RL, Mueller AM, McClintock ML (1983). Cytogenetic evaluation of bone marrow cells from rats exposed to styrene vapor for one year. Fundam Appl Toxicol 3(2):95-8.

Sinsheimer JS, Chen R, Das SK, Hooberman BH, Osorio S, You A (1993). The genotoxicity of enantiomeric aliphatic epoxides. Mutat Res 298:197-206.

SIRC (1996). 104-Week Repeat Dose Inhalation Combined Toxicity/Carcinogenicity Study in Rats. Vol.1. (unpublished report). Prepared by Huntingdon Life Sciences Ltd. for the Styrene Information Research Center, Washington, DC.

SIRC (1998). Styrene. 104-Week Repeat Dose Inhalation Combined Toxicity/Carcinogenicity Study in Mice. Vol. 1. (unpublished report). Prepared by Huntingdon Life Sciences Ltd. for the Styrene Information Research Center, Washington, DC.

Ska B, Vyskocil A, Tardif R, Carrier G, Thuot R, Muray K, Viau C (2003). Effects of

peak concentrations on the neurotoxicity of styrene in volunteers. Hum Exp Toxicol 22(8):407-15.

Solomon JJ (1999). Cyclic adducts and intermediates induced by simple epoxides. IARC Sci Publ 150:123-35.

Solveig-Walles SA, Orsen I (1983). Single-strand breaks in DNA of various organs of mice induced by styrene and styrene oxide. Cancer Lett 21:9-15.

Somorovska M, Jahnova E, Tulinska J, Zamecnikova M, Sarmanova J, Terenova A *et al*. (1999). Biomonitoring of occupational exposure to styrene in a plastics lamination plant. Mutat Res 428(1-2):255-69.

Sorsa M, Anttila A, Jarventaus H, Kubiak R, Norppa H, Nylander L, Pekari K, Pfaffli P, Vainio H (1991). Styrene revisited--exposure assessment and risk estimation in reinforced plastics industry. Prog Clin Biol Res 372:187-95.

Speit G, Henderson L (2005). Review of the in vivo genotoxicity tests performed with styrene. Mutat Res 589(1):67-79.

Spencer HC, Irish DD, Adams EM, Rowe VK (1942). The response of laboratory animals to monomeric styrene. J Ind Hyg Toxicol 24(10):295-301.

Sripaung N, Motohashi Y, Nakata K, Nakamura K, Takano T (1995). Effects of simultaneous administration of ethanol on styrene metabolism under fed and fasted conditions in the perfused rat liver. J Toxicol Environ Health 45(4):439-51.

Srivastava SP, Das M, Mushtaq M, Chendra SV, Deth PK (1982). Hepatic effects of orally administered styrene in rats. J Appl Toxicol 2(4):219-22.

Srivastava S, Seth PK, Srivastava SP (1989). Effect of styrene administration on rat testis. Arch Toxicol 63(1):43-6.

Srivastava S, Seth PK, Srivastava SP (1990). Embryo/fetotoxicity of styrene in rats. J Environ Biol 11:73-7.

Srivastava S, Seth PK, Srivastava SP (1992a). Biochemical and morphological studies in testes of rat offspring of mothers exposed to styrene during lactation. Pharmacol Toxicol 70(4):314-6.

Srivastava S, Seth PK, Srivastava SP (1992b). Effect of styrene on testicular enzymes of growing rat. Indian J Exp Biol 30(5):399-401.

Starcher BC, Kuhn C, Overton JE (1978). Increased elastin and collagen content in the lungs of hamsters receiving an intratracheal injection of bleomycin. Am Rev Respir Dis 117(2):299-305.

Stewart, RD, Dodd HC, Baretta ED, Schaffer AW (1968). Human exposure to styrene vapor. Arch Environ Health 16:656-62.

Stock BH, Schreiber J, Guenat C, Mason RP, Bend JR, Eling TE (1986). Evidence for a free radical mechanism of styrene-glutathione conjugate formation catalyzed by prostaglandin H synthase and horseradish peroxidase. J Biol Chem 261(34):15915-22.

Stoltz DR, Whitey RJ (1977). Mutagenicity testing of styrene and styrene epoxide in Salmonella typhimurium. Bull Environ Contam Toxicol 17(6):739-42.

Stoner GD (1991). Lung tumors in strain A mice as a bioassay for carcinogenicity of environmental chemicals. Exp Lung Res 17:405-23.

