FINAL

Report on Carcinogens Background Document for

Styrene

September 29, 2008

U.S. Department of Health and Human Services
Public Health Servces
National Toxicology Program
Research Triangle Park, NC 27709

This Page Intentionally Left Blank

FOREWORD

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of identified substances (i) that either are known to be human carcinogens or are reasonably be anticipated to be human carcinogens and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of Health and Human Services (HHS), has delegated responsibility for preparation of the RoC to the National Toxicology Program (NTP), which prepares the report with assistance from other Federal health and regulatory agencies and nongovernmental institutions.

Nominations for (1) listing a new substance, (2) reclassifying the listing status for a substance already listed, or (3) removing a substance already listed in the RoC are reviewed in a multi-step, scientific review process with multiple opportunities for public comment. The scientific peer-review groups evaluate and make independent recommendations for each nomination according to specific RoC listing criteria. This background document was prepared to assist in the review of styrene. The scientific information used to prepare Sections 3 through 5 of this document must come from publicly available, peer-reviewed sources. Information in Sections 1 and 2, including chemical and physical properties, analytical methods, production, use, and occurrence may come from published and/or unpublished sources. For each study cited in the background document from the peer-reviewed literature, information on funding sources (if available) and the authors' affiliations are provided in the reference section. The draft background document was peer reviewed in a public forum by an *ad hoc* expert panel of scientists from the public and private sectors with relevant expertise and knowledge selected by the NTP in accordance with the Federal Advisory Committee Act and HHS guidelines and regulations. This document has been finalized based on the peer-review recommendations of the expert panel and public comments received on the draft document. Any interpretive conclusions, comments, or statistical calculations made by the authors or peer reviewers of this document that are not contained in the original citation are identified in brackets [ ].

A detailed description of the RoC nomination review process and a list of all substances under consideration for listing in or delisting from the RoC can be obtained by accessing the 12th RoC at http://ntp.niehs.nih.gov/go/9732. The most recent RoC, the 11th Edition (2004), is available at http://ntp.niehs.nih.gov/go/19914.

CONTRIBUTORS

Project Managers, Authors, and Principal Reviewers

National Toxicology Program (NTP) and National Institute of Environmental Health Sciences (NIEHS)

Ruth Lunn, Dr.P.H.	Director, Report on Carcinogens Group
C.W. Jameson, Ph.D.	Report on Carcinogens Office (former Director; currently at CWJ Consulting, LLC)
Gloria Jahnke, D.V.M.	Health Scientist, Report on Carcinogens Group

Constella Group, LLC (Support provided through NIEHS Contract Number NO1-ES-35505)

Sanford Garner, Ph.D.	Principal Investigator
Stanley Atwood, M.S., DABT	
Greg Carter, M.E.M.	
Andrew Ewens, Ph.D.	
Dana Greenwood, B.S.	
Jennifer Ratcliffe, Ph.D.	

Consultants

Henrik Kolstad, M.D., Ph.D.	Arbejdsmedicinsk Klinic, Department of Occupational Medicine, Aarhus, Denmark
Pavel Vodicka, M.D., Ph.D.	Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Czech Republic
Joe Haseman, Ph.D.	Independent Consultant
Doug Rickert, Ph.D.	Independent Consultant

Administrative Support

Ella Darden, B.S.	Constella Group, LLC
Tracy Saunders, B.S.	Constella Group, LLC
Shawn Jeter, B.S.	Report on Carcinogens Group, NIEHS
Jenaya Brown	Report on Carcinogens Group, NIEHS

Editorial Support

Susan Dakin, Ph.D.	Independent Consultant in Technical & Scientific Writing & Editing

PEER REVIEW

The draft background document on Styrene was peer reviewed by the Report on Carcinogens (RoC) expert panel for Styrene. The panel met in a public forum at the Radisson Hotel, Research Triangle Park, NC on July 21-22, 2008. Members of the expert panel are as follows:

David Phillips, Ph.D., DSc. FRCPath
(Chair)
Institute of Cancer Research

Scot Eustis, D.V.M., Ph.D., DACVP
Independent Consultant

Peter Infante, Dr.P.H., M.PH., D.D.S.
Peter Infante Consulting, Inc.

Genevieve Matanoski, M.D., Dr.P.H.
Johns Hopkins Bloomberg School of Public Health
Department of Epidemiology

Shane S. Que Hee, Ph.D.
University of California, Los Angeles
School of Public Health, Department of Environmental Health Sciences

Thomas J. Smith, Ph.D., CIH
Harvard School of Public Health
Department of Environmental Health

Suzanne Snedeker, Ph.D.
Cornell University
College of Veterinary Medicine

Michael P. Stone, Ph.D.
Vanderbilt University
Department of Chemistry

Elizabeth M. Ward, Ph.D.
American Cancer Society
Epidemiology and Surveillance Research

Garold S. Yost, Ph.D.
University of Utah
Department of Pharmacology and Toxicology

Lauren Zeise, Ph.D.
California EPA
OEHHA Reproductive and Cancer Hazard Assessment

Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services

National Toxicology Program

The criteria for listing an agent, substance, mixture, or exposure circumstance in the RoC are as follows:

Known To Be Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans*, which indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer.

Reasonably Anticipated To Be Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans*, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded,

or

there is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset,

or

there is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to, dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub-populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals, but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

*This evidence can include traditional cancer epidemiology studies, data from clinical studies, and/or data derived from the study of tissues or cells from humans exposed to the substance in question that can be useful for evaluating whether a relevant cancer mechanism is operating in people.

This Page Intentionally Left Blank

Executive Summary

Introduction

Styrene is a viscous, highly flammable liquid used worldwide in the production of polymers, which are incorporated into products such as rubber, plastic, insulation, fiberglass, pipes, automobile parts, food containers, and carpet backing.

Styrene was nominated for possible listing in the *Report on Carcinogens* by a private individual based on its widespread use and exposure and evidence of carcinogenicity from studies in humans and experimental animals.

Human Exposure

The primary use of styrene is in the manufacture of polystyrene, which is used extensively in the manufacture of plastic packaging, thermal insulation in building construction and refrigeration equipment, and disposable cups and containers. Styrene also is used in styrene-butadiene rubber, other polymers, and resins that are used to manufacture boats, shower stalls, tires, automotive parts, and many other products. U.S. production of styrene has risen steadily over the past 70 years, with 11.4 billion pounds produced in 2006 (domestic production capacity for 2006 was estimated at 13.7 billion pounds). Styrene and styrene metabolites in blood and urine, and styrene-7,8-oxide–DNA adducts and styrene-7,8-oxide–hemoglobin adducts are generally accepted biological indices of exposure to styrene. The primary source of exposure to the general public is inhalation of indoor air; however, exposure can also occur from inhalation of outdoor air, ingestion of food and water, and potentially from skin contact. Tobacco smoke also can be a major source of styrene exposure for both active smokers and individuals exposed to environmental tobacco smoke. Outdoor and indoor air levels (including air levels in most other occupational settings) are generally below 1 ppb [0.001 ppm], although higher levels have been reported. Workers in certain occupations, including the reinforced-plastics , styrene-butadiene, and styrene monomer and polymer industries, are potentially exposed to higher levels of styrene than the general public. Air levels in the reinforced-plastics industry are generally lower than 100 ppm, [although much higher levels have frequently been measured], while levels in the styrene-butadiene industry and the styrene

monomer and polymer industries have rarely been reported to exceed 20 ppm. Numerous Federal agencies have established regulations for styrene, including the Department of Homeland Security, DOT, EPA, FDA, and OSHA, and both ACGIH and NIOSH have established guidelines to limit occupational exposure to styrene.

Human Cancer Studies

Numerous epidemiological studies have evaluated the relationship between styrene and cancer in humans. Most of the studies are cohort studies of workers in three major industries: (1) the reinforced-plastics industry, (2) the styrene-butadiene rubber industry, and (3) the styrene monomer and polymer industry. Two additional cohort studies (one on biomonitored workers, and the second on environmental exposure to styrene-butadiene), several case-control studies, and an ecological study have also been published.

The limitations of these studies include potential misclassification of styrene exposure and disease, small numbers of long-term workers, inadequate follow-up, and the potential for co-exposure to other chemicals. Thus, although more than a hundred thousand workers have been studied to assess a possible carcinogenic effect of styrene exposure, only a small fraction of well-characterized, high-level, and long-term styrene-exposed workers have been followed for a sufficiently long time. In addition, most of the available studies of occupational cohorts have focused only on male workers (who constitute the majority of exposed workers) or have not performed gender-specific risk analyses. [Thus, comparatively few data are available on cancer incidence or mortality among exposed female workers, limiting the ability to evaluate breast cancer or cancers at tissue sites specific for females.]

Workers in the reinforced-plastics industry have the highest levels of exposure and few other potentially carcinogenic exposures, but many of the workers in this industry have short-term exposure, often of less than a year. Cancer mortality or incidence was studied in the following four populations of reinforced-plastics workers: (1) in Washington state in the United States (Ruder *et al.* 2004), (2) in 30 manufacturing plants in unspecified U.S. locations (Wong *et al*. 1994), (3) in Denmark (Kolstad *et al.* 1994), and (4) in

Europe (Denmark, Finland, Italy, Norway, United Kingdom, and Sweden) (Kogevinas *et al.* 1994a). (The Danish and the European populations were partly overlapping, as 13,682 Danish male workers were included among the 36,610 male workers making up the European cohort.)

In the styrene-butadiene industry, the cohort studies are among the largest, with the longest follow-up times. The principal methodological challenge is to separate the potentially independent or synergistic effects of butadiene, a known human carcinogen, which is highly correlated with styrene in this industry. Two independent (non-overlapping populations) are available, a small cohort of 6,678 male workers at a rubber tire manufacturing plant (a subset of the workers were engaged in the production of styrene-butadiene and other rubbers) (McMichael *et al.* 1976a) and a larger cohort established by Delzell and colleagues (Delzell *et al.* 1996, 2006) of 13,130 to 16,610 styrene-butadiene rubber industry workers from multiple plants in the United States and Canada. The cohort established by Delzell includes most (but not all) of the workers from two cohorts — a 2-plant cohort (Texas) (Meinhardt *et al.* 1982) and an 8-plant cohort originally established by Matanoski and colleagues (United States and Canada) and reported in a series of previous publications (7 of the 8 plants were included in the Delzell cohort). Thus, there is considerable overlap between these populations. Two nested case-control studies (Matanoski *et al.*1997, Santos-Burgoa *et al.* 1992) of a single group of cases with lymphohematopoietic cancers were available from the Matanoski cohort. The Delzell cohort expanded the previous cohorts to include workers employed from 1943 to January 1, 1991 and followed to 1998, whereas the earlier cohort included workers employed until 1976 and followed until 1982. In addition, the individual study populations were established by different procedures and exclusion criteria (which may partly explain the lack of complete consistency in the number of study subjects across the published studies) and often used different exposure assessments, selection of study subjects, and types of analysis. Two types of analyses were conducted on the Delzell cohort: external analyses reporting on standardized mortality ratios (SMRs) for the total cohort or subsets of the cohorts for multiple cancers sites (Sathiakumar *et al.* 1998, 2005), and, secondly, internal analyses of relative risk (RR) estimates for quantitative exposure to styrene and lymphohematopoietic cancers (Delzell *et al.* 2001, 2006,

Macaluso *et al.* 2006, Graff *et al.* 2005). (Dimethyldithiocarbamate [DMDTC] was also included as a potential confounder in some analyses of lymphohematopoietic cancer in the Delzell cohort, according to the authors, because of its potential immunosuppressant activity in CD4+ lymphocytes, although its carcinogenicity has not been evaluated outside of this series of studies). Workers in the styrene monomer and polymer industry may be exposed to a variety of chemicals, including benzene, toluene, ethylbenzene, and various solvents, and the cohorts are smaller, with many short-term workers, and few cancer outcomes.

The potential effect of styrene on lymphohematopoietic cancers has been studied most extensively. Findings for lymphohematopoietic cancer and other tumor sites of interest are discussed below.

Lymphohematopoietic cancers

Statistically significant increases were observed for all lymphohematopoietic cancers combined and leukemia among rubber-tire manufacturing workers (McMichael *et al.* 1976) and statistically nonsignificant increases were observed for combined lymphohematopoietic cancers and some specific lymphohematopoietic cancers in the Meinhardt and Matanoski cohorts, but the potentially confounding effects of butadiene and other exposures were not analyzed. Two nested case-control studies (using different types of analyses and exposure assessments and the same group of cases) from the Matanoski cohort attempted to evaluate the relative contribution of styrene and butadiene to lymphohematopoietic cancer mortality. Santos-Burgoa *et al.* (1992) found no significant excess risks for combined and specific lymphohematopoietic cancers and mean exposure after controlling for butadiene exposure. Matanoski *et al.* (1997) calculated risks for both average and cumulative exposure to styrene. Taking into account butadiene exposure, and demographic and employment variables in step-down regression analyses, these models found, for an average exposure of 1 ppm vs. no exposure, significant associations for all lymphohematopoietic cancers combined, lymphomas, and myeloma, but not leukemia. For cumulative exposure, significant positive associations between styrene exposure and combined lymphohematopoietic cancers, leukemia, and

myeloma were found, with butadiene exposure dropping out of each of the final models except for leukemia.

Specific lymphohematopoietic cancers have been studied more extensively in the Delzell cohort. With respect to leukemia, statistically significant increases have been reported among subgroups of workers with longer durations of employment and longer latency, with the highest cumulative exposure, and in certain specific job groups (Sathiakumar *et al.* 2005, Delzell *et al.* 2006). Internal analyses by Delzell *et al.* involving single-chemical (styrene only), 2-chemical (styrene and butadiene), and 3-chemical (styrene, butadiene, and DMDTC) models of cumulative exposure have shown increased relative risks of leukemia with increasing cumulative styrene exposure. However, the response was attenuated when controlling for exposure to butadiene and was no longer apparent (RRs were less than or equal to one) after additionally controlling for DMDTC. Elevated risks for leukemia were also observed with increasing exposure to styrene peaks in single-chemical, 2-chemical and 3-chemical models (although it was attenuated somewhat in the 2- and 3-chemical models) (Graff *et al.* 2005, Delzell *et al.* 2006).

No statistically significant increased risks were found for other lymphohematopoietic cancers in all employees of the Delzell cohort, but statistically significant risks of NHL and CLL combined were found among workers with higher exposure in an external (SMR) analysis, and in internal analyses among ever-hourly workers, ever-hourly workers with 10+ years of employment and 20 to 29 years or 30 years since first hire, and among specific job groups. Risks of NHL or NHL and CLL combined appeared to increase with increasing cumulative styrene exposure; the risks increased when butadiene was added to the model, and were somewhat attenuated in models that included DMDTC. Exposure to butadiene did not appear to be related to NHL and CLL combined or NHL risk. [However, it should be noted that no trend analyses were performed on these data.] (Graff *et al.* 2005, Delzell *et al.* 2006). No associations were found for other types of lymphohematopoietic cancers and styrene exposure in the Delzell cohort.

In the reinforced-plastics industry, among the highest-exposure groups, the total number of observed versus expected deaths or cases across the four cohorts were comparable for

all lymphohematopoietic (52 observed vs. 52.8 expected), lymphomas (14 vs. 15.1), or leukemia (19 vs. 19.8), and were slightly higher than expected for Hodgkin's disease (11 observed vs. 7.9 expected) and multiple myeloma (4 vs. 3.4). Significantly increased risks for leukemia incidence were reported in the Danish study among workers with earlier first date of exposure, and who had worked at least 10 years since first employment, but not for workers employed for 1 year or more (Kolstad *et al.* 1994). In the European multi-country cohort (which overlaps with the Danish study), no excess of leukemia mortality was found, and no exposure-response relationships with cumulative or average exposure were observed, although a non-significant trend was observed with time since first exposure (Kogevinas *et al.* 1994a). With respect to other lymphohematopoietic cancers, non-significantly increased risks for non-Hodgkin's lymphoma were found in the Danish and European multi-country cohorts. Positive exposure-response relationships with average styrene exposure and time since first exposure was observed for lymphohematopoietic cancers ($P = 0.019$ and 0.012, respectively) and for malignant lymphoma ($P = 0.052$ and 0. 072, respectively) in the European multi-country cohort, but no relationship with cumulative exposure was observed (Kogevinas *et al.* 1994a). No excesses in mortality from any lymphohematopoietic cancers were observed in the two smaller cohort studies (Ruder *et al.* 2004 and Wong *et al*. 1994). In the styrene monomer and polymer industries, the risk of lymphohematopoietic malignancies was also increased in most of the studies (as well as the total number of observed cases across studies), but these workers might also have been exposed to benzene.

Pancreatic cancer

Among the highest styrene-exposed group in the reinforced-plastics industry, there was an excess in the total number of observed cases of pancreatic cancer across the four cohort studies compared with the total number of expected cases [corresponding to an SMR of 1.77 (95 % CI = 1.23 to 247)]. Increases in pancreatic cancer risk were observed in three of the four reinforced-plastics industry cohorts (one of which was statistically significant [Kolstad *et al.* 1995], and the other two of which were nonsignificant [Kogevinas *et al.* 1994a, Ruder *et al.* 2004]). The risk of pancreatic cancer was slightly

higher among the Danish workers with longer term employment and earlier start date, and increased with cumulative exposure in the multi-plant cohort. No indications of exposure-response relationships were found in the smaller U.S. cohorts. Statistically nonsignificant increased risks were also observed in one study in the styrene monomer and polymer industry (Frentzel-Beyme *et al.* 1978), and among biomonitored workers (10 years after the first measurement) (Anttila *et al.* 1998). However, no increased risk of pancreatic cancer was reported among styrene-butadiene workers (Sathiakumar *et al.* 2005).

Esophageal cancer

Among workers with high potential exposure to styrene, increases in esophageal cancer risk were reported in three of the four cohorts (statistically significant increases in mortality were observed among all exposed workers in the two U.S. studies of reinforced-plastics workers [Ruder *et al.* 2004, and Wong *et al.* 1994] and a statistically nonsignificant increase among a subset of laminators in the European cohort [Kogevinas *et al.* 1994a]). Risks were not elevated among the Danish reinforced-plastics workers (Kolstad *et al.* 1994). Across the industry, an approximately 2-fold excess of esophageal cancer was observed among high-exposed groups (laminators and others). A nonsignificant trend with cumulative exposure was reported in the European multi-country study. No increases in risk were reported among styrene-butadiene rubber workers or among styrene monomer and polymer workers.

Other sites

Findings were less consistent for cancer at other sites. Significantly increased risks were observed for cancers of the lung, larynx, stomach, benign neoplasms, cervix and other female tumors, prostate, rectum, and urinary system in either a single study or two studies. There were some supporting exposure-response data for cancers of the urinary system and rectum. A significant increase in breast cancer mortality was observed in a case-control study of occupational exposures among adult females (Cantor *et al.* 1995), although there was no evidence of increased risk between low- and high-exposure categories. An ecological study reported a significant increase in the risk of invasive

breast cancer in the general population, but exposure estimates were based on environmental releases of styrene, which are the least precise measures of exposure.

Studies in Experimental Animals

The carcinogenicity of styrene in rats and mice has been investigated by several routes of exposure. Other relevant studies in experimental animals include studies of mixtures (β-nitrostyrene and styrene) and studies of the major metabolite of styrene, styrene-7,8-oxide (styrene oxide).

Mice

Three strains of mice were exposed to styrene by gavage. In male $B6C3F_1$ mice, exposure to styrene for 5 days per week for 78 weeks was associated with a significantly increased incidence of alveolar/bronchiolar adenoma and carcinoma (combined) in high-dose (300 mg/kg) animals, and a significant positive dose-response trend was observed (NCI 1979a). NCI questioned the significance of these lung tumors because the incidence in the control group was unusually low compared with historical untreated controls, and only small numbers of vehicle historical controls were available from the same testing laboratory. [However, a larger number of vehicle (corn oil)-treated historical controls from this same time period (prior to 1979), with similar study duration, and from the same source as the styrene study were available from a different testing laboratory. Results from these historical vehicle controls indicated that the concurrent vehicle controls in the NCI study were not unusually low and the lung tumor incidence in the high-dose group was significantly increased compared with those historical controls.] There also was a significant dose-response trend for hepatocellular adenomas in female $B6C3F_1$ mice, but no significant pair-wise comparisons were observed. The other gavage study included a single dose of styrene administered to pregnant dams on gestation day 17 and weekly exposures of the pups after weaning (Pomomarkov and Tomatis 1978). O20 mice (a strain with a high spontaneous incidence of lung tumors) were dosed at 1,350 mg/kg and C57Bl mice were dosed at 300 mg/kg. A significantly higher incidence and earlier onset of lung tumors (adenoma and carcinoma combined) occurred in both male and female O20 mice compared with vehicle controls. Tumor incidence was not significantly increased in C57Bl mice.

Significantly increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) occurred in male CD-1 mice at inhalation exposure concentrations of 40 to 160 ppm over a period of 104 weeks and in female mice at exposure concentrations of 20, 40, and 160 ppm over a period of 98 weeks (Cruzan *et al.* 2001). Female mice in the high-dose (160-ppm) group also had increased incidences of alveolar/bronchiolar carcinoma.

No increased incidences of tumors were observed in female A/J mice (also a strain susceptible to lung tumors) treated with 20 intraperitoneal injections of styrene over 7 weeks (total dose of 200 μmol [approximately 100 mg/kg b.w.]) and evaluated 20 weeks after the last injection (Brunnemann *et al.* 1992).

Rats

Several of the studies in rats were limited because of short duration, high mortality, incomplete histopathology, or incomplete reporting. None of the carcinogenicity studies reviewed in rats showed evidence of lung tumors, and none of the gavage (NCI 1979a, Pomomarkov and Tomatis 1978, Conti *et al.* 1988), or intraperitoneal or subcutaneous injection studies (Conti *et al.* 1988) reported an increased incidence in any tumor type.

An oral gavage study in F344 rats (NCI 1979a) and an inhalation study in Sprague-Dawley rats (Cruzan *et al.* 1998) were the most robust and most completely reported carcinogenicity studies. Neither study showed an increase in tumor incidences in styrene-exposed rats, although Sprague-Dawley rats exhibited a negative trend in pituitary and mammary gland tumors and a positive trend for testicular interstitial-cell tumors. In another inhalation study in Sprague-Dawley rats, there was a dose-related increase in the incidences of malignant mammary gland tumors; treatment-related and statistically significant incidences of these tumors were seen in the top three dose groups (Conti *et al.* 1988). A drinking-water study did not report any dose-related carcinogenic effects (Beliles *et al.* 1985). However, statistical reanalyses of study data indicated a marginal increase in the incidence of mammary fibroadenoma in high-dose female rats and a significant dose-related trend. Another inhalation study (Jersey *et al.* 1978) [unpublished but reviewed in several published reports] indicated that styrene was associated with a

statistically significant increase in incidence of mammary adenocarcinoma in the low-(600-ppm) but not high-dose (1000-ppm) group and a significant increase (when compared with historical but not concurrent controls) in the combined incidence of lymphosarcoma and leukemia in female rats in both the 600-ppm and 1000-ppm dose groups. The authors did not consider the mammary adenocarcinomas to be causally associated with styrene exposure because the incidence of mammary adenocarcinoma was low compared with historical controls and there was no incidence of mammary adenocarcinoma in the high-dose group. Elevated incidences of leukemia/lymphosarcoma were observed in both treatment groups of female Sprague Dawley rats in this inhalation study.

Mixtures and Metabolite Studies

No increase in tumor incidence was observed in rats exposed by gavage (3 days per week) to a mixture of 70% styrene and 30% β-nitrostyrene over 78 weeks (NCI 1979b), but an increased incidence of lung tumors was observed in male mice in the 175 mg/kg dose group, but not in the 350 mg/kg dose group exposed to this styrene/β-nitrostyrene mixture. [However, because of poor survival of the high-dose male mice there were substantially fewer animals at risk for late-occurring tumors.]

The styrene metabolite, styrene-7,8-oxide, was previously evaluated for carcinogenicity and is listed in the Report on Carcinogens [first listed in the 10th Report on Carcinogens, 2002] as *reasonably anticipated to be a human carcinogen* based on forestomach tumors in rats and mice and liver tumors in male mice.

Absorption, Distribution, Metabolism, and Excretion

Styrene can be absorbed through inhalation, ingestion, or skin contact, but the most important route of exposure in humans in occupational settings is by inhalation, which results in rapid absorption and distribution of approximately 60% to 70% of inhaled styrene; the highest tissue concentrations are in subcutaneous fat. Food is also an important source of exposure for the general population. Metabolic activation of styrene results in formation primarily of the genotoxic metabolite styrene-7,8-oxide, which can be detoxified by glutathione conjugation or conversion to styrene glycol by microsomal

epoxide hydrolase. Styrene is metabolized in both the liver and the lung, and the Clara cells in the lung are regarded as the major cell type in styrene activation following inhalation exposure. The initial step in styrene metabolism is catalyzed by cytochromes P450; CYP2E1 and Cyp2f2 are the predominant enzymes in humans and experimental animals. In animals, CYP2E1 predominates in liver, while Cyp2f2 is the primary enzyme in mouse lung. CYP2A13, CYP2F1, CYP2S1, CYP3A5, and CYP4B1 are preferentially expressed in the lung compared with liver in humans, and the human CYP2F1 has been shown to be capable of metabolizing styrene when expressed *in vitro*. Because styrene-7,8-oxide contains a chiral carbon, this and some subsequent styrene metabolites can exist as either *R*- or *S*-enantiomers. A second metabolic pathway through styrene-3,4-oxide results in formation of 4-vinylphenol, which has been detected in humans, rats, and mice *in vivo*, but the importance of 4-vinylphenol in styrene toxicity has not been well characterized. Almost all absorbed styrene is excreted as urinary metabolites, primarily mandelic acid and phenylglyoxylic acid.

Species differences exist among rats, mice, and humans in the metabolism and toxicity of styrene, which may be related, at least in part, to interspecies differences in the stereochemistry of metabolism. The *R*-enantiomer, which has been suggested by some reports to be more toxic than the *S*-form, has been reported to be produced in relatively larger amounts in mouse lung than in rat lung, but the difference was less pronounced when microsomal preparations were used. In mice, the *R*-isomer of styrene-7,8-oxide was significantly more hepatotoxic than the *S*-isomer; the toxicity of the *R*-isomer also was greater in the lung, but the difference was not statistically significant.

Toxicity

Styrene exposure has been associated with numerous health effects in humans and laboratory animals. The acute toxicity of styrene is low to moderate with an oral LD_{50} of 320 mg/kg and an inhalation LC_{50} of 4,940 ppm (4-hour exposure) in mice and an oral LD_{50} of 5,000 mg/kg and an inhalation LC_{50} of 2,770 ppm (2-hour exposure) in rats. The primary effects of acute exposure to styrene in experimental animals and humans include irritation of the skin, eyes, and respiratory tract and CNS effects. Drowsiness, listlessness,

muscular weakness, and unsteadiness are common signs of systemic styrene intoxication. Several studies have reported effects on color vision, hearing threshold, reaction time, and postural stability following long-term occupational exposure to styrene at concentrations ranging from about 20 to 30 ppm. Reports of ischemic heart disease and hepatic, renal, hematological, and immunological effects have been inconsistent. Human data are insufficient to determine whether styrene is a reproductive or developmental toxicant, but effects of styrene to increase serum prolactin levels in humans have been reported.

Styrene toxicity in experimental animals is similar to that reported in humans. Exposure to styrene vapors can cause eye and respiratory tract irritation, CNS depression, and death. Clara cells are the main target of styrene pneumotoxicity, and the available data indicate increased susceptibility in the mouse. Glutathione depletion as a result of styrene exposures has been reported to be associated with damage to lung, liver, and kidney tissues. The cytotoxicity of styrene in the mouse lung, a tissue high in CYP2F isoforms, could be prevented by CYP2F inhibitors. Some studies have reported reproductive and developmental effects, but these effects were seen mostly at doses associated with maternal toxicity. Reported effects have included embryonic, fetal, and neonatal death, skeletal and kidney abnormalities, decreased birth weight, neurobehavioral abnormalities, and postnatal developmental delays. The possibility of polystyrene dimer and trimer extracts from food containers mimicking the physiological effects of estrogen have also been investigated, but with a mixture of positive and negative results.

Genetic Damage

In vitro studies show that styrene-7,8-oxide forms DNA adducts and causes single-strand breaks in a dose-related manner. Several studies have shown a correlation between single-strand breaks and DNA adducts and indicate that the strand breaks, which are not generally regarded as significantly lethal or mutagenic lesions, are efficiently repaired within several hours after exposure has stopped. Adducts are formed primarily at the N7-, N^2-, and O^6-positions of guanine. N7-adducts are formed in the greatest amount but are the least persistent, while O^6-adducts are formed in the least amount but are the most persistent. Styrene-7,8-oxide was mutagenic without metabolic activation in all *in vitro*

mutagenicity test systems reported and caused mutations in some studies in the presence of metabolizing enzymes. Both styrene and styrene-7,8-oxide caused cytogenetic effects (sister chromatid exchange [SCE], chromosomal aberrations, and micronuclei) in human lymphocytes or other mammalian cells *in vitro*. DNA adducts have been detected in liver and lung cells of mice and rats exposed to styrene *in vivo*, although the levels varied across studies. The majority of studies in experimental animals demonstrated an effect of both styrene-7,8-oxide and styrene exposure on single-strand breaks, while both positive and negative results for cytogenetic or clastogenic effects of styrene were reported.

DNA adducts, primarily N7- and O^6-adducts, were reported in white blood cells in all studies of styrene-exposed workers employed mainly in hand-lamination plants. In most studies in workers, single-strand breaks showed exposure-related increases; however, two studies gave negative results. The limited data on mutation frequencies in *HPRT* and *GPA* in styrene-exposed workers are inconclusive. More than half the studies measuring chromosomal aberrations have reported an increase in chromosomal aberrations in styrene-exposed workers (or subgroups of workers), and several studies have reported a positive exposure-response relationship with styrene air levels or urinary metabolites. A meta-analysis of 22 studies found a positive association between styrene exposure level and chromosomal aberration frequency when exposure levels were dichotomized as greater than or less than a threshold value of 30 ppm for an 8-hour time-weighted average. Studies of other cytogenetic markers in humans are conflicting. About half of the studies that evaluated micronucleus and SCE frequency in styrene workers were positive, and a few studies have reported significant dose-response relationships with styrene exposure. A meta-analysis of 10 micronucleus studies was inconclusive, and a meta-analysis of 14 SCE studies indicated a slight increase in SCE frequency but, again, was too small to be conclusive. A number of studies have been published on the possible modulating role of genetic polymorphisms, mainly in xenobiotic metabolism enzymes and DNA-repair genes, at the level of various biomarkers. Some authors have suggested that genetic susceptibility (probably at many loci) may be important in styrene-mediated genotoxicity.

Mechanistic Data

The proposed mechanisms for the carcinogenicity of styrene include both genotoxic and epigenetic pathways. These mechanisms, which are not necessarily mutually exclusive, include: (1) metabolic conversion of styrene to styrene-7,8-oxide and subsequent induction of DNA damage in the target tissue and (2) cytotoxic effects of styrene metabolites in the mouse lung. A variety of DNA adducts (including some at base-pairing sites on nucleotides) induced by styrene and styrene-7,8-oxide has been identified in human cells, experimental animals, and occupationally exposed workers, but the covalent binding indices for both molecules are relatively low in rats and mice. The DNA damage induced by styrene exposure, including single-strand breaks, was found to correlate significantly with markers of styrene exposure in some studies of styrene workers. Styrene is mutagenic through the formation of styrene-7,8-oxide (*in vitro*). A number of studies reported a positive association between occupational exposure to styrene and the frequency of chromosomal aberrations, with some studies reporting exposure-response relationships. Some authors have suggested that polymorphisms in DNA-repair genes could put some individuals at higher risk for styrene genotoxicity or carcinogenicity.

Many researchers have tried to explain why lung tumors were observed in mice but not in rats in long-term inhalation exposure studies. Some researchers have proposed that styrene exposure causes pulmonary hyperplasia in the mouse lung, which may play a role in the development of lung tumors. Effects of repeated styrene exposure observed in the lungs of mice, but not in rats, included focal crowding of bronchiolar cells, bronchiolar epithelial hyperplasia, and bronchiolo-alveolar hyperplasia. The Harvard Center for Risk Analysis (Cohen *et al.* 2002) considered three factors as possible explanations for the greater susceptibility of mouse lung than rat lung to development of hyperplasia leading to tumors with exposure to styrene are: (1) the presence of the styrene-metabolizing cytochromes in mouse lung tissues, (2) greater formation of the *R*-enantiomer of styrene-7,8-oxide, and (3) the susceptibility of mouse lung tissue to glutathione depletion. However, they concluded that although toxicokinetic models generally predict higher rates of metabolism by mice and rats than by humans, the models do not consistently predict a difference between the rodent species. An alternative mechanism is that

interspecies differences in styrene toxicity are most likely explained through CYP2F-generated metabolites (2f2 in mice, 2F4 in rats, and 2F1 in humans) in the mouse lung. This is based on data showing that most of the effects of cytotoxicity and tumor formation were seen in mouse respiratory tissues, which are high in CYP2F isoforms, and that CYP2F inhibitors prevented cytotoxicity. Moreover, metabolites formed from ring oxidation, including 4-vinylphenol, are about 6-fold higher in mice compared with rats, and 4-vinylphenol is more potent than styrene-7,8-oxide as a pneumotoxicant.

This Page Intentionally Left Blank

Abbreviations

ABS:	acrylonitrile-butadiene-styrene
ACGIH:	American Conference of Governmental Industrial Hygienists
ADH:	alcohol dehydrogenase
ALDH:	aldehyde dehydrogenase
AlO:	aldehyde oxidase
ALL:	acute lymphocytic leukemia
AML:	acute myelogenous leukemia
ANOVA:	analysis of variance
ASPEN:	Assessment System for Population Exposure Nationwide
ATSDR:	Agency for Toxic Substances and Disease Registry
BCF:	bioconcentration factor
BEAM:	Boston Exposure Assessment in Microenvironments
BEI:	biological exposure indices
BLS:	Bureau of Labor Statistics
BRCA1:	breast cancer 1, early onset gene
b.w.:	body weight
C:	control
C+:	centromere positive
C–:	centromer negative
CA:	chromosomal aberrations
Cal/OSHA:	California Division of Occupational Safety and Health
CBI:	covalent binding index
CC1b:	Clara-cell specific protein
CDC:	Centers for Disease Control and Prevention

CEH: *Chemical Economics Handbook*

CERHR: Center for Evaluation of Risks to Human Reproduction

CHO: Chinese hamster ovary

CLL: chronic lymphocytic leukemia

cm: centimeter

CML: chronic myeloid leukemia

CNS: central nervous system

CO: cyclohexene oxide

CPBI: cytokinesis proliferation block index

CR: creatinine

CREST: calcinosis-Raynaud's phenomenon-oesophageal dismobility-sclerodactyly-telangiectasis syndrome of scleroderma

CYP: cytochrome P450

Cyt-B: cytochalasin B

d: day

Da: Dalton

DAPI: 4',6-diamidino-2-phenylindol·2HCl

DC: decarboxylase

dm: decimeter

DMDTC: dimethyldithiocarbamate

DMSO: dimethylsulfoxide

DNA: deoxyribonucleic acid

DOT: Department of Transportation

E: exposed

EPA: Environmental Protection Agency

EPHX: epoxide hydrolase

ETS: environmental tobacco smoke

E.U.: European Union

F: female

FDA: Food and Drug Administration

FISH: fluorescence *in-situ* hybridization

g: gram

GGT: gamma-glutamyl transpeptidase

GI: gastrointestinal

GPA: glycophorin A

GSH: glutathione

GSTM1: glutathione S transferase M1

GSTT1: glutathione S transferase T1

γ-GT: gammaglutamyl transpeptidase

h: hour

HA: hydroxylapatite

HazDat: Hazardous Substances Release and Health Effects Database

HE: human erythrocytes

HEL: human embryonic lung

HFC: high-frequency cells

HIC: highest ineffective concentration

HID: highest ineffective dose

HPRT: hypoxanthine phosphoribosyltransferase

HSDB: Hazardous Substances Data Bank

Hz: Hertz

IARC: International Agency for Research on Cancer

ICD: International Classification of Diseases

i.p.: intraperitoneal

IRR: incidence rate ratio

JEM: job-exposure matrix

K+: kinetochore-positive

kg: kilogram

K_{oc}: soil organic carbon-water partitioning coefficient

K_{ow}: octanol-water partition coefficient

L: liter

LC: liquid chromatography

LD_{50}: lethal dose for 50% of the population

LEC: lowest effective concentration

LED: lowest effective dose

LH: lymphohematopoietic

LHC: lymphohematopoietic cancer

LWAE: lifetime weighted average exposure

M: male

m^3: cubic meter

MA: mandelic acid

mEH: microsomal epoxide hydrolase

mfg.: manufacturing

mg: milligram

mL: milliliter

MM: multiple myeloma

MN: micronuclei

MNBC: binucleated lymphocytes

MNMC: mononucleated lymphocytes

mol wt: molecular weight

MS: mass spectrometry

N: sample size

NA: not available

NA-AAF: *N*-acetoxy-2-acetylaminofluorene

NAcT: *N*-acetyltransferase

NADPH: nicotinamide adenine dinucleotide phosphate, reduced form

NAP: not applicable

NCEs: micronucleated normochromatic erythrocytes

NCHS: National Center for Health Statistics

NCI: National Cancer Institute

ND: not detected

NDMA: *N*-nitrosodimethylamine

NDT: not determined

NHANES: National Health and Nutrition Examination Survey

NHL: non-Hodgkin's lymphoma

NI: not identified

NIEHS: National Institute of Environmental Health Sciences

NIOSH: National Institute for Occupational Safety and Health

ng: nanogram

NLM: National Library of Medicine

NNK: 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone

No.: number

NQ: not quantified

NR: not reported

NRC: National Response Center

NS: not significant

NT: not tested

NTP: National Toxicology Program

OH: hydroxyl

OR: odds ratio

OSHA: Occupational Safety and Health Administration

PAH: polycyclic aromatic hydrocarbon

PAMA: phenacylmercapturic acid

PBL: peripheral blood lymphocytes

PBPK: physiologically based pharmacokinetic model

PC: personal computer

PCEs: micronucleated polychromatic erythrocytes

PEL: permissible exposure limit

PGA: phenylglyoxylic acid

PHA: phytohemagglutinin

PHEMA: phenylhydroxyethyl mercapturic acids

PWN: pokeweed

ppb: parts per billion

ppbv: parts per billion by volume

ppm: parts per million

r: correlation coefficient

REL: recommended exposure limit

RoC: Report on Carcinogens

RR: relative risk

RTECS: Registry of Toxic Effects of Chemical Substances

RV: recreational vehicle

s.c.: subcutaneous

S_B: styrene in blood

SBR: styrene-butadiene rubber

SCE: sister chromatid exchange

SD: standard deviation

SDH: sorbitol dehydrogenase

SE: standard error of the mean

SIR: standardized incidence ratio

SIRC: Styrene Information and Research Center

SO: styrene oxide

SOC: Standard Occupational Classification

SOCMI: Synthetic Organic Chemical Manufacturing Industry

SSB: single-strand breaks

STEL: short-term exposure limit

TDS: Total Diet Study

TK: thymidine kinase

TLV: threshold-limit value

TRI: Toxics Release Inventory

TWA: time-weighted average

U_B: styrene in urine

UDS: unscheduled DNA synthesis

USITC:	United States International Trade Commission
µg:	microgram
VOC:	volatile organic chemical
VPT:	vinylphenol
WHO:	World Health Organization
XO:	xanthine oxidase
XPC:	xeroderma pigmentosum, complementation group C
XPD:	xeroderma pigmentosum, complementation group D
XPG:	xeroderma pigmentosum, complementation group G
XRCC:	X-ray repair cross-complementing group
yr:	year

Table of Contents

1 Introduction 1
1.1 Chemical identification 1
1.2 Physical-chemical properties 2
1.3 Metabolites 3
1.4 Analogues 7
2 Human Exposure 9
2.1 Use 9
2.2 Production 11
2.3 Environmental release, fate, and occurrence 14
2.3.1 Air 16
2.3.2 Water 27
2.3.3 Soil 30
2.3.4 Food 32
2.4 General population exposure 38
2.5 Occupational exposure 44
2.5.1 The reinforced plastics industry 45
2.5.2 The styrene-butadiene rubber (SBR) industry 61
2.5.3 Styrene-butadiene rubber production exposure levels 65
2.5.4 The styrene monomer and polymer industry 68
2.5.5 Other occupational exposures 72
2.6 Biological indices of exposure 74
2.7 Regulations and guidelines 81
2.7.1 Regulations 81
2.7.2 Guidelines 82
2.8 Summary 83
3 Human Cancer Studies 85
3.1 The reinforced-plastics industry 87
3.1.1 Washington state 87
3.1.2 United Kingdom 90
3.1.3 United States 91
3.1.4 Denmark 93
3.1.5 Denmark, Finland, Norway, Italy, Sweden, and the United Kingdom. 96
3.2 The styrene-butadiene rubber industry 108
3.2.1 United States: McMichael *et al.* 109
3.2.2 United States: Meinhardt *et al.* 109
3.2.3 United States and Canada: Matanoski, Santos-Burgoa, and coworkers 110
3.2.4 United States and Canada: Delzell, Sathiakumar, Macaluso, Graff 113

3.3 The styrene monomer and polymer industry ... 136
3.3.1 Germany ... 136
3.3.2 United States- multi-plant ... 137
3.3.3 United States- single plant ... 138
3.3.4 United Kingdom ... 139
3.4 Other cohort studies ... 142
3.4.1 Styrene-exposed workers (biomarker study) ... 142
3.4.2 Environmental exposure ... 142
3.5 Case-control and ecological studies ... 146
3.5.1 Lymphohematopoietic cancers ... 146
3.5.2 Breast cancer ... 147
3.5.3 Series of studies in a Canadian population ... 149
3.5.4 Lung cancer and styrene exposure ... 150
3.6 [Strengths and limitations of the literature] ... 156
3.6.1 Utility of the studies ... 156
3.6.2 Misclassification of disease and exposure ... 158
3.6.3 Other possible biases and confounding ... 161
3.7 Summary of previous evaluations (IARC and Cohen *et al.*) ... 164
3.8 Summary of the findings for selected cancer sites ... 165
3.8.1 Esophageal cancer ... 173
3.8.2 Pancreatic cancer ... 174
3.8.3 Laryngeal cancer ... 175
3.8.4 Lung cancer ... 175
3.8.5 Lymphohematopoietic cancers ... 176
3.8.6 Other sites ... 183
3.9 Summary ... 188
4 Studies of Cancer in Experimental Animals ... 195
4.1 Mice ... 195
4.1.1 Oral ... 196
4.1.2 Inhalation ... 200
4.1.3 Intraperitoneal injection ... 202
4.2 Rats ... 202
4.2.1 Oral ... 202
4.2.2 Inhalation ... 207
4.2.3 Parenteral administration ... 211
4.3 Mixtures containing styrene ... 212
4.4 Styrene metabolites ... 213
4.5 Summary ... 214
5 Other Relevant Data ... 221
5.1 Absorption, distribution, metabolism, and excretion ... 221