Straif K, Weiland SK, Werner B, Chambless L, Mundt KA, Keil U (1998). Workplace risk factors for cancer in the German rubber industry: Part 2. Mortality from non-respiratory cancers. Occup Environ Med 55(5):325-32

Strange RC, Davis BA, Faulder CG, Cotton W, Bain AD, Hopkinson DA, Hume R (1985). The human glutathione S-transferases: developmental aspects of the GST1, GST2, and GST3 loci. Biochem Genet 23(11-12):1011-28.

Sumner SC, Cattley RC, Asgharian B, Janszen DB, Fennell TR (1997). Evaluation of the metabolism and hepatotoxicity of styrene in F344 rats, B6C3F1 mice, and CD-1 mice following single and repeated inhalation exposures. Chem Biol Interact 106(1):47-65.

Sumner SC, Cruzan G, Johanson G, Ghanayem BI, Fennell TR (2001). Styrene metabolism in rats, mice, and humans. CIIT Activities 21(3-4):1-8.

Symanski E, Bergamaschi E, Mutti A (2001). Inter- and intra-individual sources of variation in levels of urinary styrene metabolites. Int Arch Occup Environ Health 74:336-44.

Szabo E, Goheer A, Witschi H, Linnoila RI (1998). Overexpression of CC10 modifies neoplastic potential in lung cancer cells. Cell Growth Differ 9(6):475-85.

Takao T, Nanamiya W, Nazarloo HP, Asaba K, Hashimoto K (2000). Possible reproductive toxicity of styrene in peripubertal male mice. Endocr J 47(3):343-7.

Taskinen H, Anttila A, Lindbohm ML, Sallmen M, and Hemminki K (1989). Spontaneous abortions and congenital malformations among the wives of men occupationally exposed to organic solvents. Scand J Work Environ Health 15:345-52.

Taubeneck MW, Daston GP, Rogers JM, Keen CL (1994). Altered maternal zinc metabolism following exposure to diverse developmental toxicants. Reprod Toxicol 8(1):25-40.

Teixeira JP, Gaspar J, Silva S, Torres J, Silva SN, Azevedo MC *et al*. (2004). Occupational exposure to styrene: modulation of cytogenetic damage and levels of urinary metabolites of styrene by polymorphisms in genes CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1. Toxicology 195(2-3):231-42.

Thaete LG, Malkinson AM (1990). Differential staining of normal and neoplastic mouse lung epithelia by succinate dehydrogenase histochemistry. Cancer Lett 52:219-27.

Thiess AM, Schwegler H, Fleig I (1980). Chromosome investigations in lymphocytes of workers employed in areas in which styrene-containing unsaturated polyester resins are manufactured. Am J Ind Med 1(2):205-10.

Thiess AM, Fleig I (1978). Chromosome investigations on workers exposed to styrene/polystyrene. J Occup Med 20(11):747-9.

Thyssen J, Althoff J, Kimmerle G, Mohr U (1981). Inhalation studies with benzo[a]pyrene in Syrian golden hamsters. J Natl Cancer Inst 66(3):575-7.

Tomanin R, Ballarin C, Bartolucci GB, De Rosa E, Sessa G, Iannini G, Cupiraggi AR,

Sarto F (1992). Chromosome aberrations and micronuclei in lymphocytes of workers exposed to low and medium levels of styrene. Int Arch Occup Environ Health 64:209-15.

Tornero-Velez R, Rappaport SM (2001). Physiological modeling of the relative contributions of styrene-7,8-oxide derived from direct inhalation and from styrene metabolism to the systemic dose in humans. Toxicol Sci 64:151-61.

Tornero-Velez R, Waidyanatha S, Echeverria D, Rappaport SM (2000). Measurement of styrene-7,8-oxide and other oxidation products of styrene in air. J Environ Monit 2:111-7.

Tornero-Velez R, Waidyanatha S, Perez HL, Osterman-Golker S, Echeverria D, Rappaport SM (2001). Determination of styrene and styrene-7,8-oxide in human blood by gas chromatography-mass spectrometry. J Chromatog B 757:59-68.

Toppila E, Forsman P, Pyykkö I, Starck J, Tossavainen T, Uitti J, Oksa P (2006). Effect of styrene on postural stability among reinforced plastic boat plant workers in Finland. J Occup Environ Med 48(2):175-80.