5.1.1 Absorption ... 221
5.1.2 Distribution ... 223
5.1.3 Metabolism ... 225
5.1.4 Excretion ... 238
5.2 Toxicity ... 240
5.2.1 Humans ... 240
5.2.2 Experimental animals ... 246
5.2.3 Estrogenicity studies ... 256
5.3 Interspecies differences in metabolism, toxicity, and toxicokinetics ... 258
5.3.1 Styrene-7,8-oxide formation in the lung ... 258
5.3.2 Detoxification of styrene-7,8-oxide in respiratory tissue ... 259
5.3.3 Stereochemistry considerations ... 260
5.3.4 Kinetics of styrene metabolism and toxicokinetic models ... 262
5.4 Genetic and related effects ... 264
5.4.1 DNA adduct formation ... 264
5.4.2 *In vitro* studies ... 268
5.4.3 *In vivo* studies in experimental animals ... 278
5.4.4 Studies in styrene-exposed workers ... 289
5.4.5 Genetic polymorphisms and susceptibility to styrene-mediated genotoxicity ... 360
5.4.6 Summary of styrene and styrene-7,8-oxide genotoxicity ... 366
5.5 Mechanistic studies and considerations ... 368
5.5.1 Genotoxicity ... 370
5.5.2 Gene expression and apoptosis ... 373
5.5.3 Oxidative stress ... 374
5.5.4 Cytotoxic effects of styrene on mouse lung ... 375
5.5.5 Selected styrene analogues ... 377
5.6 Summary ... 380
5.6.1 Absorption, distribution, metabolism, and excretion ... 380
5.6.2 Toxicity ... 380
5.6.3 Interspecies differences in metabolism, toxicity, and toxicokinetics ... 381
5.6.4 Genetic and related effects ... 382
5.6.5 Mechanistic studies and considerations ... 383
6 References ... 385
Glossary of Terms ... 455

List of Tables

Table 1-1. Chemical identification of styrene ... 2
Table 1-2. Physical and chemical properties of styrene ... 3

Table 1-3. Urinary metabolites of styrene in rodents and humans 5
Table 1-4. Styrene analogues 7
Table 2-1. Styrene use in industrial resin 10
Table 2-2. Residual styrene-monomer levels in polymer and copolymer materials in 1980 18
Table 2-3. Historical levels of residual styrene (mg/kg) in polymer and copolymer: 1976–1980 19
Table 2-4. Concentrations of styrene in outdoor air in the United States 22
Table 2-5. U.S. levels of styrene measured in indoor air and by personal monitoring 26
Table 2-6. Levels of styrene measured in U.S. waters 29
Table 2-7. Measurements of styrene in foods packaged in polystyrene 34
Table 2-8. Food levels of styrene [source of styrene unknown] 36
Table 2-9. Summary of styrene levels in FDA's Total Diet Study (1991–2003[a]) 38
Table 2-10. Daily styrene intakes for the general public from various sources 39
Table 2-11. Estimated daily intake of styrene from various media for Canadians of different ages 40
Table 2-12. Estimated annual and lifetime exposures for the general public 42
Table 2-13. Summary of measured styrene exposure levels in the reinforced plastics industry. 55
Table 2-14. Summary of occupational styrene exposure levels in the styrene-butadiene rubber industry 67
Table 2-15. Summary of occupational styrene exposure levels in the styrene monomer and polymer industry in the United States 71
Table 3-1. Epidemiologic studies of cancer risk following styrene exposure in the reinforced-plastics industry, 1985–2004 (results of the most recent follow-up[a]) 99
Table 3-2. Risk of leukemia with cumulative and peak exposure[a] to styrene, butadiene, and DMDTC[b] 123
Table 3-3. Cumulative exposure to styrene, butdadiene and DMDTC and risk of NHL and NHL+CLL. 126
Table 3-4. Epidemiologic studies of cancer risk following styrene exposure in the styrene-butadiene rubber industry, 1976–2005 127
Table 3-5. Cohort studies of cancer risk following styrene exposure in the styrene monomer and polymer industry, 1978–1992 140
Table 3-6. Other cohort studies evaluating cancer risk and exposure to styrene 144
Table 3-7. Case-control and ecological studies evaluating cancer risk and exposure to styrene 151
Table 3-8. Relative occurrence of cancer in 12 cohort studies of populations exposed to styrene (total study populations) 168
Table 3-9. Mortality or incidence of selected cancers among all workers in the reinforced-plastics industry 186

Table 3-10. Mortality or incidence of selected cancers among workers in high-styrene–exposure groups (laminators and others)* in the reinforced-plastics industry 187
Table 4-1. Tumor incidences in B6C3F$_1$ mice exposed to styrene by gavage for 78 weeks and surviving for at least 52 weeks 198
Table 4-2a. Lung tumor incidences in O20 mice exposed to styrene *in utero* and weekly by gavage for 16 weeks after weaning 199
Table 4-2b. Tumor incidences in C57Bl mice exposed to styrene *in utero* and weekly by gavage for 120 weeks after weaning 199
Table 4-3. Lung tumor incidence in CD-1 mice exposed to styrene by inhalation for 98 or 104 weeks[b] 201
Table 4-4. Mammary gland tumor incidence in Sprague-Dawley rats exposed to styrene in drinking water for 104 weeks 205
Table 4-5. Summary of carcinogenicity studies in rats exposed to styrene by oral administration 206
Table 4-6. Incidence of mammary tumors in Sprague-Dawley rats exposed to styrene by inhalation for 52 weeks 208
Table 4-7. Mammary tumors and leukemia or lymphosarcoma in Sprague-Dawley rats exposed to styrene by inhalation for 18 to ~21 months 210
Table 4-8. Tumor incidences in Sprague-Dawley rats exposed to styrene by inhalation for 104 weeks 211
Table 4-9. Tumor incidences in B6C3F$_1$ mice exposed to a mixture of β-nitrostyrene and styrene for 79 weeks 213
Table 4-10. Summary of neoplastic lesions in mice and rats exposed to styrene-7,8-oxide by gavage 214
Table 4-11. Summary of studies in mice 217
Table 4-12. Summary of studies in rats[a] 218
Table 5-1. Production of *R*- and *S*-enantiomers of styrene-7,8-oxide by cell preparations enriched in either Clara cells or type II cells from rat and mouse lungs[a] 229
Table 5-2. Styrene-7,8-oxide DNA adducts formed in mammalian cells *in vitro* 269
Table 5-3. DNA damage in mammalian cells exposed to styrene-7,8-oxide 272
Table 5-4. Mutagenicity of styrene and styrene-7,8-oxide *in vitro* 274
Table 5-5. Cytogenetic effects of styrene *in vitro* 277
Table 5-6. Cytogenetic effects of styrene-7,8-oxide *in vitro,* without metabolic activation 279
Table 5-7. Formation of styrene-7,8-oxide DNA adducts in animals exposed to styrene 282
Table 5-8. DNA damage in experimental animals exposed to styrene or styrene-7,8-oxide 286
Table 5-9. Cytogenetic effects of styrene and styrene-7,8-oxide in experimental animals 289
Table 5-10. Studies of DNA adducts in white blood cells of workers occupationally exposed to styrene in Bohemia, the United States, and Germany 293

Table 5-11. DNA damage (single-strand breaks or alkali-labile sites) in workers occupationally exposed to styrene 299
Table 5-12. Mutation frequencies in workers exposed to styrene 306
Table 5-13. Chromosomal aberrations in lymphocytes from workers occupationally exposed to styrene 309
Table 5-14. Micronuclei in lymphocytes from workers occupationally exposed to styrene 332
Table 5-15. Sister chromatid exchange in lymphocytes from workers occupationally exposed to styrene 347
Table 5-16. Genotype analyses in *in vitro* studies with styrene and styrene-7,8-oxide 362
Table 5-17. Genotype analyses *in vivo* in workers occupationally exposed to styrene in association with biomarkers of genotoxicity 365
Table 5-18. Genetic and related effects of styrene 367

List of Figures

Figure 1-1. Chemical structures of styrene and polystyrene 1
Figure 1-2. Stereoisomers of styrene-7,8-oxide (epoxyethylbenzene) 4
Figure 2-1. Synthesis of styrene from ethylbenzene and polymerization of styrene to form polystyrene 12
Figure 2-2. U.S. styrene production (1960–2006) 13
Figure 2-3. U.S. imports and exports for styrene 14
Figure 2-4. Temporal decline in styrene exposure scores (short-term samples [< 1 h]) estimated for reinforced plastics workers 48
Figure 2-5. Temporal decline in styrene exposure scores short-term samples [< 1 h]) estimated for reinforced plastics workers 49
Figure 2-6. Typical continuous emulsion styrene-butadiene rubber polymerization process 63
Figure 2-7. Typical emulsion styrene-butadiene rubber finishing process 64
Figure 2-8. Solution styrene-butadiene rubber manufacture by continuous process 65
Figure 2-9. Polymerization of polystyrene by the continuous process 69
Figure 5-1. Styrene metabolism in humans 226
Figure 5-2. Pneumotoxicity and hepatotoxicity of styrene-7,8-oxide enantiomers in male non-Swiss albino mice at 24 hours after i.p. administration 254
Figure 5-3. Styrene-7,8-oxide binding sites in DNA (from Vodicka *et al.* 2002a) 266

1 Introduction

Styrene is a very important monomer used worldwide in the production of polymers, which are incorporated into products such as rubber, plastic, insulation, fiberglass, pipes, automobile parts, food containers, and carpet backing. Most of these products contain both free styrene monomer and styrene polymerized in long chains (polystyrene or mixed polymers) (ATSDR 1992).

Styrene was nominated for possible listing in the *Report on Carcinogens* by a private individual based on its widespread use and exposure and evidence of carcinogenicity from studies in humans and experimental animals. The International Agency for Research on Cancer (IARC) currently classifies styrene as possibly carcinogenic to humans (Group 2B) based on limited evidence in humans and limited evidence in experimental animals (IARC 1994a, 2002). Styrene-7,8-oxide, a major metabolite of styrene, has been classified by the *Report on Carcinogens* as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals (NTP 2004). IARC (1994b) also classifies styrene-7,8-oxide as probably carcinogenic to humans (Group 2A) based on sufficient evidence in experimental animals (forestomach tumors in rats and mice and liver tumors in male mice) and mechanistic data.

1.1 Chemical identification

Styrene is an aromatic hydrocarbon with the structure illustrated in Figure 1-1. Styrene can polymerize to form polystyrene (Figure 1-1). Table 1-1 contains chemical identification information for styrene.

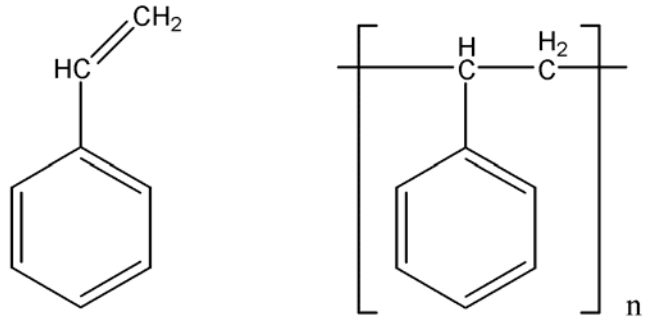


Figure 1-1. Chemical structures of styrene and polystyrene

Table 1-1. Chemical identification of styrene

Characteristic	Styrene	Styrene-7,8-oxide
Chemical Abstracts index name	styrene	phenyloxirane
CAS Registry number	100-42-5	96-09-3
Molecular formula	C_8H_8	C_8H_8O
Synonyms	cinnamene, cinnamol, ethenylbenzene, phenylethylene, styrol, styrolene, vinylbenzene, vinylbenzol, phenylethene, styrole, NSC 62785, cinnamenol, stirolo, styreen, styren, styrene monomer, styron, styropol, styropor, vinylbenzen, FEMA Number 3234, NCI-CO2200, UN 2055, IMO 3.3, Standard Transportation No. 49072 65 1.	epoxyethylbenzene; 1,2-epoxyethylbenzene; 1,2-epoxy-1-phenylethane; 1-phenyl-1,2-epoxyethane; epoxystyrene; NCI-C54977; NSC 637

Source: ChemIDPlus 2008a, 2008b, HSDB 2008a, 2008b, IARC 1994b.

1.2 Physical-chemical properties

Styrene is a colorless or yellowish, viscous liquid with a sweet, floral odor (HSDB 2008a). It has a flash point of 34°C (closed cup), lower explosive limit of 0.9% to 1.1% (v/v), upper explosive limit of 6.1% to 6.8% (v/v), and an autoignition temperature of 490°C. Styrene is highly flammable and easily ignited by heat, sparks, or flames and its vapors may form explosive mixtures with air due to the formation of peroxides. Styrene may polymerize when contaminated by oxidizing agents, halides, or when heated, and it emits acrid fumes upon decomposition (NSC 2004, SPA 2008). Usually styrene is stabilized for safe storage, transport, and use by an inhibitor, commonly *p*-tert-butylcatechol (HSDB 2008a). Typical impurities are ethylbenzene (85 ppm maximum), polymer content (10 ppm maximum), *p*-tert-butylcatechol (10 to 15 ppm or 45 to 55 ppm), aldehydes (as benzaldehyde) (200 ppm), peroxides (as H_2O_2) (0.0015% by weight or 100 ppm maximum), benzene (1 ppm maximum), sulfur (25 ppm maximum), and chlorides (as chlorine) (50 ppm maximum). The physical and chemical properties of styrene are summarized in Table 1-2.

Polystyrene is a colorless solid with a melting point of 240°C and a relative density of 1.04 to 1.13 (NIOSH 2008). It is insoluble in water and has a flash point of 345°C to 360°C. When burned or heated above 300°C, polystyrene decomposes and releases toxic fumes, including styrene.

Table 1-2. Physical and chemical properties of styrene

Property	Information
Molecular weight	104.2
Melting point (°C)	–31
Boiling point (°C)	145
Specific gravity	0.906 at 20°C
Solubility water at 25°C acetone, alcohol, carbon tetrachloride, carbon disulfide, diethyl ether, ethanol, methanol, *n*-heptane, toludene benzene, petroleum ether	 310 mg/L at 25°C soluble very soluble
Octanol-water partition coefficient (log K_{ow})	2.95
Dissociation constant (pK_a)	NA
Vapor pressure (mm Hg)	6.4 at 25°C
Vapor density	3.6 (air = 1)
Critical temperature (°C)	363.7
Henry's law constant	0.00275 atm-m^3/mol at 25°C
Hydroxyl radical reaction rate constant	5.8 x 10^{-11} cm^3/molecule-sec at 25°C

Sources: HSDB 2008a, IARC 1994a.

1.3 Metabolites

This section provides a brief overview of styrene metabolism in mammals and identifies the major and minor metabolites detected in humans and rodents exposed to styrene. Section 5 provides a more detailed discussion of styrene metabolism.

Metabolism of styrene to styrene-7,8-oxide by cytochromes CYP2E1, CYP2B6, and CYP2A13 has been reported to be the primary metabolic pathway in humans (Fukami *et al.* 2008, Manini *et al.* 2002b, Manini *et al.* 2002a); however, as discussed in Section 5.1.3, other cytochromes, including CYP2F1, have been shown to be able to convert styrene to styrene glycol when expressed *in vitro*. In addition, another toxic metabolite of styrene, 4-vinylphenol, has been detected in small amounts in rats and humans and is postulated to result from a ring-oxidation reaction forming styrene-3,4-oxide as an intermediate.

The primary metabolite, styrene-7,8-oxide, is an epoxide that exists in two enantiomeric forms (stereoisomers, or chemical compounds with asymmetric centers whose molecules

are nonsuperimposable mirror images): the *R*- and *S*-isomers (Figure 1-2). Epoxides are oxygen-containing heterocyclic compounds that are highly reactive because of the strain associated with the three-membered ring structure (Melnick 2002). The oxide forms are further metabolized to styrene glycol (phenylethylene glycol) by microsomal epoxide hydrolase. Styrene glycol is then oxidized by alcohol and aldehyde dehydrogenases to form mandelic acid and phenylglyoxylic acid and their conjugates, the main urinary metabolites. Known and hypothesized urinary styrene metabolites are shown in Table 1-3.

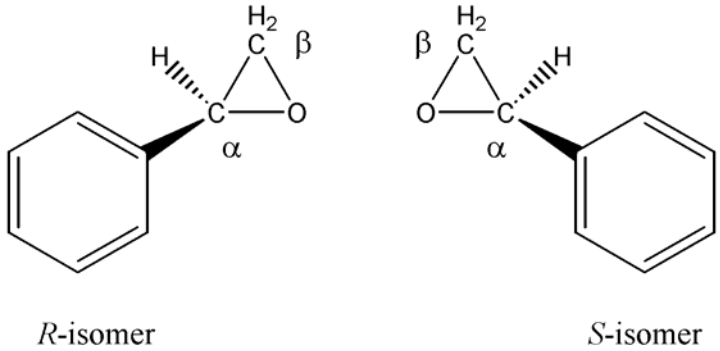
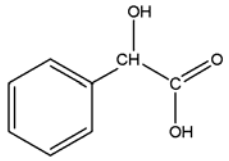
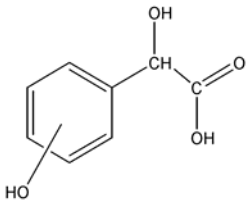
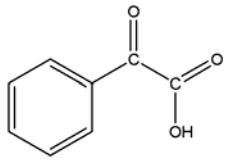
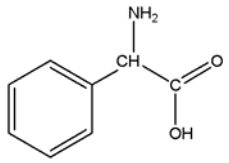
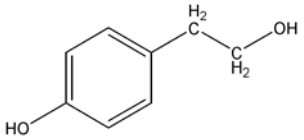
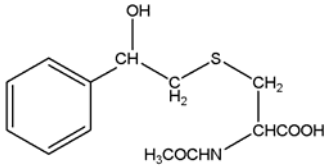
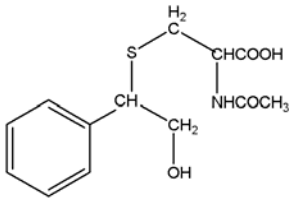
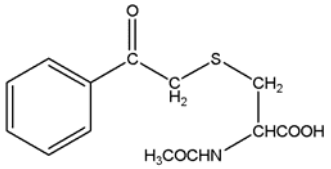
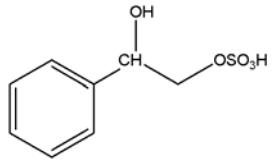
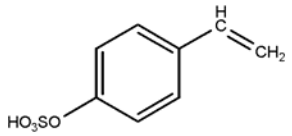
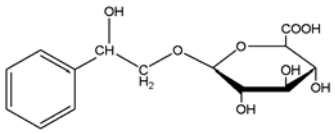
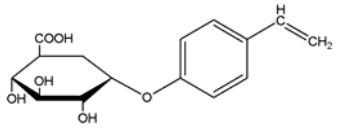


Figure 1-2. Stereoisomers of styrene-7,8-oxide (epoxyethylbenzene)

The stereoisomers of styrene-7,8-oxide are designated as *R*- or *S*- based on ordering the molecule with the lowest priority atom (hydrogen) away from the viewer as designated by the dashed line. The order of the remaining substituents from highest to lowest (i.e., oxygen, carbon in the benzene ring, carbon in CH_2) is oriented either clockwise (*R*-isomer) or counterclockwise (*S*-isomer). Also, the two carbons attached to the benzene ring are identified as the α- and β-carbons based on their order of attachment to the ring.

Table 1-3. Urinary metabolites of styrene in rodents and humans

Metabolite	Molecular weight	Structure
mandelic acid	152	
hydroxymandelic acid	168	
phenylglyoxylic acid	150	
phenylglycine[a]	151	
2-(4-hydroxy-phenyl)ethanol	138	
phenylhydroxyethyl mercapturic acids[b] (PHEMAs): M1	283	
PHEMAs: M2[b]	283	
phenacylmercapturic acid (PAMA)	281	

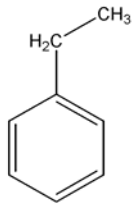
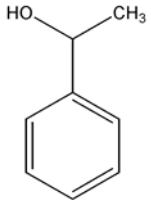
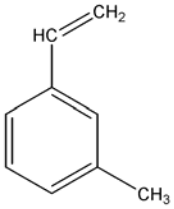
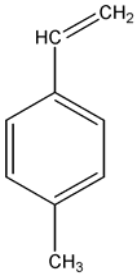
Metabolite	Molecular weight	Structure
styrene glycol sulfate	218	
4-vinylphenol sulfate	200	
styrene glycol glucuronide	314	
4-vinylphenol glucuronide	296	

Source: Linhart *et al.* 2000, Manini *et al.* 2002b.
[a]Manini *et al.* (2002b) reported that this metabolite is expected to be formed, but it has never been demonstrated in urine following styrene exposure.
[b]Two diasteroisomers of each PHEMA exist (see Figure 5-1).

1.4 Analogues

Many chemical analogues of styrene exist. Table 1-4 shows the structures of four analogues that are discussed in Section 5. Ethyl benzene is a chemical precursor for styrene (see Section 2), and it and 1-phenylethanol are metabolites of styrene (see Figure 5-1). 3-Methylstyrene and 4-methylstyrene are often used as a mixture called vinyltoluene.

Table 1-4. Styrene analogues

Metabolite	Molecular weight	Structure
Ethyl benzene	106.2	
1-Phenylethanol	122.2	
3-Methylstyrene	118.2	
4-Methylstyrene	118.2	

ChemIDPlus 2008c, 2008d, 2008e, 2008f.

This Page Intentionally Left Blank

2 Human Exposure

Styrene is used primarily in the manufacture of polystyrene; it is also used to manufacture styrene-butadiene latex, styrene-butadiene rubber, unsaturated polyester resins, and numerous other copolymers. The primary sources of exposure to the general public include inhalation (including inhalation of indoor and outdoor ambient air, active smoking, and exposure from environmental tobacco smoke), and ingestion of foods. Workers may be exposed to high levels of styrene through inhalation and dermal exposure. The industries with the largest numbers of highly-exposed workers are the reinforced-plastics , styrene-butadiene rubber, and styrene monomer and polymer industries. This section describes data important in evaluating human exposure to styrene, including uses (Section 2.1), production (Section 2.2), the release, chemical fate, and levels of styrene in various environmental media (Section 2.3), general population exposures (Section 2.4), occupational exposures (Section 2.5), biological indices of exposure (Section 2.6), and U.S. regulations and guidelines that are intended to reduce exposure to styrene (Section 2.7). A summary of the human exposure section is provided in Section 2.8.