Toth B, Rappaport H, Shubik P (1963). Influence of dose and age on the induction of malignant lymphomas and other tumor by 7,12-dimethyl[a]anthracene in Swiss mice. JNCI 30:723-40.

Triebig G, Schaller K-H, Valentin H (1985). Investigations on neurotoxicity of chemical substances at the workplace. VII. Longitudinal study with determination of nerve conduction velocities in persons occupationally exposed to styrene. Int Arch Occup Environ Health 56:239-47.

Truchon G, Gerin M, Brodeur J (1990). Urinary excretion of mandelic, phenylglyoxylic, and specific mercapturic acids in rats exposed repeatedly by inhalation to various concentrations of styrene vapors. Can J Physiol Pharmacol 68(5):556-61.

Tulinska J, Dusinska M, Jahnova E, Liskova A, Kuricova M *et al.* (2000). Changes in cellular immunity among workers occupationally exposed to styrene in a plastics lamination plant. Am J Ind Med 38(5):576-83.

Turk PW, Laayoun A, Smith SS, Weitzman SA (1995). DNA adduct 8-hydroxyl-2'-deoxyguanosine (8-hydroxyguanine) affects function of human DNA methyltransferase. Carcinogenesis 16(5):1253-5.

Tursi F, Samaia M, Salmoma M, Belvedere G (1983). Styrene oxidation to styrene oxide in human erythrocytes catalyzed by oxyhemoglobin. Experientia 39:593-4.

Tuynman A, Spelberg JL, Kooter IM, Schoemaker HE, Wever R (2000). Enantioselective epoxidation and carbon-carbon bond cleavage catalyzed by *Coprinus cinereus* peroxidase and myeloperoxidase. J Biol Chem 275(5):3025-30.

U.S. EPA (1985). Mutagenicity and carcinogenicity assessment of 1,3-butadiene. Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. EPA/600/8-85/004F.

U.S. EPA (1986). Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC. EPA/630/R-00/004. Fed Reg 51(185):33992-34003.

U.S. EPA (1988). Reference Physiological Parameters in Pharmacokinetic Modeling. Exposure Assessment Group, Final Report. Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, Washington, D.C. EPA/600/6-88/004, February.

U.S. EPA (1991). Drinking water criteria document for styrene. Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency, Cincinnati, OH. ECAO-CIN-409. January 1991.

U.S. EPA (1992). A cross-species scaling factor for carcinogen risk assessment based on equivalence of $mg/kg^{3/4}$/day. Draft Report. June 5, 1992. Fed Reg 57:24152-73. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA (1994). Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC. EPA/600/8-90/066F.

U.S. EPA (1995). Summary of State and Federal Drinking Water Standards and Guidelines, dated 9/12/95. Federal-State Toxicology and Risk Analysis Committee (FSTRAC). Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA (1998). Acrylic Acid, Styrene, α-Methyl Styrene Copolymer, Ammonium Salt; and Styrene, 2-Ethylhexyl Acrylate, Butyl Acrylate Copolymer; Exemption from the Requirements of a Tolerance. U.S. Environmental Protection Agency, Washington, DC. Fed Reg 63(188):51835-51840. September 29, 1998. Accessed at: www.epa.gov/fedrgstr/EPA-PEST/1998/September/Day-29/p26003.htm.

U.S. EPA (2000). Methodology for deriving ambient water quality criteria for the protection of human health. Office of Water, U.S. Environmental Protection Agency, Washington, DC. EPA-822-B-00-004, Oct., 2000.

U.S. EPA (2001). National Contaminant Occurrence Database (NCOD) for Styrene. U.S. Environmental Protection Agency, Washington, DC. Accessed Sept 14, 2001 at: http://oaspub.epa.gov/ncod/rpt_options_pws_pkg.national_state_selection.

U.S. EPA (2002). National Primary Drinking Water Standards. Last updated July 18, 2002. U.S. Environmental Protection Agency, Washington, DC. Accessed 7/31/2002 at: www.epa.gov/safewater.

U.S. EPA (2003). Draft Final Guidelines for Carcinogen Risk Assessment. National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC. EPA/630/P-03/001A. Accessed 1/31/2005 at: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=55445.

U.S. EPA (2005a). Guidelines for Carcinogen Risk Assessment. March, 2005. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC. Accessed at: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283. EPA/630/P-03/001F.

U.S. EPA (2005b). Supplemental Guidance for Assessing Susceptibility from Early-Life Exposures to Carcinogens. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC. EPA/630/R-03/003F, March, 2005.