The information reported in this section was obtained from several peer-reviewed panel evaluations or reviews for styrene, and from literature published since these reviews or evaluations. The most recent panel evaluations include (1) an IARC monograph for styrene (1994a, 2002), (2) the NTP's Center for the Evaluation of Risks to Human Reproduction (CERHR) *Expert Panel Report on the Reproductive and Developmental Toxicity of Styrene* by Luderer *et al.* (2005), (3) the *Evaluation Of The Potential Health Risks Associated With Occupational And Environmental Exposure To Styrene* by the Harvard Center for Risk Analysis authored by Cohen *et al.* (2002) [sponsored by the Styrene Information and Research Center], (4) the European Union's 2002 *Risk Assessment Report* for styrene, and (5) the ATSDR *Toxicological Profile for Styrene* (1992).

2.1 Use

IARC (2002) reported that styrene was first isolated in 1831 through the distillation of a natural balsam called storax. Styrene did not become commercially important, however,

until World War II when the United States initiated a major program to develop synthetic rubber (Miller *et al.* 1994, Steele *et al.* 1994). Since that time, styrene has become an important chemical used in the synthesis and manufacture of polystyrene and hundreds of different copolymers, as well as numerous other industrial resins (Guest 1997). Styrene producers sell styrene monomer to companies (resin manufacturers and compound producers) who use the styrene to make resins. Fabricators then process the resins into a wide variety of products (Cohen *et al.* 2002). Roughly 99% of the industrial resins produced from styrene can be grouped into six major categories. These six categories of resins (including unsaturated polyester resins with and without reinforcement), and some representative products made from the resins, are presented in Table 2-1.

Table 2-1. Styrene use in industrial resin

Resin Type	Estimated resin production (%)	Typical products produced from resins
Polystyrene	50	Construction materials, cups, plates, egg cartons, audio-visual equipment (e.g., cassettes), packaging, dairy containers, toys, furniture, industrial moldings (e.g., medical dental), insulation
Styrene-butadiene rubber	15	Tires, automobile parts (e.g., hoses, belts, seals, wire insulation)
Unsaturated polyester resins (glass reinforced)	12	Boats, tubs, shower stalls, spas, hot tubs, cultured marble products, building panels, trucks
Styrene-butadiene latexes	11	Backing for carpets and upholstery, paper coatings, floor tile, adhesives
Acrylonitrile-butadiene-styrene	10	Appliances, automobile parts, business equipment, construction materials, drains, ventilation pipes, hobby equipment, casings
Styrene-acrylonitrile	1	Appliances, automobile parts, housewares, battery casings, packaging
Unsaturated polyester resins (not reinforced)	Not reported	Liners, seals, putty, adhesives

Source: Luderer *et al.* 2005.

The largest single use for styrene is in the manufacture of polystyrene (accounting for roughly half of styrene use). Polystyrene is used extensively in the manufacture of plastic packaging, thermal insulation in building construction and refrigeration equipment, and disposable cups and containers. Styrene polymers and copolymers also are increasingly used in the production of various housewares, including food containers, toys, and

electrical devices, in the production of automobile body parts, corrosion-resistant tanks and pipes, in various construction items, carpet backings, house paints, paper processing, computer printer cartridges, insulation products, wood floor waxes and polishes, adhesives, putties, personal care products, and other items (IARC 2002, Luderer *et al.* 2005, NLM 2008).

Styrene is used as a cross-linking agent in polyester resins used in gel-coating and laminating operations in the production of glass fiber–reinforced plastic products such as boats, bathtubs, shower stalls, tanks, and drums (EPA 1997a, Miller *et al.* 1994). The resins generally contain between 30% and 50% styrene by weight (EPA 1997a). Methyl methacrylate may be used as a cross-linking agent instead of, or in addition to, styrene; however, styrene is by far the most common agent used.

2.2 Production

There are two commercially viable methods to produce styrene (ATSDR 1992, HSDB 2008a). The most common process, which accounts for over 90% of the total world styrene production, involves catalytic dehydrogenation of ethylbenzene. In the Dow Process, superheated steam is injected with ethylbenzene over a fixed catalytic reactor. The catalyst is iron-oxide based and contains Cr_2O_3 and KOH or K_2CO_3 as promoters (Cheresources 2008b). Ethylbenzene conversion is typically 60% to 65%, and there are three significant byproducts: toluene, benzene, and hydrogen. After the reaction, the products are cooled and the product stream, which contains styrene, toluene, benzene, and unreacted ethylbenzene, is fractionally condensed. After adding a polymerization inhibitor, the styrene is vacuum distilled to reach the required purity (noted as 99.8%). The second process involves oxidation of ethylbenzene to its peroxide, which is then reacted with propylene to produce propylene oxide and alpha-methylphenyl carbinol. The carbinol is then dehydrated to produce styrene.

As noted above, production of polystyrene is the single largest use of styrene. In one process, an inert organic solvent environment provides the medium for the polymerization reaction (Cheresources 2008a). 1,2-Dichloroethane is the most common solvent used, although carbon tetrachloride, ethyl chloride, methylene dichloride, benzene, toluene, ethylbenzene, and chlorobenzene are suitable. The preferred initiator

for the reaction is a mixture of boron trifluoride and water. The typical feed stream to the reactor consists of 50 weight percent styrene monomer, 100 ppm water and 200 ppm boron trifluoride (based on styrene weight) with the remainder being organic solvent.

The chemical reaction for synthesis of styrene from ethylbenzene and the polymerization process for production of polystyrene, the most common product made from styrene, are illustrated in Figure 2-1.

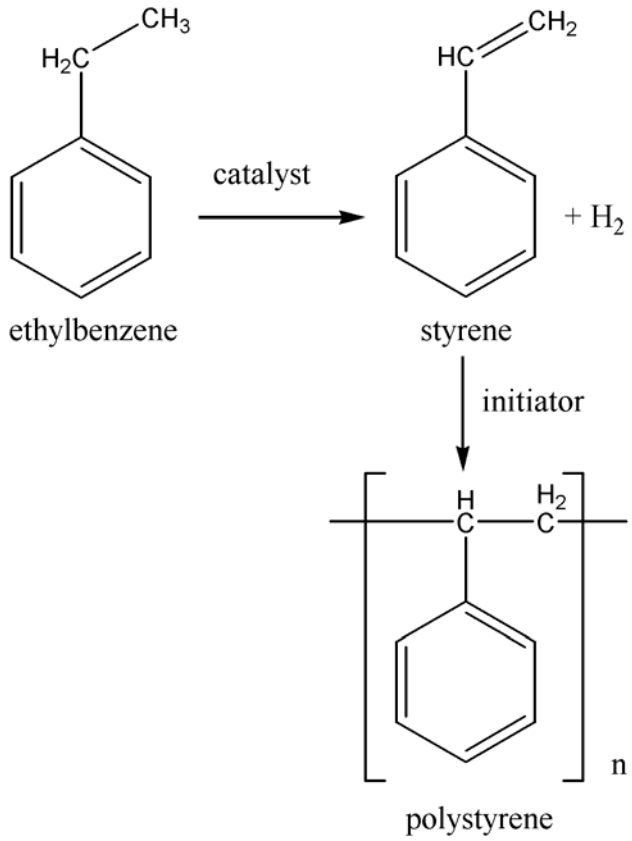


Figure 2-1. Synthesis of styrene from ethylbenzene and polymerization of styrene to form polystyrene

U.S. production of styrene has risen steadily since 1960 with a few dips from one year to the next. In the *Chemical Economics Handbook (CEH) Marketing Research Report* for styrene, Berthiaume and Ring (2006) estimated U.S. styrene production to be 1,740 million pounds in 1960, rising to a maximum of 11,897 million pounds in 2000, and production of 11,387 million pounds in 2006. Figure 2-2 summarizes the historical production data presented in *CEH*. Other sources, such as the U.S. International Trade

Commission (USITC), have estimated similar production levels. In 2002, Cohen *et al*. reported that U.S. styrene production exceeded 10 billion pounds [10,000 million pounds] annually and that from this, over 13 billion pounds [13,000 million pounds] per year of styrene-containing resins were produced.

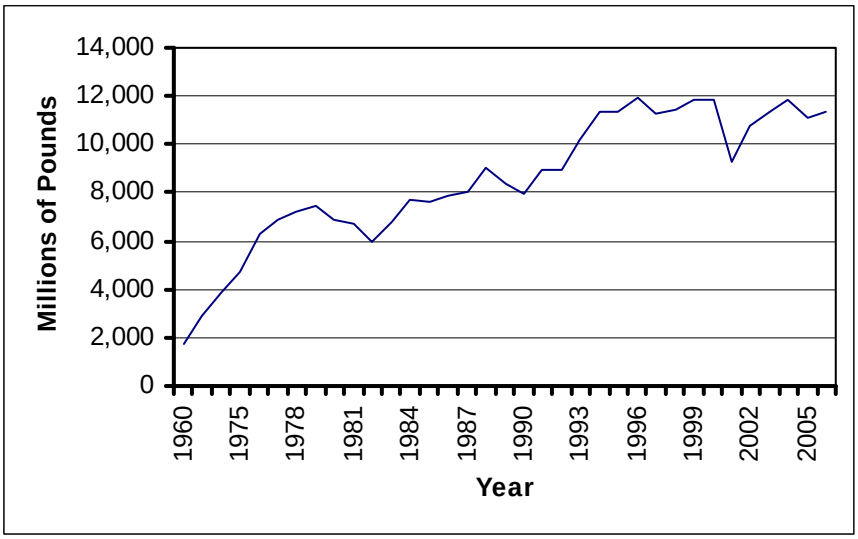


Figure 2-2. U.S. styrene production (1960–2006)
Source: (Berthiaume and Ring 2006).

As of 2006 there were eight active producers of styrene in the United States. The three largest of these producers accounted for 54% of domestic production in 2006 (Berthiaume and Ring 2006).

Import and export data are presented in Figure 2-3. *CEH* (Berthiaume and Ring 2006) and USITC data (USITC 2008a, 2008b) each showed a steadily increasing trend in both imports and exports from 1975 through 2007. The minimum level for imports was 7 million pounds in both 1975 and 1977, and the maximum level was 1,475 million pounds in 2007. The minimum level for exports was 574 million pounds in 1975 and the maximum level was 4,200 million pounds in 2007.

During the 1990s, styrene consumption in the United States increased at an average annual rate of 2.2% with over 99% consumed in the production of polymers and copolymers (Berthiaume and Ring 2006). U.S. consumption of styrene in 2006 was 9,600 million pounds and was anticipated to reach a level of 10,800 million pounds by 2011.

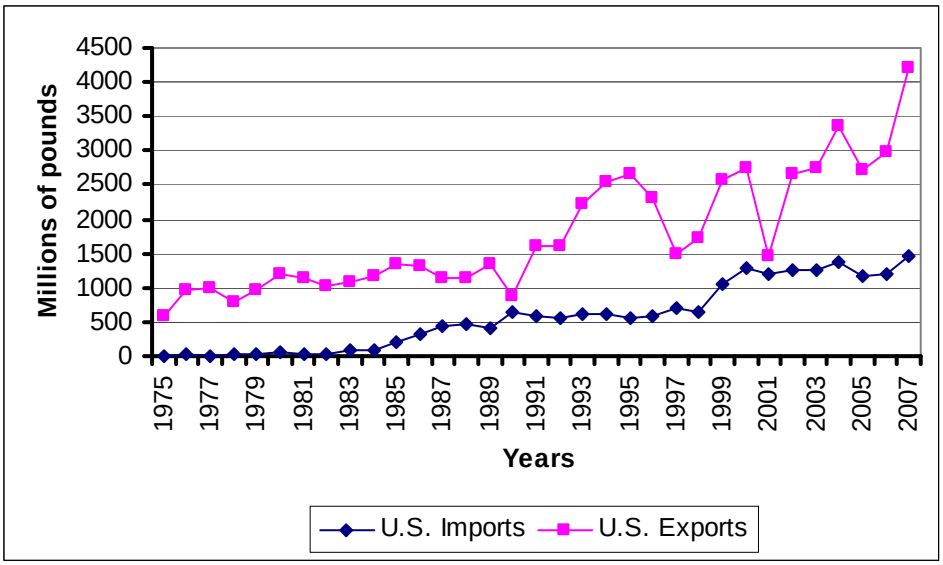


Figure 2-3. U.S. imports and exports for styrene
Sources: Berthiaume and Ring 2006 and USITC 2008a,b.

2.3 Environmental release, fate, and occurrence

Styrene has been measured in both outdoor air and indoor air, with generally higher levels found in indoor air. Styrene has been detected in a small percentage of U.S. drinking water samples, generally at low levels, and it has also been detected in both surface and ground waters in the United States. It has been found in soils of U.S. hazardous waste sites. It can occur in food both naturally and through migration from packaging materials containing residual styrene monomer. Numerous spills containing styrene have been reported to the National Response Center (NRC) since 1990, and these spills have the potential to contaminate air, water, soil, and even food supplies. This section discusses the release, environmental fate, and occurrence of styrene in air, water, soil, and food. The exposure level data presented in this section provides general information on exposure levels and can be considered "semi-quantitative." It is not intended to provide an estimate of the level of exposure for the general population or for any particular subpopulation. Section 5.2.1 discusses the possible estrogenicity of styrene as an environmental contaminant.

For environmental sampling, styrene is usually collected on solid sorbents (such as charcoal), either directly for air samples or after purging in a gas stream for water, soil, or solid waste samples (IARC 2002, ATSDR 1992). ATSDR noted that styrene from such

samples can be measured very sensitively by capillary column gas chromatography with flame ionization detector (GC/FID), and very specifically by gas chromatography with mass spectrometric detection (GC/MS). ATSDR also noted that relatively low detection limits and high accuracy can be achieved for the determination of styrene in environmental samples. IARC reported that estimated detection limits for GC/FID analysis of air samples ranged from 0.001 to 0.01 mg/sample. IARC also reported that the practical quantitation limits are 5 μg/L for groundwater samples, 250 μg/L for water-miscible liquid waste, 2,500 μg/L for non-water–miscible waste, 5 μg/kg for low-level soil and sediment samples, and 625 μg/L for high-level soil and sludge samples.

[While GC/FID and GC/MS are the most commonly used methods for the assessment of styrene in environmental media, other methods are available and have been used in the past. For data presented in this section and in the section on occupational exposure (Section 2.5) the analytical methods often were not identified in the studies reviewed. It is likely that different methods were used across these studies resulting in variations in the quality of the data presented. The data that are presented span several decades, and analytical methods are continually refined to obtain lower detection limits and to improve accuracy and precision. Therefore, in some cases, the analytical methods used to obtain these data may be outdated. There also will be differences in the quality of data presented based on the purpose and strategy of the sample collection and the study design. The environmental and occupational studies reviewed varied in the type of data provided. In general, if arithmetic means and ranges were available, those data are presented here. However, geometric means, medians, maximum values, standard deviations, and other summary statistics may be provided if those data were presented rather than arithmetic means and ranges in the cited document.]

The information reported for air, water, and soil is limited to data from the United States. However, for styrene levels in food, information obtained from other countries are provided in addition to U.S. data, as much of the food consumed in the U.S. is imported.

2.3.1 Air

Styrene can be emitted in the air from industrial production and use of styrene and styrene-based polymers and copolymers, motor vehicle emissions and other combustion processes, off-gassing of building materials and consumer products, and cigarette smoking (ATSDR 1992, IARC 1994a). For the general public, significant exposure to styrene can result from both outdoor and indoor sources. The remainder of this section discusses outdoor and indoor releases of styrene to air, its fate and transport in air, and measured levels in outdoor and indoor air.

2.3.1.1 Outdoor release

Major sources of styrene in outdoor air include industrial sources, automobile emissions, and combustion processes such as waste incineration and the burning of wood (ATSDR 1992, IARC 1994a). Typical sources of industrial styrene emissions include facilities producing styrene, polystyrene, other plastics, glass fiber–reinforced plastic products, synthetic rubber, and resins (ATSDR 1992). For 2006 [the most recent data available], the U.S. EPA's Toxics Release Inventory (TRI) reported styrene fugitive air emissions of 9.9 million pounds, and point-source air emissions of 37.4 million pounds (TRI 2008a). These air emissions combined [47.2 million pounds] accounted for roughly 93% of the total TRI styrene releases for all reported environmental media in 2005. Between 1988 (the first year of TRI reporting) and 2005, the smallest reported total air release (point-source plus fugitive emissions) was 30.3 million pounds in 1991 and the largest was 59.5 million pounds in 1999. Among the 519 TRI 2001 Core Chemicals, styrene had the 6th highest level of point-source air emissions and the 5th highest level of fugitive air emissions in 2005 (TRI 2008b, 2008c). [Note that since EPA's reporting requirements are for those facilities that produce or use large amounts of a chemical, actual emissions probably are greater than those reported.]

Styrene has been identified in motor vehicle emissions from both gasoline- and diesel-powered engines. The U.S. EPA estimated that in 1990, 32.9% of total U.S. styrene emissions were from on-road vehicles (IARC 2002). In 1999, it was estimated that in the U.S. 14,284 tons [28.6 million pounds] of styrene were emitted from highway vehicles and 3,055 tons [6.1 million pounds] from non-road equipment (EPA 2007). Emissions in

2010 are expected to fall to 7,652 tons [15.3 million pounds] for highway vehicles and 2,297 tons [4.6 million pounds] for non-road equipment primarily as a result of reductions due to the Mobile Source Air Toxics rule (see Section 2.7 for regulations and guidelines).

Glass fiber–reinforced plastic composites production and boat manufacturing are other major sources of styrene emissions. The U.S. EPA estimated that in 1990, 39.8% of U.S. styrene emissions were from these sources (IARC 2002) (see Section 2.5.1 for further discussion). [More recent data on national emissions levels from this industry were not found.]

Another source of outdoor styrene emissions is thermal degradation of styrene-containing polymers. IARC (2002) reported that results from one study showed styrene monomer to be the main volatile product of the thermal decomposition of polystyrene, constituting up to 100% of the volatiles. Styrene also has been measured in the air near open burning of scrap tires. The EPA (1997b) reported a median concentration of 85 $\mu g/m^3$ (20 ppb), with a 90th percentile concentration of 2,320 $\mu g/m^3$ (540 ppb) for ambient concentrations measured within 1,000 feet downwind of 14 uncontrolled fires.

2.3.1.2 Indoor release

Indoor sources of styrene emissions include off-gassing of building materials and consumer products and tobacco smoke (ATSDR 1992, IARC 2002). Styrene from adhesives used in the construction and finishing of buildings has been identified in indoor air. Polystyrene products such as packaging materials, toys, housewares, appliances, computers, and other plastic and rubber items can also contribute small amounts of the monomer to indoor air levels (ATSDR 1992, Bako-Biro *et al.* 2004).

EU (2002) noted that polystyrene and styrene copolymers are resistant to biodegradation, and therefore, decomposition to the monomer is unlikely. However, polymers can contain residual styrene monomer, and off-gassing of styrene from household products such as carpet glues, construction adhesives, and polyester-containing flooring materials are potential sources of styrene in indoor air (Luderer *et al.* 2005, EU 2002, ATSDR 1992).

Table 2-2 provides data on styrene monomer levels in various types of styrene polymer and copolymer materials [more recent data were not found].

Table 2-2. Residual styrene-monomer levels in polymer and copolymer materials in 1980

Polymer or copolymer	Residual styrene levels (ppm)	
	Typical	Maximum
Polystyrene	300–1,000	2,500
Acrylonitrile-butadiene-styrene (for food containers)	200–300	600
Acrylonitrile-butadiene-styrene (for other uses)	300–1,000	2,000
Styrene-acrylonitrile	600–1,200	2,000
Methyl methacrylate-butadiene-styrene	ND–10	30
Glass-reinforced plastic	20–200	1,000
Styrene-acrylic copolymers	60 in latex	NR
Styrene-butadiene - raw polymer	10–30	NR

Source: EU 2002.
ND = not detected; NR = not reported.

Historical levels of styrene from 1976 to 1980 are presented in Table 2-3. These data show some reduction in residual styrene monomer levels from 1976 to 1980. Residual levels have been further reduced since this time due to improvements in production methods (EU 2002). Luderer *et al.* (2005) noted that the residual monomer data from 1980 may overestimate residual levels of styrene in polymers currently manufactured in the United States because of changes in regulations and production methods since 1980.

Table 2-3. Historical levels of residual styrene (mg/kg) in polymer and copolymer: 1976–1980

Year	Polystyrene		Expanded polystyrene		High-impact polystyrene		Acrylonitrile-butadiene-styrene		Styrene-acrylonitrile	
	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max
1976	870	970	–	–	800	1,270	700[b]	1,600[b]	–	–
1977	700; 800[a]	1,020; 1,100[a]	–	–	600	990	300[b]	1,060[b]	3,400	5,000
1978	380	580	1,400	–	420	840	300[b]	800[b]	1,000	1,550
1979	400	790	1,400	–	380	600	300[b]; 600[c]; 1,220[d]	700[b]; 790[c]; 1,220[d]	950	1,300
1980	410	600	1,000	–	360	490	300[b]; 600[c]; 700[d]	600[b]; 1,000[c]; 870[d]	950	1,250

Source: EU 2002.
Max = maximum level reported.
[a]Two values were reported in original source without explanation.
[b]Intended for food containers.
[c]Intended for refrigerator applications.
[d]Intended for household appliances.

There is limited information on losses of styrene to air from finished articles. EU (2002) reported that based on an examination of three types of polystyrene to determine any loss of residual monomer, no change was seen in flexible or rigid polystyrene cold drinks cups over a six-month interval, although there appeared to be some loss (from 104 ppm to 71 ppm residual styrene) from foam hot drinks cups. Another study reviewed by the EU reported that the residual monomer content of polystyrene and styrene copolymers (300 to 500 ppm) did not reveal any losses over 2 years. The EU also reported that typical levels of styrene in expanded polystyrene molding will decrease from an initial level of 500 mg/kg to an equilibrium level of around 200 mg/kg over a period of 2 to 5 years depending on use.