U.S. EPA (2009). Styrene. Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency, Washington, DC. Accessed 10/28/09 at: http://www.epa.gov/iris/subst/0104.htm.

U.S. FDA (1999). Food additives permitted for direct addition to food for human consumption. CFR 3 (Title 21):52-9. Chap I, Part 172, Subpart F, Sec. 172.515. Revised April 1, 1999. Accessed 11/10/99 at: http://frwebgate.access.gpo.gov/cgi-bin/get-cfr.cgi..

Uusküla M, Järventaus H, Hirvonen A, Sorsa M, Norppa H (1995). Influence of GSTM1 genotype on sister chromatid exchange induction by styrene-7,8-oxide and 1,2-epoxy-3-butene in cultured human lymphocytes. Carcinogenesis 16(4):947-50.

Vaghef H, Hellman B (1998). Detection of styrene and styrene oxide-induced DNA damage in various organs of mice using the comet assay. Pharmacol Toxicol 83(2):69-74.

Vainio H, Jarvisalo J, Taskinen E (1979). Adaptive changes caused by intermittent styrene inhalation on xenobiotic biotransformation. Toxicol Appl Pharmacol 49(1):7-14.

Vainio H, Paakkonen R, Ronnholm K, Raunio V, Pelkonen O (1976). A study on the mutagenic activity of styrene and styrene oxide. Scand J Work Environ Health 2:147-51.

Van Hummelen P, Severi M, Pauwels W, Roosels D, Veulemans H, Kirsch-Volders M (1994). Cytogenetic analysis of lymphocytes from fiberglass-reinforced plastics workers occupationally exposed to styrene. Mutat Res 310(1):157-65.

van Zeeland AA, Vrieling H (1999). Mutation in mammalian cells in vitro and in vivo as indicators of exposure of carcinogens. IARC Sci Publ 146:355-65.

Verplanke AJW, Herber RFM (1998). Effects on the kidney of occupational exposure to styrene. Int Arch Occup Environ Health 71(1):47-52.

Viaene MK, Pauwels W, Veulemans H, Roels HA, Masschelein R (2001). Neurobehavioural changes and persistence of complaints in workers exposed to styrene in a polyester boat building plant: influence of exposure characteristics and microsomal epoxide hydrolase phenotype. Occup Environ Med 58(2):103-12.

Viau C, Bernard A, De Russis R, Ouled A, Maldague P, Lauwerys R (1987). Evaluation of the nephrotoxic potential of styrene in man and in rat. J Appl Toxicol 7(5):313-6.

Vodicka P, Bastlova T, Vodickova L, Peterkova K, Lambert B, Hemminki K (1995). Biomarkers of styrene exposure in lamination workers: levels of O6-guanine DNA adducts, DNA strand breaks and mutant frequencies in the hypoxanthine guanine phosphoribosyltransferase gene in T-lymphocytes. Carcinogenesis 16(7):1473-81

Vodicka P, Koskinen M, Vodickova L, Stetina R, Smerak I, Barta A *et al.* (2001a). DNA adducts, strand breaks and micronuclei in mice exposed to styrene by inhalation. Chem-Biol Interact 137:213-27.

Vodicka P, Soucek P, Tates AD, Dusinska M , Sarmanova J, Zamecnikova M *et al.* (2001b). Association between genetic polymorphisms and biomarkers in styrene-exposed workers. Mutat Res 482:89-103.

Vodicka P, Tuimala J, Stetina R, Kumar R, Manini P, Naccarati A *et al.* (2004). Cytogenetic markers, DNA single-strand breaks, urinary metabolites, and DNA repair

rates in styrene-exposed lamination workers. Environ Health Perspect 112(8):867-71.

Vodicka P, Tvrdik R, Osterman-Golkar S, Vodickova L, Peterova K, Soucek P *et al.* (1999). An evaluation of styrene genotoxicity using several biomarkers in a 3-year follow-up of hand-lamination workers. Mutat Res 445:205-24.

Vodicka P, Vodickova L, Hemminki K (1993). 32P-postlabeling of DNA adducts of styrene-exposed lamination workers. Carcinogenesis 14(10):2059-61.

Vodicka P, Vodicková L, Trejbalová K, Srám RJ, Hemminki K (1994). Persistence of O6-guanine DNA adducts in styrene-exposed lamination workers determined by 32P-postlabelling. Carcinogenesis 15(9):1949-53.