The European Union (EU 2002) reported that an emission factor of 0.03% [0.3 g/kg] would be appropriate for long-term use (*e.g.*, insulation in buildings) but would over-estimate losses for short-term applications such as packaging. Rates of styrene emission from glued carpet have been estimated at 98 ng/min per m^2 (ATSDR 1992). In a chamber test of cork parquet flooring applied on concrete, styrene emissions were measured at 3

μg/h-m^2 after 24 hours, 2 μg/h-m^2 after 168 hours, and < 1 μg/h-m^2 after 576 hours [the source of the styrene was not specified] (Uhde and Salthammer 2007).

Infiltration of outdoor air has been proposed to be a potentially important source of styrene levels in indoor air (Guo *et al.* 2004a). Infiltration of gasoline-related volatile organic compounds (VOCs) from attached garages into houses is another potential source of styrene in indoor air (Adgate *et al.* 2004b, Batterman *et al.* 2006, Batterman *et al.* 2007). In Minnesota residences, Adgate *et al*. found significantly higher levels of styrene in homes with attached garages than in homes without attached garages. However, in assessing styrene levels in houses and in garages of 15 Michigan residences with attached garages, Batterman *et al*. found mixed results for styrene, with 8 of the 15 houses having higher concentrations than the attached garages. The authors were unable to conclude that styrene from the attached garages contributed to indoor air levels.

Increased styrene air concentrations have also been measured in the homes of smokers versus non-smokers (IARC 2002, HSDB 2008a).

2.3.1.3 Fate

Styrene, with a vapor pressure of 6.4 mm Hg at 25°C, is expected to exist solely as a vapor in the ambient atmosphere if released to air (HSDB 2008a). In its vapor phase, it is expected to react rapidly with hydroxyl radicals and with ozone. Half-lives based on these reactions have been estimated to range from 0.5 to 17 hours (Luderer *et al.* 2005). Atmospheric washout is not expected to be an important process because of these rapid reaction rates and styrene's high Henry's law constant.

Styrene levels in indoor air can be altered due to chemical reactions with other indoor pollutants. Uhde and Salthammer (2007) reviewed how styrene and its aldehyde degradation products fluctuated due to chemical surface interactions. They reported that in a study that measured VOCs in a newly carpeted stainless-steel chamber in the presence of ozone, the gas-phase concentrations of styrene, 4-vinyl-cyclohexene, and 4-phenyl-cyclohexene decreased significantly while the concentrations of aldehydes increased. The authors reported that both 4-vinyl-cyclohexene and 4-phenyl-cyclohexene

were suspected secondary pollutants from styrene-butadiene rubber, which was used for foam backing for carpets.

2.3.1.4 Outdoor occurrence

The primary sources of styrene in outdoor air include emissions from industrial processes involving styrene and its polymers and copolymers, vehicle emissions, and other combustion processes (IARC 2002). ATSDR (1992) noted that styrene levels in outdoor air are likely to be higher in urban areas than rural areas, and Alexander (1997) noted that concentrations in outdoor air are generally higher in winter than summer months. The Hazardous Substances Data Bank (HSDB 2008a) noted that except in highly polluted areas, styrene concentrations in outdoor air generally are less than 1 $\mu g/m^3$ [0.23 ppb]; although much higher levels have been reported. Table 2-4 summarizes reported concentrations of styrene in outdoor air in the United States.

The U.S. EPA monitors ambient air concentrations of numerous air pollutants, including styrene, throughout the United States, and these data are available on their AirData web site (http://www.epa.gov/air/data/). Outdoor ambient air monitoring data for 259 monitoring sites were reported for 2007 (EPA 2008a). Based on 13,432 observations, the mean concentrations for these sites ranged from 0.028 to 5.74 ppb and the maximum concentrations ranged from 0.05 to 206.47 ppb. HSDB (2008a) and the European Union (EU 2002) provided additional information on general or non-specific air concentrations in the United States.

IARC (2002), HSDB (2008a), European Union (2002), and Luderer *et al*. (2005) have reported data on U.S. air levels of styrene in the vicinity of known sources (Table 2-4). IARC (2002) reported ambient air levels of styrene in the vicinity of seven U.S. reinforced-plastics processors. Higher levels were measured at distances of under 500 m compared with levels at distances of 500 to 1,000 m.

High air levels have been reported in the vicinity of styrene-related industries, such as reinforced plastic processors, or hazardous waste sites (see Table 2-4). Since the 1980s, ATSDR has measured levels of various contaminants at hazardous waste sites during site investigations, and summary data for these investigations are available through an online

database called Hazardous Substance Release and Health Effects Database (HazDat) (HazDat 2008). Between 1980 and 2005, outdoor air styrene concentrations measured on the waste sites ranged up to 17,000 μg/m^3 [4,000 ppb] and offsite concentrations ranged up to 122 μg/m^3 [28.6 ppb]. [Only maximum data are presented in the HazDat online database.]

IARC (2002), Luderer *et al*. (2005), European Union (EU 2002), and HSDB (2008a) have presented results of monitoring studies from U.S. urban areas (Table 2-4). Payne-Sturges *et al*. (2004a) found that mean and median outdoor monitoring levels were less than indoor air monitoring levels and personal monitoring levels (see "Indoor Occurrence," below); however, outdoor levels were higher than modeling results predicted by the U.S. EPA's Assessment System for Population Exposure Nationwide (ASPEN) model. Similarly, Adgate *et al*. (2004a, 2004b) found higher styrene levels for indoor air monitoring and personal monitoring than for outdoor ambient air monitoring. For Table 2-4 and the remainder of the tables presenting environmental levels, the number of samples is presented when it was available in the referenced source; otherwise, the number of samples is not addressed.

Table 2-4. Concentrations of styrene in outdoor air in the United States

Location (year)	Measurement	Concentration (ppb)	Source
General or unspecified locations			
Ambient air monitoring throughout United States (2007)	mean levels maximum levels (based on 13,432 measurements)	0.028–5.74 0.05–206.47	EPA 2008a
California (1965)	mean (range)	4.9 (1.9–14.8)	EU 2002
Contra Costa County, CA (NR)	single measurement	0.09	HSDB 2008a
New Jersey, California residential areas (NR)	range of medians of 6 sets of samples	0.07–1.0	EU 2002
Four unspecified states (1981–1984)	range	ND–0.89	EU 2002
Unspecified locations (NR)	average of samples above detection limit from various studies (6,117 total samples)	0.14[a]	HSDB 2008a

Location (year)	Measurement	Concentration (ppb)	Source
Unspecified location(s) (NR)	median concentration from 135 samples	2.1	HSDB 2008a
Measurements in the vicinity of a known source			
Vicinity of reinforced-plastics processors in multiple states (NR)	< 500 m from facility 500 to 1,000 m from facility	0.07–690 0.4–5.6	HSDB 2008a, IARC 2002
Houston, TX, industrial complex close to major transport route (1987–1988)	mean of 135 samples	0.5[a]	EU 2002
Vicinity of sanitary and hazardous waste landfills (NR)	maximum range of means	15.5 0.12–1.53	HSDB 2008a
Hazardous waste sites (1980–2005)	onsite measurements offsite measurements	up to 4,000 up to 28.6	HazDat 2008
Allegheny mountain tunnel, Pennsylvania (NR)	range	0.3–1.6	HSDB 2008a
Caldecott Tunnel, San Fransciso, CA (NR)	range	9.83–36.73	HSDB 2008a
Pennsylvania turnpike tunnel (NR)	range	0.25–1.5	Luderer *et al.* 2005
Urban locations			
Baltimore, MD (2000–2001)	mean median	0.12[a] 0.06[a]	Payne-Sturges *et al.* 2004a
Minneapolis, MN (1997)	median, winter median, spring	0.023[a] 0.0	Adgate *et al.* 2004a
Minneapolis, MN (2000)	mean	0.12[a]	Adgate *et al.* 2004b
Phoenix, AZ (1994–1996)	range of means	0.49–5.64	HSDB 2008a
Tucson, AZ (1994–1996)	range of means	0.09–0.23	HSDB 2008a
Los Angeles, CA (1981)	range; 16 of 17 samples positive	0.5–3.0[a]	EU 2002
Three New Jersey cities (NR)	range of means, summer range of means, winter	0.07–0.13 0.15–0.23	Luderer *et al.* 2005
Twenty urban test stations in California (1989–1995)	1 d per month monitoring average maximum	 0.2 2.9	IARC 2002
Four unspecified cities (NR)	range	1–15	HSDB 2008a
One unspecified city (NR)	median	0.14	HSDB 2008a

ND = not detected; NR = not reported.
[a] Reported in units of $\mu g/m^3$ in source document.

2.3.1.5 Indoor occurrence

Indoor air concentrations of styrene typically exceed outdoor air concentrations (Miller *et al.* 1994). IARC (2002) reported that median residential air concentrations collected by personal sampling have generally ranged from 1 to 3 μg/m^3 [0.2 to 0.7 ppb]. ATSDR (1992) noted that mean indoor air levels of styrene have been reported in the range of 0.1 to 9 μg/m^3 [0.02 to 2.1 ppb] and can be attributed to off-gassing from building materials and consumer products and from tobacco smoke. Fishbein (1992) reported typical indoor levels ranging from 0.3 to 50 μg/m^3 [0.07 to 11.7 ppb]. Based on a U.S. EPA national VOCs database compiled in the early- to mid-1980s from various sources of U.S. indoor air concentration data, Miller *et al*. (1994) reported a mean indoor styrene air level of 1.413 ppb and a median level of 0.305 ppb based on 2,125 data points. Styrene levels measured in indoor air in the United States are presented in Table 2-5.

Increased styrene air concentrations have been measured in the homes of smokers versus non-smokers. In a screening-phase study of 284 Minnesota homes, Adgate *et al*. (2004b) found statistically significant increases in styrene levels in homes with smokers compared with homes without smokers. Another study showed that styrene concentrations were approximately 0.5 μg/m^3 [0.1 ppb] higher in homes with smokers than in homes without smokers (IARC 2002). Tobacco use by adults also resulted in elevated styrene exposure levels for children (Adgate *et al.* 2004a, Adgate *et al.* 2004b) (see below). Based on a styrene emission factor of 235 μg/cigarette, Nazaroff and Singer (2004) estimated exposure concentrations ranging from 0.6 to 1.4 μg/m^3 [0.14 to 0.33 ppb] in U.S. private residences.

Payne-Sturges *et al.* (2004a) noted that exposure research has consistently shown personal exposure levels for most VOCs are very different from outdoor air concentrations and this may result in over- or under-estimates of risks when outdoor air concentrations are used exclusively. The authors examined the extent of exposure misclassification and its effect on risk as estimated by the U.S. EPA's ASPEN model relative to monitoring results from a community-based exposure assessment conducted in Baltimore, MD. For styrene, monitoring data were consistently higher than the levels predicted by the ASPEN model. The ASPEN model predicted mean and median air

concentrations for styrene of 0.12 μg/m^3 [0.03 ppb]; however, mean monitoring values were 2.72 μg/m^3 [0.64 ppb] for indoor air, and 2.51 μg/m^3 [0.59 ppb] for personal monitoring. The authors noted that indoor exposures were the dominant source of styrene exposure.

In a study of exposure to VOCs in microenvironments, breathing zone styrene air concentrations were measured for a non-random sample of 71 non-smoking adults living in three urban neighborhoods in Minneapolis-St. Paul, MN and compared with concurrent area measurements taken inside the participants' residences and in outside air (Sexton *et al.* 2007). The participants maintained time-activity logs during the sampling period, recording the amount of time spent in seven microenvironments: (1) indoors at home, (2) indoors at work or school, (3) indoors in other locations (any indoor location other than home, work, or school); (4) outdoors at home, (5) outdoors at work or school, (6) outdoors in other locations (any outdoor location other than home, work, or school); and (7) in transit. The authors reported that the highest estimated concentrations (presented graphically only) were found for "indoors in other locations" followed by "indoors at work/school" and "indoors at home." The means for "outside" and "in transit" were only slightly greater than zero. Batterman *et al.* (2002) also reported low levels of styrene in a study of VOCs in microenvironments related to transportation (buses and cars), and suggested that industrial emissions contributed to variation in the levels measured.

Loh *et al.* (2006) characterized the distribution of VOCs, including styrene, in non-residential microenvironments in stores, restaurants, and transportation modes in the Boston, MA metropolitan area as part of the Boston Exposure Assessment in Microenvironments (BEAM) study. They reported that styrene levels were higher in stores, particularly hardware, housewares, and multipurpose stores, compared with transportation. Styrene varied significantly ($P < 0.05$; Wilcoxon rank sum test) by season, with levels being higher in summer compared with winter; however, only hardware and multipurpose stores were sampled in both seasons. The authors also concluded that concentrations of styrene were strongly influenced by smoking in the dining

microenvironment. Uhde and Salthammer (2007) noted that numerous chemical interactions can impact indoor air levels of styrene.

In a study assessing VOC exposures to children in Minneapolis, MN, Adgate *et al.* (2004a) noted that styrene levels were more frequently detectable in home and personal samples than in outdoor samples or samples taken in the children's schools. The authors reported slightly higher styrene air levels for home monitoring than for personal monitoring, and an 8-fold to almost 10-fold increase in home levels compared with school levels. In another study of VOC exposures in households with children (N = 284) in Minneapolis, MN, households with smokers, households with attached garages, and non-urban residences (which had a greater prevalence of smokers and attached garages) all had significantly higher levels of styrene (Adgate *et al.* 2004b).

ATSDR measures indoor air concentrations as part of their hazardous waste site investigations (HazDat 2008). Between 1980 and 2005, maximum concentrations measured in buildings onsite at hazardous waste sites were much higher than maximum concentrations measured in off-site buildings.

Table 2-5. U.S. levels of styrene measured in indoor air and by personal monitoring

Location (year)	Measurement	Concentration (ppb)	Source
General data			
Nationwide (compiled from data before mid-1980s)	mean median	1.413 0.305	Miller *et al.* 1994
Studies assessing microenvironments			
Minneapolis, MN (2000)	*School monitoring* winter (median) spring (median) *Home monitoring* winter (median) spring (median) *Personal monitoring* winter (median) spring (median)	 0.02[a] (N = 39) 0.02[a] (N = 47) 0.16[a] (N = 93) 0.19[a] (N = 88) 0.12[a] (N = 93) 0.12[a] (N = 88)	Adgate *et al.* 2004a
Minneapolis, MN (1997)	*Screening assessment* indoor monitoring (mean) *Intensive-phase assessment* personal monitoring (mean)	 0.28[a] (N = 284) 0.28[a] (N = 73)	Adgate *et al.* 2004b

Location (year)	Measurement	Concentration (ppb)	Source
	indoor monitoring (mean)	0.33[a] (N = 101)	
Baltimore, MD (2000–2001)	indoor monitoring (mean) personal monitoring (mean)	0.64[a] (N = 33) 0.59[a] (N = 31)	Payne-Sturges *et al.* 2004a
Minneapolis-St. Paul, MN (1999)	home (mean) school/work (mean) other indoor (stores, restaurants) (mean) outside (mean) in transit (mean) (N = 333 personal exposure samples (including duplicates) with matched time activity data for 70 participants)	0.14[a,b] 0.19[a,b] 0.26[a,b] < 0.01[a,b] < 0.01[a,b]	Sexton *et al.* 2007
Detroit, MI commuting routes (1999)	*Pilot study* range *Three day study* mean and range	0.07–0.26[a] (N = 16) 0.26[a] (0.02–0.82) (N = 48)	Batterman *et al.* 2002
Boston, MA (2003–2004)	stores (geometric mean) restaurants (geometric mean)	0.71[a] (N = 89) 0.28[a] (N = 20)	Loh *et al.* 2006
Hazardous waste sites			
Hazardous waste sites (1980–2005)	On-site buildings (maximum) Off-site buildings (maximum)	1,276[a] 41.5[a]	HazDat 2008

[a] Reported in units of μg/m^3 in source document.
[b] Estimated from graph.

2.3.2 Water

2.3.2.1 Release

The primary source of styrene in surface waters is industrial discharges (ATSDR 1992). Styrene has been detected in effluents from chemical, textile, latex, and coal-gasification plants at levels up to 970 μg/L. The daily styrene loading from a single chemical plant into the St. Clair River (Michigan) was estimated at 133 kg [293 lb]. Styrene also has been detected in the leachate from an industrial landfill and in surface water and groundwater at U.S. hazardous waste sites.

For the year 2006, a reported 4,043 lb of styrene were released to U.S. surface waters based on TRI data (TRI 2008a). The TRI data have fluctuated widely since 1988, with a maximum release of 243,148 lb reported in 1998 and a minimum of 3,004 lb in 2001. The second-highest reported release to surface water was 59,069 lb in 1988. Styrene also

has been detected in both the groundwater and surface water at hazardous waste sites (ATSDR 1992).

2.3.2.2 Fate

Volatilization and biodegradation are expected to be the major fate and transformation processes in water. Based on its Henry's law constant, styrene is expected to volatilize rapidly from environmental waters: the extent of volatilization depends on water depth and turbulence with no volatilization occurring in stagnant deep water (ATSDR 1992, Luderer *et al.* 2005). The estimated volatilization half-life of styrene from a river 1 m deep with a current speed of 1 m/s and a wind velocity of 3 m/s is roughly 3 hours. Half-lives have been estimated from 1 hour in a shallow body of water to 13 days in a lake, and from 4 to 30 weeks in groundwater (Luderer *et al.* 2005). Some biological oxygen demand studies have shown styrene to be biodegradable. Hydrolysis is not expected to be an important degradation process. Adsorption to particulate matter and sediment may have some significance, based on an organic carbon adsorption coefficient (K_{oc}) of 270 to 550 (Howard 1989). Styrene generally does not persist in water, because of its biodegradability and volatility (Cohen *et al.* 2002).

2.3.2.3 Occurrence

Limited data are available on styrene levels in water. When styrene has been detected in waters, it has generally been at low levels. This section discusses styrene levels in drinking water and environmental waters, and levels that have been detected in various waters at hazardous waste sites. Table 2-6 presents monitoring results for styrene in U.S. waters.

Drinking water

Extensive studies of U.S. drinking-water supplies indicate that if styrene is present, it generally is at very low concentrations (< 1 μg/L [1 ppb]) (Cohen *et al.* 2002). Miller *et al.* (1994) reported that in surveys of drinking-water supplies in the United States and Canada, styrene has been detected in a small percentage of drinking-water samples at concentrations generally less than 1 μg/L [1 ppb]. Styrene was not detected in several U.S. drinking-water surveys (Miller *et al.* 1994, EU 2002); detected but not quantified in other studies (Howard 1989); and reported as detected in some studies styrene, but no

levels were reported (Miller *et al.* 1994, Howard 1989). Levels have been reported in drinking-water supplies in Cincinnati, OH, in Iowa well water, and in Connecticut in well water adjacent to a landfill that contained styrene buried in drums (Howard 1989).

Environmental water

Styrene has been found in the lower Tennessee River, the Kanawha River in West Virginia, the Great Lakes, and detected but not quantified in the Delaware River (EU 2002, Howard 1989)

Hazardous waste sites

In the ATSDR *Toxicological Profile for Styrene*, it was reported that the geometric mean levels of styrene at U.S. hazardous waste sites were 9.3 μg/L [9.3 ppb] for surface water and 5.3 μg/L [5.3 ppb] for groundwater (ATSDR 1992). Styrene has been measured as part of ATSDR's hazardous waste site investigations, which includes monitoring of on-site and off-site groundwater, on-site and off-site surface waters, and on-site tap water (HazDat 2008).

Table 2-6. Levels of styrene measured in U.S. waters

Water type, location (year)	Additional information	Concentration (ppb)	Source
Drinking water			
Drinking water, unspecified location (1975–1981)	3 surveys and over 1,000 samples	ND	Miller *et al.* 1994
Drinking water, Cincinnati, OH (NR)	no additional information provided	0.024	Howard 1989
Drinking water, unspecified location (1977–1981)	102 surface water sources 12 groundwater sources	ND ND	EU 2002
Drinking water, KS, MO, and NB (1982)	drinking water collected from 272 sites	ND	Miller *et al.* 1994
Drinking water, Evansville, IN (NR)	no additional information provided	NQ	Howard 1989
Drinking water, Cleveland, OH (NR)	no additional information provided	NQ	Howard 1989
Drinking water, New Orleans, LA (NR)	contamination might have come from the filter	NR	Howard 1989
Well water, IA (NR)	no additional information provided	1.0	Howard 1989

Water type, location (year)	Additional information	Concentration (ppb)	Source
Well water, CT (1962)	well water adjacent to a landfill containing styrene buried in drums	100–200	Howard 1989
Well water, WI (early 1980s)	detected in only 1 of 1,791 private and community wells	NR	Miller *et al.* 1994
Groundwater, unspecified locations (NR)	945 groundwater samples in a drinking-water survey	ND	Miller *et al.* 1994
Environmental water			
Surface water, lower Tennessee River (NR)	single water sample	4.2	Howard 1989
Surface water, Kanawha River, WV (NR)	no additional information provided	1.0	Howard 1989
Great Lakes (1982–1983)	winter (average) spring (average) summer (average)	0.2 0.5 < 0.1	EU 2002
Delaware River (NR)	no additional information provided	NQ	Howard 1989
Hazardous waste sites			
Tap water, nationwide (1980–2005)	onsite tap water (one measurement)	0.8	HazDat 2008
Surface water, nationwide (NR)	geometric mean level at hazardous waste sites	9.3	ATSDR 1992
Surface water, nationwide (1980–2005)	onsite levels (maximum) offsite levels (maximum)	26,000 0.4	HazDat 2008
Groundwater, nationwide (NR)	geometric mean level at sites	5.3	ATSDR 1992
Groundwater, nationwide (1980–2005)	onsite groundwater monitoring wells (maximum) offsite groundwater monitoring wells (maximum) onsite private wells (maximum) offsite private wells (maximum) municipal groundwater well near a hazardous waste site (maximum)	55,000 40,000 5,000 30 1.6	HazDat 2008

ND = not detected, NQ = detected but not quantified, NR = level not reported.