Voet D, Voet JG (1990). Biochemistry. John Wiley & Sons, New York.

Vogie KM, Mantick NA, Carlson GP (2004). Metabolism and toxicity of the styrene metabolite 4-vinylphenol in CYP2E1 knockout mice. J Toxicol Environ Health (A) 67:145-52.

Vyskocil A, Emminger S, Malir F, Fiala Z, Tusl M, Ettlerova E, Bernard A (1989). Lack of nephrotoxicity of styrene at current TLV level (50 ppm). Int Arch Occup Environ Health 61(6):409-11.

Wallace L, Buckley T, Pellizzari E, Gordon S (1996). Breath measurements as volatile organic compound biomarkers. Environ Health Perspect 104(Suppl 5):861-9.

Walles SA, Edling C, Anundi H, Johanson G (1993). Exposure dependent increase in DNA single strand breaks in leucocytes from workers exposed to low concentrations of styrene. Br J Ind Med 50(6):570-4.

Walles SA, Norppa H, Osterman-Golkar S, Mäki-Paakkanen J (1988) . Single-strand breaks in DNA of peripheral lymphocytes of styrene-exposed workers. IARC Sci Publ 89:223-6.

Ward EM, Fajen JM, Ruder A (1995). Mortality study of workers in 1,3-butadiene production units identified from a chemical workers cohort. Environ Health Perspect 103:598-603.

Ward EM, Fajen JM, Ruder A (1996). Mortality study of workers employed in 1,3-butadiene production units identified from a large chemical workers cohort. Toxicology 113:157-68.

Watabe T, Hiratsuka A, Aizawa T, Sawahata T, Ozawa N, Isobe M *et al.* (1982). Studies on metabolism and toxicity of styrene. IV. 1-Vinylbenzene 3, 4-oxide, a potent mutagen formed as a possible intermediate in the metabolism in vivo of styrene to 4-vinylphenol. Mutat Res 93(1):45-55.

Watabe T, Hiratsuka A, Sone T, Ishihama T, Endoh K (1984). Hepatic microsomal oxidation of styrene to 4-hydroxystyrene 7,8-glycol via 4-hydroxystyrene and its 7,8-oxide as short-lived intermediates. Biochem Pharmacol 33(19):3101-3.

Watabe T, Isobe M, Sawahata T, Yoshikawa K, Yamada S, Takabatake E (1978). Metabolism and mutagenicity of styrene. Scand J Work Environ Health 2:142-55.

Watabe T, Ozawa N, Yoshikawa K (1981). Stereochemistry in the oxidative metabolism

of styrene by hepatic microsomes. Biochem Pharmacol 30(12):1695-8.

Watanabe T, Endo A, Kumai M, Ikeda M (1983). Chromosome aberrations and sister chromatid exchanges in styrene-exposed workers with reference to their smoking habits. Environ Mutagen 5(3):299-309.

Watanabe T, Endo A, Sato K, Ohtsuki T, Miyasaka M, Koizumi A, Ikeda M (1981). Mutagenic potential of styrene in man. Ind Health 19(1):37-45.

Weiland SK, Mundt KA, Keil U, Kraemer B, Birk T, Person M, Bucher AM, Straif K, Schumann J, Chambless L (1996). Cancer mortality among workers in the German rubber industry: 1981-91. Occup Environ Med 53(5):289-98

Weisburger JH, Williams GM (1991). Critical effective methods to detect genotoxic carcinogens and neoplasm-promoting agents. Environ Health Perspect 90:121-6.

Welp E, Partanen T, Kogevinas M, Andersen A, Bellander T, Biocca M *et al.* (1996). Exposure to styrene and mortality from nonmalignant diseases of the genitourinary system. Scand J Work Environ Health 22(3):223-6.

Wenker MAM, Kezic S, Monster AC, deWolff FA (2000). Metabolism of styrene-7,8-oxide in human liver in vitro: Interindividual variation and stereochemistry. Toxicol Appl Pharmacol 169:52-8.

Wenker MAM, Kezic S, Monster AC, deWolff FA (2001a). Metabolism of styrene in the human liver in vitro: Interindividual variation and enantioselectivity. Xenobiotica 31:61-72.

Wenker MAM, Kezic S, Monster AC, deWolff FS (2001b). Metabolic capacity and interindividual variation in toxicokinetics of styrene in volunteers. Hum Exp Toxicol 20:221-8.