2.3.3 Soil

2.3.3.1 Release

Soil may become contaminated through spills or discharges of styrene-containing materials and through land disposal of styrene-containing wastes (ATSDR 1992).

Sediment may become contaminated through disposal of styrene-containing wastes to surface waters or through overland transport of contaminated materials to surface waters. For 2006, TRI data showed that 11,242 lb of styrene were released to land (on-site and off-site land treatment and other land disposal) (TRI 2008a).

2.3.3.2 Fate

Styrene in soils is subject to biodegradation. Degradation of 87% to 95% has been observed in sandy loam and landfill soil over a 16-week period, and degradation of 2.3% to 12% per week has been observed in two subsurface aquifers (Howard 1989). K_{oc} values ranging from 260 to 550 have been calculated (Howard 1989, Luderer *et al.* 2005). These K_{oc} values indicate moderate to low soil mobility. It has been demonstrated that styrene buried in soil can leach into underlying groundwater. Styrene that leaked into surrounding soil from buried drums persisted for up to two years. Relatively strong adsorption of styrene was observed in a sand aquifer, as the breakthrough time for styrene was about 80 times that of a nonadsorbing tracer (Howard 1989). Varying rates of volatilization from soils have been reported in the literature; however, all studies agree that volatilization rates decrease with increasing soil depth (Luderer *et al.* 2005).

2.3.3.3 Occurrence

There are limited data on styrene levels in soil. ATSDR has measured sediment and soil concentrations of styrene as part of numerous hazardous waste-site investigations (HazDat 2008). The maximum concentrations measured in sediment were 70 ppm on-site and 0.37 ppm off-site. Soil concentrations were obtained at differing soil depths both on-site and off-site. For samples taken from the top three inches of soil, concentrations on-site were up to 14,000 ppm and off-site concentrations were up to 0.14 ppm. Surface top-soil concentrations on-site were measured at levels up to 2,900 ppm. Subsurface soil (deeper than 3 inches) was only measured on-site with a maximum concentration of 4,600 ppm. Because styrene is volatile, it is also present in soil gas and was measured during the waste-site investigations both on-site and off-site. On-site soil gas concentrations were up to 8,082,000 $\mu g/m^3$ [1,896 ppm], and off-site concentrations were up to 690 ppb [0.69 ppm]. Sediment from the lower Tennessee River contained styrene at 4.2 ppb [0.0042 ppm] (Howard 1989).

2.3.4 Food

2.3.4.1 Sources of styrene in food

Styrene has been detected as a constituent of a wide range of foods and beverages, with the highest measured levels occurring in unprocessed, raw cinnamon (IARC 1994a). Styrene is known to occur in the exudates from damaged trunks of certain trees, probably from the natural degradation of the cinnamic acid derivatives that occur in large quantities in the exudates, and this has been proposed as the source of styrene in cinnamon (IARC 1994a). Pinches and Apps (2007) demonstrated that in the presence of cinnamic acid, the molds *Trichoderma viride* and *T. koningii* produced styrene in foods. Styrene is also known to occur at very low concentrations in many agricultural foods, although it is not known whether the styrene is produced endogenously or is the result of environmental contamination (Tang *et al.* 2000). The presence of styrene in packaged foods is reported to be due primarily to monomer leaching from polystyrene containers (ATSDR 1992, Howard 1989). The primary factors that determine the rate of migration of styrene from polystyrene containers include the lipophilicity of the food, surface area of the container per volume of food, and the duration of contact (ATSDR 1992, EU 2002, Lickly *et al.* 1995a).

ATSDR (1992) reported that the rate at which styrene migrates from polystyrene containers into food is mainly a function of the diffusion coefficient of the monomer in the polymer and of the lipophilicity of the food. For example, 4% to 6% of the free monomer in polystyrene packaging migrated into corn oil or sunflower oil within 10 days, while only 0.3% to 0.6% migrated into milk, beef, or water. Stoffers *et al*. (2004) found the mean styrene migration rate from polystyrene into olive oil stored at 40°C for 10 days to be 0.013 mg/dm^2 [130 ng/cm^2]. The authors noted that the migration was quite low: only 1.8% of the initial styrene migrated. ATSDR (1992) reported styrene migration from foam cups into liquids such as water, tea, or coffee to be about 8 ng/cm^2, while migration into 8% ethanol, as might be encountered in wine or other alcoholic drinks, was 36 ng/cm^2.

Lickly *et al.* (1995a) found that styrene migration from polystyrene foam used for food-contact materials (styrofoam plates, bowls, cups, egg cartons, meat trays, and hinged

carryout containers) was proportional to the square root of the time of exposure. Others have noted that styrene concentrations increase in foods packaged in polystyrene with increasing duration of contact (ATSDR 1992, Lozano *et al.* 2007, Miller *et al.* 1994).

Temperature also has an impact on styrene migration. Lickly *et al*. (1995a) reported that the log of the mean diffusion coefficient was linearly related to the inverse of the absolute temperature of exposure from 70°F to 150°F [21°C to 66°C]. The mean diffusion coefficients ranged from 4.5×10^{-11} cm^2/s at 70°F to 3.4×10^{-9} cm^2/s at 150°F. Choi *et al*. (2005) examined the migration behavior of styrene monomer and oligomers from polystyrene to the food simulants water and heptane, which are used to simulate aqueous and fatty foods, respectively. Higher temperatures yielded faster migration rates, and the higher molecular weight oligomers had slower migration rates than the styrene monomer. Styrene can migrate into food from plastic containers during heating or cooking in microwave or conventional ovens. Nerín and Acosta (2002) estimated the migration of styrene and several other VOCs into food from five commercially available types of plastic containers: polycarbonate, polypropylene copolymer, polypropylene random, polypropylene-20% talcum, and styrene-acrylonitrile. Styrene migration was estimated at levels ranging from 1.8×10^{-6} to 6.7×10^{-4} mg/kg of food. The experiment was conducted in 120°C to 150°C [250°F to 300°F] ovens for 30 minutes. The maximum migration level was from a styrene-acrylonitrile container, and the minimum migration level was from a container made of polypropylene copolymer.

In an assessment of the effects of cold storage and packaging material on the migration of a number of chemicals, including styrene, into sweet-cream butter, Lozano *et al*. (2007) found that the relative abundance of styrene in foods increased as a function of time and storage temperatures. Styrene levels were found to be lower for fresh and frozen butter products when compared with refrigerated butter products (see Table 2-7). Styrene levels were also found to be higher for butter products wrapped in parchment when compared with butter wrapped in foil.

Styrene has a log K_{ow} of 2.95, indicating moderate potential for bioaccumulation (Howard 1989). Howard (1989) suggested that styrene's solubility ("relatively high water

solubility") to be high enough to make bioconcentration in biological organisms unlikely. Based on a bioconcentration factor (BCF) of 13.5, bioconcentration of styrene in aquatic organisms is expected to be low (HSDB 2008a). EU (2002) did an extensive review and analysis of the BCF and similarly concluded that it is unlikely that styrene will accumulate in aquatic organisms. However, styrene has been detected in fish and other aquatic organisms (see Section 2.3.4.2 below).

2.3.4.2 Styrene levels in food

Styrene levels in foods have been extensively documented. As noted above, styrene can leach from containers and wrapping materials into food, and it also can occur naturally in foods. This section first presents data on styrene levels in food due to migration from packaging materials. This is followed by a discussion of levels in food not believed to be due to migration from packaging materials, or the source of styrene is not known. Lastly, data are provided for the U.S. FDA's Total Diet Study, which simply measures levels of contaminants, including styrene, in table-ready food, irrespective of the source of the contaminant.

Miller *et al.* (1994) and HSDB (2008a) summarized the results of several studies that measured styrene concentrations in foods packaged in polystyrene. Table 2-7 summarizes these data.

Table 2-7. Measurements of styrene in foods packaged in polystyrene

Food	Concentration, mean or range[a] (μg/kg, or ppb)
Dairy Products	
Butter (range from fresh to 12 months of storage)	
Wrapped in parchment	22.7–1,174
Wrapped in foil	0–277
Refrigerator-stored after 12 months	277–1,174
Freezer-stored after 12 months	101–607
Sour cream	143–246
Yogurt	trace–34.6
Butter-fat cream	59.2
Milk	17.2
Soft cheese	16

Food	Concentration, mean or range[a] (μg/kg, or ppb)
Cream	11
Margarine table spreads	10
Cottage cheese	9.3
Beverages	
Orange drink	47
Lime drink	25
White coffee	21
Cold lemon drink	17
Hot chocolate	13
Desserts	
Cream dessert products	30
Other unspecified desserts	22
Fruit	
Glacé fruit	< 10
Strawberries	< 10
Other Products	
Chopped peel (unspecified fruit)	180
Gravy	64
Honey	22.7
Coleslaw	< 10
Fish	< 10
Fresh meat	< 10
Takeout food	< 10
Eggs	ND
Wine	ND

Sources: HSDB 2008a, Lozano *et al.* 2007, Miller *et al.* 1994.
ND = not detected (levels of detection not provided).
[a]A range is provided if the source document provided a range or if data are combined across sources.

Based on a literature review, Cohen *et al.* (2002) presented data on styrene levels measured in various foods (Table 2-8). Styrene occurs naturally in some foods and beverages (Miller *et al.* 1994, Steele *et al.* 1994, Tang *et al.* 2000), [and although it was not specified whether levels presented by Cohen *et al.* were measured by a process that avoided contact with styrene, it is likely that these data represent naturally occurring food levels].

Table 2-8. Food levels of styrene [source of styrene unknown]

Food	Concentration, mean or range[a] (μg/kg, or ppb)
Fruits	
Black currants	60
Bilberries	25
Kiwi	2
Soursop	0.17
Papaya	0.1
Sapodilla fruit	0.01
Vegetables	
Peas, southern	0–20
Lentils	5
Peas, split	5
Beans	4
Fish	
Whitefish	1
Meat	
Turkey sausage	100
Guinea hen, roasted (in skin)	1
Eggs	1–6
Alcoholic beverages	
Beer	10–200
Red wine	0–10
Bilberry wine	< 10
Hot beverages	
Roasted coffee	20–360

Source: Cohen *et al.* 2002.
[a] A range is provided if data were provided as a range or as multiple entries in the source.

Using a process that avoided contact with styrene or any type of plastic, Steele *et al.* measured styrene concentrations in 12 types of raw agricultural products, with results suggesting that styrene may be a natural constituent of many foods. Of 12 foods, 8 had detectable styrene levels, from a low of 0.233 ng/g [ppb] for Oregon peaches to a high of 39,200 ng/g [ppb] for cinnamon from Indonesia. [It is noteworthy that three cinnamon samples from three different sources were analyzed, and concentrations ranged from 179 ng/g to the high of 39,200 ng/g.] Other studies that have measured natural levels in foods (*i.e.*, without contact with polystyrene) have yielded similar results (Miller *et al.* 1994).

Styrene also is produced naturally as a metabolite in the process of making some foods, such as wine, beer, and cheese (Cohen *et al.* 2002); it has been measured in wines, with the majority of samples showing concentrations of 1 to 3 μg/L; the maximum concentration observed was 8 μg/L (Tang *et al.* 2000).

Whole body concentrations of styrene ranging between 15 and 100 μg/kg have been measured in splake and walleye fish caught in the St. Clair River, Canada. Styrene was also detected, but not quantified, in several other fish from the St. Clair River (EU 2002). Edible shellfish from Atlantic Canada were reported to contain styrene at levels less than 10.0 μg/kg. In a Japanese survey in 1986, styrene was found in 28 of 131 samples of fish at concentrations ranging from 0.5 to 2.3 μg/kg (limit of detection 0.5 μg/kg).

Since 1991, the U.S. FDA has measured styrene in U.S. foods in its Total Diet Study (TDS). The TDS measures levels of various contaminants and nutrients in foods that are prepared as they would be consumed, so the results can be used to provide a realistic measure of intake. Foods are purchased from supermarkets in selected U.S. cities, generally three to four times per year, and shipped to a central FDA laboratory, where they are prepared and analyzed (foods are measured as raw commodities if they are generally consumed as such). Table 2-9 summarizes styrene levels detected in TDS food samples from 1991 through 2003 (the most recent year for which data were available).

Table 2-9. Summary of styrene levels in FDA's Total Diet Study (1991–2003[a])

Food group (number detected/number of samples)	Range of means[b] (ppb)[c]	Full range across samples (ppb)[c]
Fruits and vegetables (raw oranges, bananas, avocados, strawberries, tomatoes, raisins; frozen strawberries; canned corn), and fruit juices (57/359)	0.1–119.26	< 2.0–1,980
Breads (white bread, fruit or plain muffins) (38/88)	5.23–29.07	< 2.0–510
Desserts and sweets (ice cream, cookies, cakes, Danish pastry, fruit pies, candy, brownies, chocolate, popsicle, doughnut, toaster pastry, soda pop, sandwich cookies) (287/792)	0.05–50.77	< 2.0–199
Dairy (cheese, cream cheese, butter, milk, sour cream) (51/308)	0.05–11.11	< 2.0–196
Snacks (roasted nuts and sunflower seeds, peanut butter, oil-popped and microwave popcorn, tortilla and potato chips, crackers) (134/352)	0.25–37.65	< 2.0–116
Fast food and takeout (hamburger, hotdog, pizza, taco, beef chow mein, French fries, fried chicken) (170/484)	0.28–17.95	< 2.0–94
Meat, fish, eggs (cooked ground beef, chuck roast, pork sausage and bacon, lamb, turkey, bologna, frankfurter, salami, tuna, fish sticks, scrambled eggs) (152/572)	0.23–7.59	< 2.0–85
Infant products (soy-based and milk-based formula, teething biscuits, apple juice, carrots, beef and broth/gravy) (8/264)	0.05–1.82	< 2.0–80
Oil products (olive, safflower, and vegetable oil; margarine) (45/92)	1.25–46.5	< 2.0–115
Breakfast cereals (fruit flavored, granola with raisins) (10/88)	0.48–1.77	< 2.0–50
Salads (macaroni and potato salad, coleslaw, buttermilk-type salad dressing) (11/56)	0.25–4.5	< 2.0–8.0

Source: FDA 2006.
[a] The most recent year for which data were available as of April 2008.
[b] In calculating the means, FDA assigned a level of 0 to results below the limit of detection.
[c] Data presented in ppm in source document.

2.4 General population exposure

This section provides information related to exposure to styrene for the general population. Because most exposure estimates are not specific to a particular country, and international data often are utilized in the assessments, some exposure estimates are presented that are not specific to the United States. This information may be useful in identifying the factors that impact exposure for the general population in the United States and elsewhere.

Sources of exposure to styrene include inhalation (including indoor and outdoor ambient air, smoking, and inhalation of environmental tobacco smoke), dermal exposure, and consumption of contaminated food, water, and other beverages. Increased exposures

could occur for persons living in urban areas or close to major sources of styrene (*e.g.*, highly trafficked areas, industrial production facilities, or hazardous waste sites). Another potential source of exposure to the general public is exposures from inadvertent chemical spills (NRC 2008).

Exposure from ingestion of municipal drinking-water supplies probably is negligible, as styrene has been detected in drinking-water monitoring surveys infrequently, and when it has been detected, it has generally been at low levels. Ingestion of contaminated groundwater, however, could result in significant exposure (ATSDR 1992, Howard 1989). Because of the low occurrence and levels of styrene in water, Cohen *et al*. (2002) suggested that dermal exposures from water could be assumed to be negligible.

Fishbein (1992) estimated the relative significance of different routes of exposure to styrene to illustrate the importance of both indoor air exposures and occupational exposures. The results of this analysis are presented in Table 2-10. These results show that occupational exposures result in the highest styrene intakes; however, the general public also is exposed to styrene.

Table 2-10. Daily styrene intakes for the general public from various sources

Exposure situation	Styrene concentration (ppb)[a]	Nominal daily intake (μg)	Daily intake for 70 kg adult (μg/kg bw/day)
Within 1 km of the production unit[b]	7.0	600	9
Polluted urban atmosphere[b]	4.7	400	6
Urban atmosphere[b]	0.07	6	0.09
Indoor air[b]	0.07–11.7	6–1,000	0.09–14
Polluted drinking water (2 L per day)	0.2	2	0.03
Cigarette smoke (20 cigarettes per day)	4.7–11.3	400–960	6–14

Source: Fishbein 1992, Luderer *et al.* 2005.
[a] Presented in units of $\mu g/m^3$ in source document.
[b] Based on the assumption of a daily breathing volume of 10 m^3 at work or 20 m^3 at home or in an urban environment.

Health Canada estimated daily styrene intakes from various media for different age groups of the Canadian general population (Table 2-11). As seen in Table 2-11, food and indoor air are the largest contributors to exposure for non-smokers. In this assessment, an

indoor air concentration of 0.28 μg/m^3 was used, which is similar to the low-end value used by Fishbein (1992) (above). Estimates for exposure from smoking assumed that styrene content in mainstream cigarette smoke is 10 μg/cigarette [which was half the level of the minimum of the range of values presented by Fishbein above], and that 20 cigarettes per day are smoked. [Note that the exposure values in this table are presented in units of μg/kg b.w. and are not directly comparable to most of the data in this section, which are presented in units of μg/d.]

Table 2-11. Estimated daily intake of styrene from various media for Canadians of different ages

Estimated intake (μg/kg b.w./day)					
Medium	**0–6 mo**	**7 mo–4 yr**	**5–11 yr**	**12–19 yr**	**20–70 yr**
Air ambient	0.004– 0.11	0.006 – 0.15	0.007–0.17	0.006–0.14	0.005–0.13
Indoor	0.07	0.09	0.10	0.09	0.08
Drinking water	< 0.005–0.03	< 0.00 –0.02	< 0.002–0.08	< 0.001 – 0.006	< 0.001–0.005
Soil	< 0.00005	< 0.0004	< 0.00001	< 0.000004	< 0.00003
Food	< 0.58	< 0.53	< 0.30	< 0.15	< 0.11
Total intake (not including cigarettes)	< 0.66– < 0.79	< 0.63– < 0.79	< 0.41– < 0.58	< 0.25– < 0.39	< 0.20– < 0.33
Intake by cigarette smokers	NA	NA	NA	3.51	2.86

Source: HealthCanada 1993.
NA = not assessed.

Smoking can result in styrene exposure both directly for smokers and indirectly through environmental tobacco smoke (ETS) (*i.e.*, side-stream smoke and exhaled cigarette smoke). Exposure to styrene has been estimated to be six times higher for smokers than for nonsmokers (Cohen *et al.* 2002). As noted in Table 2-10, Fishbein (1992) estimated a styrene exposure of 400 to 960 μg/day based on 20 cigarettes per day and inhalation of 20 to 48 μg of styrene per cigarette. Tang *et al.* (2000) estimated an additional styrene intake (above the daily intake from air and food) of 100 μg/day, based on 20 cigarettes per day and inhalation of 5 μg styrene per cigarette. In a study on toxic compounds in ETS, Bi *et al.* (2005) presented styrene levels in ETS for three types of cigarettes: ultra low tar (146 μg/cigarette), full flavor low tar (159 μg/cigarette), and full flavor (119

μg/cigarette). Charles *et al*. (2007) found similar levels in ultra-low–nicotine (90 μg/cigarette), standard nicotine (160 μg/cigarette), and low nicotine (162 μg/cigarette) cigarettes. Miller *et al.* (1998) assessed the contribution of ETS to total styrene exposure. The results of this study showed that for the study population, 15% of a passive smoker's and 8% of a non-smoker's daily intake of styrene was attributable to ETS. (A passive smoker does not smoke but spends at least some time in a closed area with a smoker.) Charles *et al*. (2007) found that side-stream smoke emissions greatly exceeded main-stream smoke emissions. Analysis of emissions from a low-nicotine cigarette showed main-stream smoke emissions of 11 μg/cigarette and side-stream smoke emissions of 147 μg/cigarette.

Exposure to styrene from food ingestion has been estimated in a number of studies. Lickly *et al.* (1995b) estimated U.S. dietary styrene exposure at 9 μg/day. A study of residents of the United Kingdom in 1983 showed styrene intake from food ingestion of 1 to 4 μg/day (Lickly *et al.* 1995b). Another study of U.K. residents, which employed a probabilistic modeling approach, estimated median daily intake of styrene from food contaminated with food contact materials to be 0.039 μg/kg b.w. per day for adults, 0.048 μg/kg b.w. per day for youths, and 0.035 μg/kg b.w. per day for seniors (Holmes *et al.* 2005). Another study, based on the average *per capita* consumption figures of the general population in Germany, estimated the average annual styrene intake via food consumption to be roughly 0.8 to 4.5 mg/person [2.2 to 12.3 μg/d] (Tang *et al.* 2000). Using the same data and applying a U.S. FDA consumption factor based on the assumption that only 10% of foods are packaged in polystyrene, an annual intake of 0.08 to 0.45 mg/person [0.22 to 1.23 μg/d] was estimated. Other studies have estimated annual per-person styrene intake via food ingestion ranging from 0.26 to 14.8 mg [0.7 to 40.5 μg/d] (Tang *et al.* 2000).

In an exposure and risk assessment for styrene, Cohen *et al*. (2002) based their assessment only on inhalation and food ingestion exposures, assuming that exposure from ingestion and dermal contact with water is negligible due to its limited occurrence and low levels. Estimated airborne concentrations for this study are presented in Table 2-12.

Table 2-12. Estimated annual and lifetime exposures for the general public

Exposure scenario	Maximum annual average (ppb)	Lifetime average (ppb)
Typical ambient exposure	1	1
High-end ambient exposure	5	5
Exposure to styrene from smoking	6	< 6
Living 100 m from a 100,000-lb/yr emission facility (high-exposure scenario, 95th percentile individual)	12	2.8
Living at the point of greatest exposure in the vicinity of a 1 million-lb/yr emission facility (high-exposure scenario, 95th percentile individual)	700	219

Source: Cohen *et al.* 2002.

In assessing exposure from food, the authors first estimated exposure from naturally occurring substances. Using upper-end concentration data from the literature the authors estimated that exposure for the U.S. population would be less than 0.2 μg/kg of food ingested. They then assumed food consumption of 3 kg/day and arrived at a daily styrene ingestion rate of 0.6 μg/day. The authors used the exposure level of 9 μg/day presented by Lickly *et al*. (1995b) (see above) for ingestion of food contaminated through migration from polystyrene packaging. The authors concluded that 10 μg/day is a reasonable upper bound estimate for total dietary intake, which they noted corresponds to 0.2 μg/kg b.w. for a 70-kg adult.

Several studies have confirmed styrene exposure to the general public through the use of biological monitoring. In one study, styrene was detected in all eight human breast milk samples from women in four U.S. cities (Howard 1989). In a National Human Adipose Tissue Survey by the U.S. EPA in 1982, styrene was detected in wet adipose tissue with a frequency of 100% at concentrations ranging from 8 to 350 ppb. Styrene also has been detected in the general population in blood at a mean concentration of 0.4 μg/L and in exhaled breath at mean concentrations of 0.7 to 1.6 $\mu g/m^3$ (ATSDR 1992).

Blood styrene levels were assessed in the Priority Toxicant Reference Range Study conducted as part of the Centers for Disease Control and Prevention's (CDC) Third National Health and Nutrition Examination Survey (NHANES III). The Priority Toxicant

Reference Range Study assessed blood levels of numerous VOCs among a nonstatistical subsample of NHANES III participants aged 20 to 59 (NCHS 2000). Samples in which styrene was below the detection limit were assigned a value of 0.013 μg/L, which is equal to the lower detection limit (0.019 μg/L) divided by the square root of 2. Of 624 samples, styrene levels were below the detection limit in 78 samples (12.5%), and ranged from 0.019 to 4.006 μg/L in 546 samples. The mean styrene level for all 624 samples was 0.07 μg/L, the median was 0.04 μg/L, and the 95th percentile value was 0.18 μg/L (Ashley *et al.* 1994, Sexton *et al.* 2005). [The means obtained by assigning a value of 0.013 μg/L to samples below the detection limit or by assigning a value of 0.00 μg/L to these samples were the same after rounding.] It is important to note that because this study was conducted with a nonstatistical subsample of NHANES III participants, statistical weights cannot be assigned, and estimates for the total U.S. population therefore cannot be calculated (NCHS 2000).

Sexton *et al.* (2005) examined blood levels of styrene and several other VOCs over a two-year period in more than 150 children from two poor, minority neighborhoods in Minneapolis, MN. For styrene, the mean concentration was 0.17 μg/L, the median concentration was 0.12 μg/L, and the 95th percentile concentration was 0.50 μg/L. The authors compared these levels with the NHANES styrene levels for adults (0.07 for mean, 0.04 for median, 0.18 for 95th percentile) and noted that the elevated levels of styrene in children were unexpected. The authors noted that the children's VOC exposures and related blood levels were the product of concentrations in the air, water, soil, dust, food, beverages, and consumer products with which they came into contact through everyday activities and behaviors, but they were unable to explain the elevated styrene levels for the children. The authors made note of the fact that the NHANES data included smokers, which made the elevated styrene levels in children even more surprising, and they ultimately concluded that the source of the children's exposure to styrene needed further investigation. In a follow-up study, Sexton *et al*. (2006) measured blood levels of several chemicals, including styrene, in 43 children aged 3 to 6 from a socioeconomically disadvantaged neighborhood in Minneapolis, MN. The mean and

median for the group was 0.07 ng/mL (μg/L) and the 95th percentile was 0.11; levels that the authors noted were similar to the NHANES levels.

2.5 Occupational exposure

In some workplace settings, styrene air levels can exceed by several orders of magnitude the levels generally found in outdoor and indoor air. [Because of this, air levels in this section are presented in parts per million [ppm] rather than parts per billion [ppb], which were used in the outdoor air and indoor air sections above.] Workers in a number of different industries can be exposed to styrene. Workers can be exposed during the production and use of styrene monomer, polystyrene, glass fiber–reinforced plastics, styrene-butadiene rubber and other styrene-based polymers, and in other miscellaneous occupations (ATSDR 1992, IARC 2002). The National Occupational Hazard Survey, conducted by the National Institute for Occupational Safety and Health (NIOSH) from 1972 to 1974, estimated that 292,018 employees were occupationally exposed to styrene at 16,394 facilities. The National Occupational Exposure Survey, conducted by NIOSH from 1981 to 1983, estimated that 333,212 employees (including 86,902 women) were occupationally exposed to styrene at 24,702 facilities in 154 industries. The U.S. Bureau of Labor Statistics (BLS) uses the Standard Occupational Classification (SOC) system to classify workers into occupational categories for labor statistics analyses. Workers are classified into one of over 820 occupations according to their occupational definitions. In May 2006, the BLS estimated that 32,510 workers were employed in SOC code 51-2091 — Fiberglass Laminators and Fabricators (defined as “laminate layers of fiberglass on molds to form boat decks and hulls, bodies for golf carts, automobiles, or other products”). “Ship and boat building” was the largest subcategory in this SOC segment, with 12,910 employees (BLS 2007). No information was found on the numbers of workers in the other industrial segments mentioned above.

Based on the breakdown of industrial sectors used for the review of the human cancer data in Section 3, this section provides information on the following three major industrial settings: the reinforced-plastics industry, the styrene-butadiene rubber industry, and the styrene monomer and polymer industry. The section concludes with a discussion of other miscellaneous occupational exposures. Section 3 of this document reviews

epidemiologic studies from the United States and other countries; therefore, this section reports information identified for occupational exposures either in the United States or in other countries.

2.5.1 The reinforced plastics industry

IARC (2002) has noted that the highest occupational exposures to styrene, with respect to the number of employees and exposure levels, occur in the fabrication of objects such as boats, car and truck parts, tanks, tubs, and shower stalls from glass fiber–reinforced polyester composite plastics. [“Reinforced plastics” is the term generally used in this document, but other terms used to describe this industry include fiberglass-reinforced plastics, fiberglass-reinforced polyester resin, reinforced plastic composites, and laminated plastics.] Styrene has been noted to be the principal VOC present in resins used in the reinforced plastics industry (Hillis 1997, Hillis and Davis 1995, MnTAP 2007, Säämänen 1998), and according to the U.S. EPA, styrene is the main hazardous air pollutant in the reinforced plastic composites industry (EPA 2003). Table 2-13 at the end of this section provides both styrene air levels and levels of biological markers for the studies where they were assessed. The text discusses the major issues related to these studies. This section presents information on worker exposures in the reinforced plastics industry. Because much of the discussion on exposure levels involves process and job descriptions, the section begins with an overview of two of the main processes used in the production of glass fiber–reinforced plastic products. This is followed by historical industry-wide exposure levels, levels based on the product being manufactured or the manufacturing process employed, and levels based on specific jobs or tasks. Studies that assessed respirator use are then discussed briefly followed by a short discussion of studies that measured styrene-7,8-oxide concurrently with styrene. The section concludes with a discussion of studies that have assessed dermal exposure in the reinforced plastics industry.

2.5.1.1 Process description

Two main processes are used to produce glass fiber–reinforced plastic composite products: an open-mold process and a closed-mold process. In general, large glass fiber–reinforced plastic composite products are built using an open-mold process. With this

process, a mold of the desired final product is sprayed with a layer of gel coat, which is pigmented polyester resin that hardens and becomes the smooth outer surface of the product (CDC 2007, EPA 1997a). After the gel coat has hardened, it is coated with a "skin coat" of chopped glass fibers and polyester resin and then rolled with a roller to compact the fibers and remove air bubbles. After the skin coat has hardened, additional layers of fiberglass cloth and chopped glass fibers saturated with resin are added until the desired final thickness is obtained. These layers of resin and chopped glass fibers are usually applied with either spray equipment (spray-up), such as a chopper gun, or by hand using a bucket and brush or paint-type roller (lay-up). The layers are compressed by rolling the surface, usually by hand. After the resin has cured, the part is removed from the mold and the edges are trimmed to the final dimensions. Exposure to styrene can occur at all steps of the open-mold process, as both the gel coat and the polyester resin contain substantial levels of styrene.

Closed molding is the name given to fabrication techniques in which reinforced plastic parts are produced between the halves of a two-part mold or between a mold and a flexible membrane (EPA 1997a). There are a number of different processes that are considered a closed-mold process. One example, called "resin transfer molding," uses half molds that are closed before resin injection and curing, thereby potentially reducing exposure during this stage (CDC 2007, EPA 1997a). However, prior to injection of the resin, the process is similar to the open-mold process. First, a gel coat is applied to the interior surface of both molds to provide a smooth finish on all external surfaces after the cure. Following application of the gel coat, dry fiber reinforcement mat is placed into the mold before closing. After the mold is closed, resin and initiator are pumped into the mold cavity by a pressure pump. Curing takes place while the mold is closed. While it is expected that exposures will be limited during the final resin transfer and curing inside the closed mold, the closed-mold process does not control emissions and potential exposures during gel-coat application. In this process, gel coating is generally done inside a spray booth. Usually, one worker sprays the gel coat inside the spray booth, and another worker applies the fiberglass inside the mold, closes the mold, and sets up the mold for resin injection. Both of these jobs have the potential for exposure to styrene. Other closed-mold processes include vacuum bagging, vacuum-assisted resin transfer molding,

and compression molding (EPA 1997a). The common feature of these processes is that at least part of the process occurs in a closed system, thereby potentially allowing for the control of styrene emissions and exposure.

2.5.1.2 Historical industry-wide exposure levels

Historically, the highest styrene exposure levels for reinforced-plastics workers has been in the range of several hundred parts per million, although declining levels have been reported to have occurred over the past several decades. In a study published in 1981 of 12 plants manufacturing fiberglass in Washington state, 40% of 8-hour samples contained styrene at over 100 ppm (IARC 2002). Kolstad *et al.* (1994) presented styrene exposure level data based on 2,473 personal air samples taken at workplaces in Denmark between 1964 and 1988 (see Section 3.1.4). Mean styrene levels were 180 ppm for 1964 to 1970, 88 ppm for 1971 to 1975, and 43 ppm for 1976 to 1988. In an extension of the 1994 study, Kolstad *et al*. (2005) used data from 2,454 personal measurements of airborne styrene taken by the Danish National Institute of Occupational Health between 1960 and 1996 to develop a semi-quantitative method to assess occupational exposure to styrene in the reinforced plastics industry when individual data are not available. Calendar year was reported to be a strong and consistent predictor of styrene exposure levels; along with product produced (boats) and process (hand and spray lamination). For the time period between 1960 and 1990, styrene exposure levels were reported to have declined by 7% annually. Kolstad *et al*. calculated exposure scores for individuals based on estimated exposure probability and exposure levels. Styrene exposure scores for 1,519 subjects based on short-term and long-term samples are presented in Figures 2-4 and 2-5 below. Exposure scores declined by about 10-fold from the 1960s to the 1990s, and the authors noted that this reflected a decline in styrene exposure levels. This study did not assess dermal exposures.

Similarly, Kogevinas *et al.* (1994a) reported that in Denmark, average exposure levels among laminators were about 200 ppm in the late 1950s, about 100 ppm in the late 1960s, and about 20 ppm in the late 1980s (see Section 3.1.5). In a review of 16 studies by Pfäffli and Säämänen (1993), a similar temporal decline in exposure levels was seen

in air concentration data from the United States, Canada, Japan, and Europe from the 1950s through 1992.

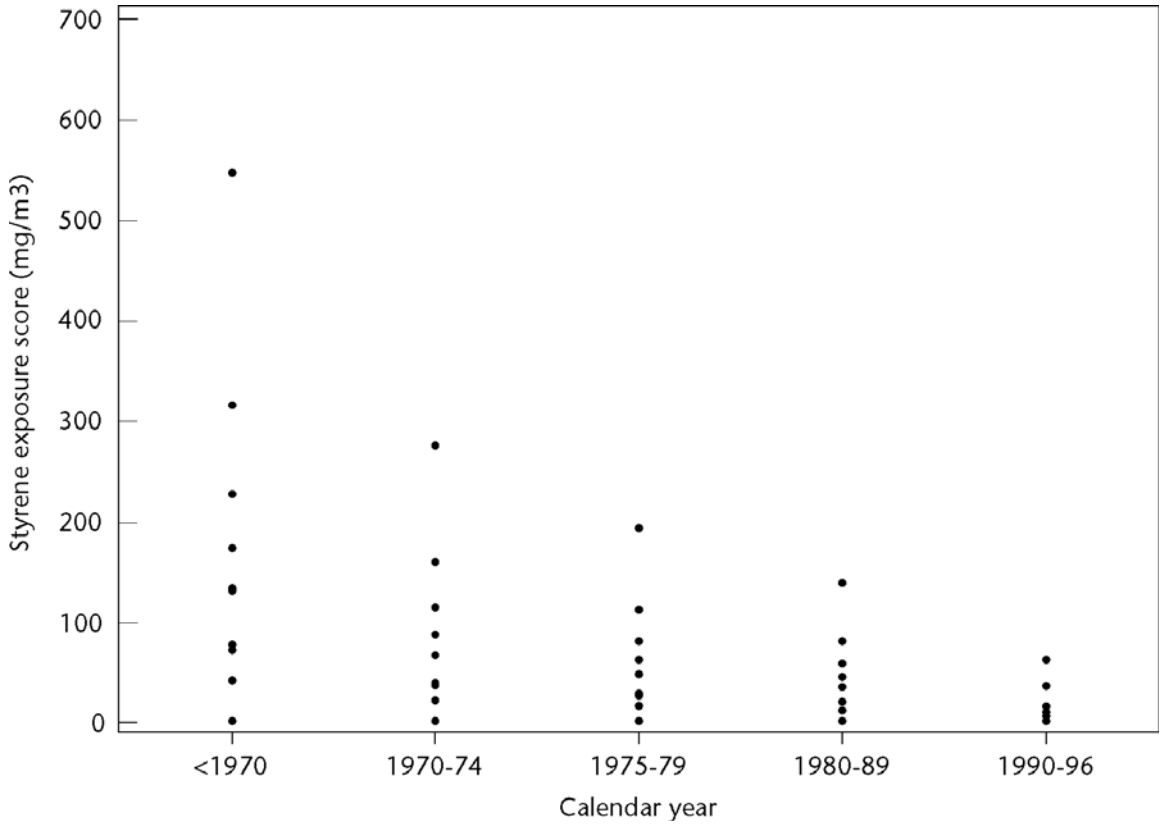


Figure 2-4. Temporal decline in styrene exposure scores (short-term samples [< 1 h]) estimated for reinforced plastics workers
Source: Kolstad *et al.* 2005.

Jensen *et al.* (1990) presented data based on 2,528 measurements of styrene at 256 workplaces in Denmark between 1955 and 1988. Annual mean concentrations decreased from a high of 1,005 mg/m^3 [236 ppm] in 1964, to a low of 88 mg/m^3 [21 ppm] in 1988. Period-specific mean concentrations were 714 mg/m^3 [168 ppm] for 1955 to 1970, 274 mg/m^3 [64 ppm] for 1971 to 1980, and 172 mg/m^3 [40 ppm] for 1981 to 1988. For the entire 1955 to 1988 period, the mean concentration was 265 mg/m^3 [62 ppm].

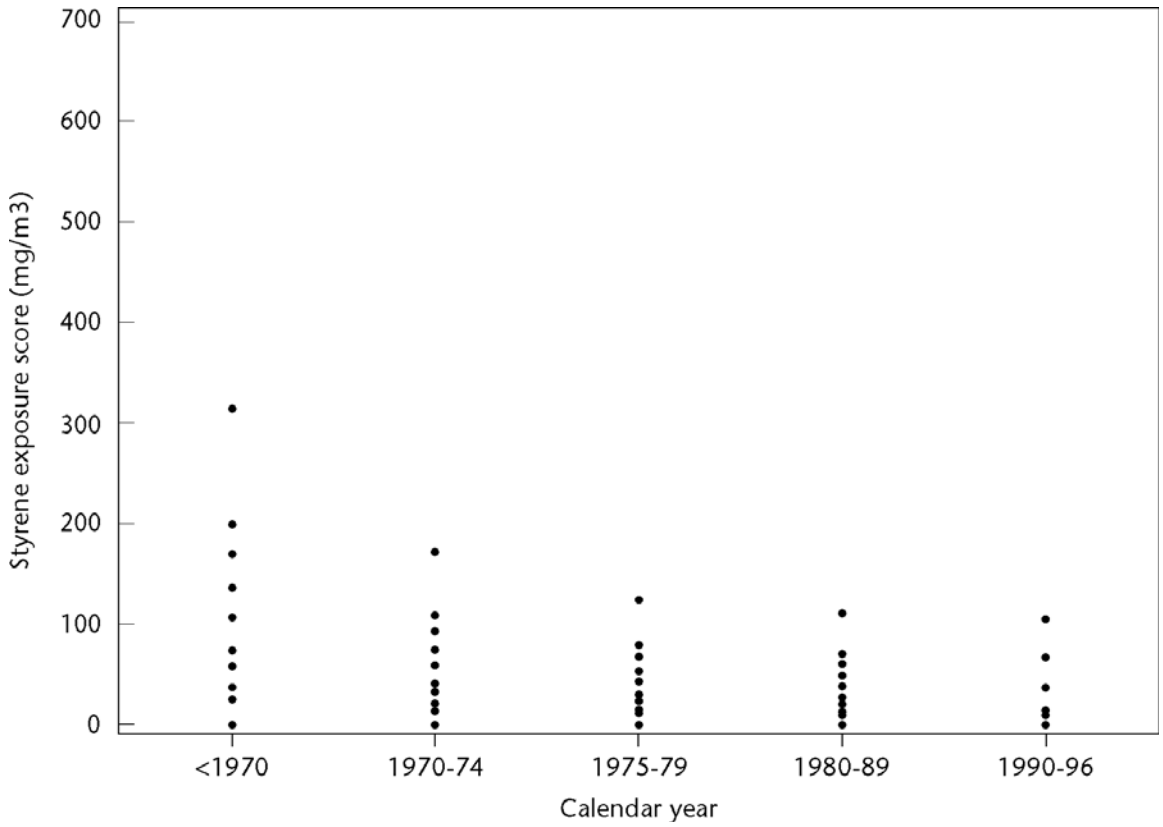


Figure 2-5. Temporal decline in styrene exposure scores short-term samples [< 1 h]) estimated for reinforced plastics workers
Source: Kolstad *et al*. 2005.

Serdar *et al.* (2006) noted that in general, air levels of styrene (and styrene-7,8-oxide) appear to have decreased substantially in this industry from the 1980s through the early 2000s. Although styrene exposures have been reduced substantially through improved work practices and products (Kolstad *et al.* 1994), Miller *et al.* noted in 1994 that peak concentrations could still exceed 100 ppm, especially during the manufacture of large items, and this can be seen in Table 2-13 for measurements taken through the 1990s.

2.5.1.3 Exposure levels based on product or manufacturing process

Several factors influence the level of styrene in workplace air in the reinforced plastics industry. Chief among these are the surface area of the product being manufactured, and the manufacturing process used. In general, the manufacture of products with large surface areas, such as boats, truck parts, and shower stalls, by the open-mold process results in higher exposures than manufacture of smaller products by a closed-mold process. Lemasters *et al.* (1985) found average styrene exposure levels associated with

open-mold processes (24 to 82 ppm) to be 2 to 3 times those associated with closed-mold processes (11 to 26 ppm).

[There does not appear to be a clear distinction as to what products result in the highest exposure levels.] Although boat building has often been associated with higher levels, this has not always been the case. IARC (2002) reported that boat building involves higher styrene exposures than any other industrial sector. Kolstad *et al.* (2005) reported that product (boats), process (lamination), and calendar year were the major determinants of styrene exposure, and the authors noted that styrene exposure levels were higher by a factor of 1.6 to 1.7 at companies producing boats than at companies producing other products. As discussed below, other studies often reported higher levels for the manufacture of products other than boats.

A survey by the State of California Division of Occupational Safety and Health ranked the highest worker exposure levels by industry based on geometric mean exposure levels. Tub or shower-stall manufacture was highest at 53.6 ppm, followed by camper manufacturing (41.0 ppm), boat manufacturing (29.1 ppm), spa manufacturing (25.8 ppm), miscellaneous manufacturing (22.0 ppm), and tank manufacturing (12.7 ppm).

In an assessment of 328 fiberglass-reinforced plastics workers in 13 similar sized plants in the Pacific Northwestern United States, Serdar *et al*. (2006) noted that exposures to styrene varied greatly based on the product being manufactured. Air levels of styrene decreased with product categories in the order of RVs > pipe and tank > hot tub > boat building ~ truck manufacture. In an assessment of 17 U.S. reinforced plastics workplaces, Luderer *et al.* (2004) reported mean air levels by products manufactured in the order of truck and RV > bathtub > pipe, tank > boat.

2.5.1.4 Exposure levels based on job or task

Exposure to styrene also varies with the type of job or task performed. (See Section 2.6 for a description of biological indices used to measure styrene exposure.) Using 4,689 urine samples obtained from reinforced plastics workers in the region of Emilia Romagna, Italy, Galassi *et al.* (1993) found that hand laminators had the highest mean mandelic acid (MA) levels (682 mg/g creatinine), followed by spray laminators (404

mg/g creatinine), rollers (327 mg/g creatinine), semiautomatic process operators (243 mg/g creatinine), and non-process workers (186 mg/g creatinine). Similarly, mean styrene air levels were highest for hand laminators (227 mg/m^3 [53 ppm]); however, rollers were exposed to the next highest levels (163 mg/m^3 [38 ppm]), followed by spray laminators (134 mg/m^3 [31 ppm]), and semiautomatic process operators (85 mg/m^3 [20 ppm]). The authors noted a clear positive correlation between air levels and urinary mandelic acid (MA) levels. The authors also noted that air levels generally decreased with time.