Wheeler CW, Wrighton SA, Guenthner TM (1992). Detection of human lung cytochromes P450 that are immunochemically related to cytochrome P450IIE1 and cytochrome P450IIIA. Biochem Pharmacol 44:183-6.

WHO (1983). Styrene. Environmental Health Criteria 27. World Health Organization, Geneva.

Wieczorek H (1985). Evaluation of low exposure to styrene. II. Dermal absorption of styrene vapours in humans under experimental conditions. Int Arch Occup Environ Health 57(1):71-5.

Wieczorek H, Piotrowski JK (1985). Evaluation of low exposure to styrene. I. Absorption of styrene vapours by inhalation under experimental conditions. Int Arch Occup Environ Health 57(1):57-69.

Wieczorek H, Piotrowski JK (1988). Kinetic interpretation of the exposure test for styrene. Int Arch Occup Environ Health 61(1-2):107-14.

Wigaeus E, Lof A, Bjurstrom R, Nordqvist MB (1983). Exposure to styrene. Uptake, distribution, metabolism and elimination in man. Scand J Work Environ Health 9:479-88.

Wigaeus E, Lof A, Nordqvist MB (1984). Uptake, distribution, metabolism and elimination of styrene in man: a comparison between single exposure and co-exposure

with acetone. Br J Ind Med 41(4):539-46.

Willey JC, Coy E, Brolly C, Utell MJ, Frampton MW, Jammersley J *et al.* (1996). Xenobiotic metabolism enzyme gene expression in human bronchial epithelial and alveolar macrophage cells. Am J Respir Cell Mol Biol 14:262-71.

Wilson HK, Robertson SM, Waldron HA, Gompertz D (1983). Effect of alcohol on the kinetics of mandelic acid excretion in volunteers exposed to styrene vapour. Br J Ind Med 40(1):75-80.

Withey JR, Collins PG (1979). The distribution and pharmacokinetics of styrene monomer in rats by the pulmonary route. J Environ Pathol Toxicol 2:1329-42.

Wolf MA, Rowe VK, McCollister DD *et al*. (1956). Toxicological studies of certain alkylated benzenes and benzene: Experiments on laboratory animals. AMA Arch Ind Health 14:387-98.

Wong O (1990). A cohort mortality study and case-control study of workers potentially exposed to styrene in the reinforced plastics and composites industry. Br J Ind Med 47:753-62.

Wong O, Trent LS, Whorton MD (1994). An updated cohort mortality study of workers exposed to styrene in the reinforced plastics and composites industry. Occup Environ Med 51:386-96.

Wynder EL, Fritz L, Furth N (1957). Effect of concentration of benzopyrene in skin carcinogenesis. J Natl Cancer Inst 19(3):361-70.

Yager JW, Paradisin WM, Symanski E, Rappaport SM (1990). Sister chromatid exchanges induced in peripheral lymphocytes of workers exposed to low concentrations of styrene. Prog Clin Biol Res 340C:347-56.

Yager JW, Paradisin WM, Rappaport SM (1993). Sister-chromatid exchanges in lymphocytes are increased in relation to longitudinally measured occupational exposure to low concentrations of styrene. Mutat Res 319(3):155-65.

Yano BL, Dittenber DA, Albee RR, Mattsson JL (1992). Abnormal auditory brainstem responses and cochlear pathology in rats induced by an exaggerated styrene exposure regimen. Toxicol Pathol 20(1):1-6.

Yao R, Wang Y, Lubet RA, You M (2003). Differential gene expression in chemically induced mouse lung adenomas. Neoplasia 5(1):41-52.

Zaidi NF, Agrawal AK, Srivastava SP, Seth PK (1985). Effect of gestational and neonatal styrene exposure on dopamine receptors. Neurobehav Toxicol Teratol 7(1):23-8.

Zhang H, Xu Y, Kamendulis LM, Klaunig JE (2000). Morphological transformation by 8-hydroxy-2'-deoxyguanosine in Syrian hamster embryo (SHE) cells. Toxicol Sci 56(2):303-12.

Zhang XX, Chakrabarti S, Malick AM, Richer CL (1993). Effects of different styrene metabolites on cytotoxicity, sister-chromatid exchanges and cell-cycle kinetics in human whole blood lymphocytes in vitro. Mutat Res 302(4):213-8.