Based on U.S. data published in 1985, IARC (2002) reported mean personal breathing-zone air concentrations for four boat fabrication tasks as follows: hull lamination, 331 mg/m^3 [77 ppm]; deck lamination, 313 mg/m^3 [73 ppm]; small parts lamination, 193 mg/m^3 [45 ppm]; and gel coating, 202 mg/m^3 [47 ppm]. Measured concentrations across all jobs or tasks ranged from 7 to 780 mg/m^3 [1.6 to 183 ppm].

Based on results from one study that assessed 8-hour TWA exposure levels across job categories and tasks within the reinforced plastics industry, spray-up/lay-up operators had the highest exposure levels with a mean of 256 mg/m^3 [60 ppm] and a range of 21 to 511 mg/m^3 [5 to 120 ppm] (IARC 2002). For other jobs, which included gel coating and 7 other job categories, the mean exposure levels ranged from ≤ 43 to 192 mg/m^3 [≤ 10 to 45 ppm], and overall exposure levels ranged from 0 to 362 mg/m^3 (0 to 85 ppm). Based on a study of 237 workers in 30 Finnish reinforced plastics plants, Nylander-French *et al*. (1999) reported that the highest 8-hour TWA styrene exposure level across 6 categories of tasks was for hand lamination of large objects (156 mg/m^3 [37 ppm]) and that these levels were approximately 4-fold higher than exposure levels for foremen (42.6 mg/m^3 [10 ppm]), which was the group with the lowest exposure levels. Overall, the mean 8-hour TWA concentration of styrene was 122 mg/m^3 [28.6 ppm] with a range of 3.2 to 608 mg/m^3 [0.75 to 142.7 ppm].

In an assessment of 48 workers in a U.S. reinforced plastics boat manufacturing facility, Rappaport *et al*. (1996) grouped workers into 10 categories based on the job performed. They reported that spray operators had the highest exposure levels with a mean of 141

mg/m^3 [33 ppm] while laminators had the second highest levels at 130 mg/m^3 [30.5 ppm]. Overall, the mean styrene air level was 64.3 mg/m^3 [15.1 ppm] with a range of 0.978 to 235 mg/m^3 [0.23 to 55.14 ppm].

In Finnish factories that produced boats, car parts, and building materials from polyester-based reinforced plastics, average styrene concentrations in personal air samples were 133 ppm for hand applicators and 130 ppm for spray applicators (IARC 1994b). Based on 2,528 measurements of styrene at 256 workplaces in Denmark between 1955 and 1988, Jensen *et al*. reported that the work processes associated with the highest concentrations were spray-up and unspecified lay-up operations.

Fairfax and Swearngin (2005) reported the results of a 2003 planned inspection by OSHA of a facility that produced bathtubs and shower stalls by spray application of styrene-based gel coat and polyester resin. The OSHA inspection consisted of full-shift personal air monitoring of two gel-coat operators and two chopper gun operators and showed TWA styrene levels ranging from 64 ppm (chopper gun operator) to 318 ppm (gel-coat operator). Follow-up measurements (personal and area) were made in June and October of 2003. June TWA levels ranged from 66 ppm to 100 ppm (both were gel-coat operators), and October levels ranged from 45 ppm (gel-coat operator) to 110 ppm (chopper gun operator). Ultimately, switching to a product containing less styrene and operational changes were needed to reduce exposure levels below the regulatory limit of 100 ppm.

2.5.1.5 Studies assessing respirator use

Nakayama *et al.* (2004) evaluated the efficiency of various types of respiratory protective equipment by comparing styrene exposure levels to urinary levels of mandelic acid among 39 workers in 5 fiberglass-reinforced plastics factories. For the 39 workers, the area monitoring results ranged from not detected (< 0.5 ppm) to 67.4 ppm. (The authors noted, however, that in a gel-coating operation that used an agent containing 40% to 50% styrene, styrene levels as high as 2,000 ppm were present for short periods of time.) Personal monitoring levels for the 39 employees ranged from 0.7 ppm to 318.8 ppm, and creatinine-adjusted mandelic acid levels ranged from 10 to 1,606 mg/g creatinine. The authors concluded that the efficiency of disposable gauze type and dust-proof respirators

was nearly zero and that the efficiency of half-mask respirators was highly dependent on the frequency of cartridge replacement.

Inaoka *et al*. (2002) conducted a study to examine winter-summer levels and associations in airborne styrene exposure concentrations and end-of-shift urinary mandelic acid levels, and the protective effect of disposable particulate respirators containing charcoal fiber (charcoal mask) and a charcoal granule cartridge mask (gas mask). The study was conducted in the winter of 1997 to 1998 and involved 105 workers in 10 small-sized fiberglass-reinforced plastics production facilities in Japan. Airborne styrene was measured using passive samplers attached to the workers' collars, near the neck, and urine samples were collected at the end of the shift for MA analysis. The authors concluded that the charcoal mask provided little protection from styrene exposure, but that the gas mask prevented 45% to 49% of styrene from being inhaled. The authors also concluded that individual exposures to styrene and urinary mandelic acid levels did not differ by season.

In a study to assess the capacity of negative-pressure half-mask respirators to protect workers from styrene exposure, personal sampling of styrene air levels and urinary styrene levels was performed on seven fiberglass-reinforced plastics workers over two successive weeks: one week without respirators and the following week with respirators (Gobba *et al.* 2000). During the study period, mean TWA workplace air concentrations of styrene were estimated for Monday, Wednesday, and Friday for both morning shifts and afternoon shifts. These six mean TWA air concentrations ranged from 169.2 to 335.7 mg/m^3 [39.6 to 78.6 ppm] with a range across all measurements of 70.9 to 488.1 mg/m^3 [16.6 to 114.5 ppm]. Mean urinary styrene levels ranged from 80 to 96.1 μg/L without the use of respirators and from 31.5 to 47.2 μg/L with the respirators. The estimated reduction of urinary styrene levels due to the respirators ranged from 30% to 90% with a mean of 60%.

2.5.1.6 Styrene-7,8-oxide exposures

Workers in the reinforced plastics industry can potentially be exposed to styrene-7,8-oxide as well as styrene, and several studies have measured exposure levels for both

substances showing styrene levels at two to three orders of magnitude higher than styrene-7,8-oxide levels. Serdar *et al*. (2006) (discussed above) noted that styrene levels in full-shift personal breathing-zone samples were roughly 500-fold higher than styrene-7,8-oxide levels.

For 237 workers in 30 Finnish reinforced plastics plants, the mean 8-hour TWA concentration of styrene was 122 mg/m^3 [28.6 ppm] with a range of 3.2 to 608 mg/m^3 [0.75 to 142.7 ppm], while the mean concentration of styrene-7,8-oxide was 0.183 mg/m^3 [0.04 ppm] with a range of 0 to 0.883 mg/m^3 [0 to 0.21 ppm] (Nylander-French *et al.* 1999). The authors found that styrene-7,8-oxide levels were positively correlated with styrene exposure levels.

In a boat manufacturing factory in the United States, Rappaport *et al*. (1996) reported a mean styrene air level of 64.3 mg/m^3 [15.1 ppm] with a range of 0.978 to 235 mg/m^3 [0.23 to 55.14 ppm] and a mean styrene-7,8-oxide level of 0.159 mg/m^3 [0.037 ppm)] with a range 0.0134 to 0.525 mg/m^3 [0.003 to 0.12 ppm]. IARC (1994b) reported that for the 19 most heavily exposed workers in a boat manufacturing company, the mean styrene exposure level was 64 mg/m^3 [15 ppm] while the mean styrene-7,8-oxide level was 0.14 mg/m^3 [0.03 ppm]. In Finnish factories that produced boats, car parts, and building materials from polyester-based reinforced plastics, average styrene concentrations in personal air samples were 133 ppm for hand applicators and 130 ppm for spray applicators; the corresponding average styrene-7,8-oxide levels were 0.04 ppm and 0.12 ppm.

Table 2-13 presents styrene levels in ambient air in the reinforced plastics industry. For this table and the remainder of the tables presenting occupational exposure levels, the number of samples is presented when it was available in the referenced source.

Table 2-13. Summary of measured styrene exposure levels in the reinforced plastics industry.

Industrial segment (year measured)	Specific job, process, or production area	Styrene air levels Mean (range) (ppm)	Biological levels Mean (range)	Reference (Location)
Boat, hot tub, pipe and tank, RV, and truck mfg. (1996–1999)	*Tasks across all categories* *Production area* boat building hot tub pipe and tank RV truck	9.14[a] (< 1–117) (N = 328) 4.41[a] (< 1.0–68.6) (N = 138) 6.85[a] (< 1.0–62.9) (N = 13) 16.0[a] (1.67–79.0) (N = 50) 45.1[a] (6.74–117) (N = 48) 4.22[a] (< 1.0–46.3) (N = 76)	S_B: 0.083 (< 0.001–2.05) mg/L (N = 295) SO_B: 0.069 (< 0.05–0.135) µg/L (N = 212)	Serdar *et al.* 2006 (USA)
Small sized facilities, products not specified (1997–1998)	Numerous tasks including hand/spray laminators, rollers, semiautomatic process workers, and non-process workers	10.3–35.9 (NR)	MA: 70-350 mg/g	Inaoka *et al*. 2002 (Japan)
Industry wide (1960–1996)	*Overall short-term*[b] *Product* boats other products *Task* hand or spray lamination other *Year* before 1970 1970–1974 1975–1979 1980–1989 1990–1996	59.7 (ND–639)[c] (N = 2,208) 100.5 (ND–639)[c] (N = 670) 42.0 (ND–563)[c] (N = 1,537) 61.0 (ND–639)[c] (N = 2,074) 39.0 (0.7–177)[c] (N = 133) 173.3 (11.7–639)[c] (N = 113) 94.2 (2.3–587)[c] (N = 425) 71.9 (0.9–403)[c] (N = 360) 41.1 (ND–456)[c] (N = 954) 19.8 (0.2–171)[c] (N = 355)	–	Kolstad *et al.* 2005 (Denmark) [note that this study is an extension of Kolstad *et al*. 1994 presented below]
Boat mfg. (NR)	hand-spraying lamination	8.71 (0.47 to 126) (N = 45)	MA+PGA: 300 (10.2 to 1,856) mg/g CR (N = 95) PHEMA: 0.9 (0.01 to 3.29) mg/g CR (N = 45)	Migliore *et al.* 2006a (Italy)

Industrial segment (year measured)	Specific job, process, or production area	Styrene air levels Mean (range) (ppm)	Biological levels Mean (range)	Reference (Location)
			VPT: 1.9 (0.1 to 7.74) mg/g CR (N = 45)	
Tub/shower mfg. (2001–2003)	chopper gun and gel coating operations	34–318 (NR) (N = 49)	–	Fairfax and Swearngin 2005 (USA)
Boat mfg. (1998)	not specified	52.3 (0.3–133.5) (N = 73)	MA: 288.5 (1.0–1,813.2) mg/g CR (N = 73) PGA: 123.8 (4.4–481.5) mg/g CR (N = 73)	Ma *et al.* 2005 (Japan)
Not specified (1998–1999)	low-exposure jobs (N = 55) high-exposure jobs (N = 53)	4.1 (0.07–22.5)[c] 9.3 (1.1–13.3)[c] (LWAE) 3.7 (0.07–15.1)[c] 22.6 (13.6–30.2)[c] LWAE	MA: 1.0 (0.1–2.7) mmol/g CR [152 (15.2–410) mg/g CR] MA: 0.8 (0.1–2.1) mmol/g CR [121.6 (15.2–319) mg/g CR]	Iregren *et al.* 2005a (Sweden)
Boat mfg. (NR)	fibrous glass department lamination department	42.5 (7.27–84.7) (N = 53) 71.6 (10.32–183) (N = 67)	–	Okun *et al.* 1985, Ruder *et al.* 2004 (USA)
Various types of products (NR)	various processes	NR (ND–67.4) (area) (N = 29) NR (0.7–318.8) (personal) (N = 39)	MA: NR (10–1,606) mg/g-creatinine (N = 39)	Nakayama *et al.* 2004 (Japan)
Boat, tub, truck/RV, pipe/tank mfg., and boat repair (NR)	not specified	9[a] (< 1–142) (N = 402)	S_B: 0.0089 (< 0.001–2.05) mg/L (N = 302)	Luderer *et al.* 2004 (USA)
Tubs/showers, sheet paneling, and other unspecified products at 4 facilities (NR)	open-mold, closed-mold, and press-methods using spray/chopper guns, sheet press, and hand lay-up and die molding	9.2–55 (0.1–140.3) (N = 99)	MA: 190–1,740 (< 10–6,980) mg/g CR PGA: 80–490 (< 10–2,250) mg/g CR (N = 104 for both MA and PGA)	Dalton *et al.* 2003, Lees *et al.* 2003 (USA)
Boats, tanks,	various processes and jobs	1.1–15.6[d] (NR)	MA: 24.6–227 (NR) mg/g CR	Liljelind *et al.* 2003

Industrial segment (year measured)	Specific job, process, or production area	Styrene air levels Mean (range) (ppm)	Biological levels Mean (range)	Reference (Location)
bathroom fixtures (NR)	(N = 12 workers with 3 to 4 matched samples)			(Sweden)
Industry-wide large open-mold spray-up/lay-up operations (NR)	*Overall* *Product specific mfg.* tub/shower camper boat spa miscellaneous tank	43 (0.2–288) 53.6[d] (NR) 41.0[d] (NR) 29.1[d] (NR) 25.8[d] (NR) 22.0[d] (NR) 12.7[d] (NR)	–	IARC 2002 (USA)
Boat mfg. (NR)	hull lamination deck lamination small parts lamination gel coating	77.7 (1.64–183) (N = 168) 73.4 (12.2–160) (N = 114) 45.3 (7.98–130) (N = 70) 47.4 (5.4–103) (N = 45)	–	IARC 2002 (USA)
Not specified (1967–1978)	spray-up/lay-up 8 other job categories	60[e] (5–120) ≤ 10–45 (0–85)	–	IARC 2002 (USA)
Not specified (NR)	hand rolling, spraying, finishing	39.6–78.6 (16.6 to 114.5) [N = 84]	S_U: 31.5–96.1 (7.4–133.3) µg/L [N = 41]	Gobba *et al.* 2000 (Italy)
Various industries, primarily reinforced-plastics production (1973–1983)	primarily laminators		MA: 2.3[a] (0–47) mmol/L [350 (0–7,144 mg/L)] (N = 10,336)	Anttila *et al.* 1998 (Finland)
Boats, containers, pipes and tubes, small parts, sheets, and vehicle parts (1988–1990)	*Jobs* hand lamination, large objects hand lamination, small objects spraying and gel coating automated lamination	 36.6 (NR)[c] [N = 216] 35.2 (NR)[c] [N = 98] 30.5 (NR)[c] [N = 22] 13.1 (NR)[c] [N = 46]	–	Nylander-French *et al.* 1999 (Finland)

Industrial segment (year measured)	Specific job, process, or production area	Styrene air levels Mean (range) (ppm)	Biological levels Mean (range)	Reference (Location)
	assembly and mold preparation foreman *Products* boats small and form parts sheets, elements, car parts containers and tubes	11.7 (NR)[c] [N = 56] 10.0 (NR)[c] [N = 28] 30.97 (NR)[c] [N = 274] 31.44 (NR)[c] [N = 68] 21.19 (NR)[c] [N = 78] 24.87 (NR)[c] [N = 56]		
Boat mfg. (1987–1988)	laminator (16 workers) service (6 workers) mold repair (3 workers) patcher (8 workers) painter (6 workers) spray operator (1 workers) mechanic (4 workers) deck rigger (2 workers) assembly (1 workers) supervisor (1 workers)	30.5 (NR)[c] 6.55 (NR)[c] 27.45 (NR)[c] 3.19 (NR)[c] 6.5 (NR)[c] 33.08 (NR)[c] 1.95 (NR)[c] 0.99 (NR)[c] 0.41 (NR)[c] 5.89 (NR)[c] (N = 2 to 7 samples per worker)	– exhaled styrene = 1.76 (0.007– 8.12) mg/m^3 (N = 1 to 7 measurements per worker)	
Industry-wide (1964–1970) (1971–1975) (1976–1988)	not specified (N = 2,473 personal air samples 1964–1988)	180 (NR) 88 (NR) 43 (NR)	–	Kolstad *et al.* 1994 (Denmark)
Industry-wide	overall TWA concentrations spray-up/lay-up operators TWA	1–200 60[e] (5–120)	–	Wong *et al.* 1994 (USA)
Industry-wide (NR)	*Process workers* hand laminators rollers spray laminators semiautomatic process	48[c] (NR) (N = 1,305) 53[c] (NR) (N = 1,028) 38[c] (NR) (N = 40) 31[c] (NR) (N = 166) 20[c] (NR) (N = 71)	MA: 631 (NR) mg/g CR (N = 2,820) MA: 682 (NR) mg/g CR (N = 2,386) MA: 327 (NR) mg/g CR (N = 63) MA: 404 (NR) mg/g CR (N = 250) MA: 243 (NR) mg/g CR (N = 121)	Galassi *et al.* 1993 (Italy)

Industrial segment (year measured)	Specific job, process, or production area	Styrene air levels Mean (range) (ppm)	Biological levels Mean (range)	Reference (Location)
	operators *Non-process workers*	16.7[c] (NR) (N = 159)	MA: 186 (NR) mg/g CR (N = 762)	
Industry-wide (1955–1970) (1971–1980) (1981–1988) (1955–1988)	not specified	168 (NR) (N = 227) 64 (NR) (N = 1,117) 40 (NR) (N = 1,184) 62 (21–236) (N = 2,528)	–	Jensen *et al.* 1990 (Denmark)
Industry-wide (1969–1981)	open-mold press-mold	3–82 (NR) (N = 1,084) 4–26 (NR) (N = 402)	–	Lemasters *et al.* 1985 (USA)

CR = creatinine; LWAE = lifetime weighted average exposure; MA = mandelic acid; mfg. = manufacturing; NA = not assessed; ND = not detected; NR = not reported; PGA = phenylglyoxylic acid; PHEMA = phenylhydroxyethylmercapturic acids; S_B = blood styrene level; S_U = urinary styrene level; VPT = vinylphenols; SO_B = blood styrene-7,8-oxide level.
[a]Median.
[b]Data also presented in source document for long-term samples showing levels that were generally around one-half the levels reported here.
[c]Presented in mg/m^3 in source document.
[d]Geometric mean(s).
[e]Reported as typical level.

2.5.1.7 Dermal exposure

[The potential exists for dermal exposure in the workplace to styrene or styrene-containing materials in either aqueous or vapor form. While dermal exposure can occur in any industry that uses styrene, the potential is especially high in the reinforced-plastics industry during lamination operations and is thus discussed in this section.]

In a study assessing the potential routes of exposures to styrene in the glass fiber–reinforced-plastics industry, workers were equipped with various types of protective equipment: total protection with an insulating suit and mask, respiratory equipment only, skin protection only, and no protection (Limasset *et al.* 1999). Urinary styrene excretion levels did not differ significantly between the group with total protection and the group with only respiratory equipment. The authors concluded that percutaneous absorption was not a particularly important pathway for styrene absorption in the glass fiber–reinforced polyester industry. These results were similar to those of Brooks *et al*. (1980). Although Limasset *et al*. and Brooks *et al*. concluded that dermal absorption was not a particularly important pathway for styrene exposure, Brown (1985) noted that when factors such as skin hydration and its condition, individual and anatomical site variations, and the permeability-enhancing effects of other compounds are considered, skin absorption can be seen as a significant exposure route for pollutants. Luderer *et al.* (2005) similarly noted that although some studies reported limited skin absorption of styrene in workers, prolonged and repeated exposure to liquid styrene could result in exposures equivalent to the lower range of doses received by inhalation.

In an experiment to assess skin absorption of the hand and forearm of liquid styrene or styrene in aqueous solution, Dutkiewicz and Tyras (1968) reported that very short exposure of the hands to liquid styrene (a few minutes) or longer exposure (about one hour) to styrene in aqueous solution can result in the absorption of as much styrene as an 8-hour average air concentration of 0.05 mg/L [50 mg/m^3 or 11.7 ppm]. They also noted that urinary mandelic acid does not provide a reliable index of absorption if there is simultaneous skin and lung exposures.

Eriksson and Wiklund (2004) used a patch sampling technique to study potential dermal exposure to styrene in the glass fiber–reinforced-plastics industry. The legs, arms, and

upper back had the highest exposures. Potential total-body styrene exposure ranged from 544 to 17,100 mg/hour, with a geometric mean of 3,780 mg/hour. Wieczorek (1985) investigated dermal absorption of styrene vapors in four volunteers exposed to styrene at 1,300 to 3,200 mg/m^3 in a study chamber. The authors calculated that the dermal absorption of styrene vapors contributed about 5% to the amount absorbed in the respiratory tract under the same experimental conditions based on comparative mandelic acid and phenylglyoxylic acid urinary levels. The authors presented a dermal vapor absorption coefficient of 0.022 m^3/hour based on the results of this study.

Minamoto *et al*. (2002) performed patch tests on 29 workers (22 of whom had reported having skin problems) employed in small-to-medium-sized reinforced-plastics plants in Japan. The authors reported one positive test result for styrene.

See Section 5.1.1.1 for more information on dermal absorption of styrene.

2.5.2 The styrene-butadiene rubber (SBR) industry

Styrene-butadiene rubber is a copolymer of butadiene and styrene in which the styrene units (approximately 25%) are distributed at random among butadiene units (75%) in molecular chains (IISRP 1973). Styrene-butadiene rubber is the most widely used synthetic rubber in the world, accounting for 46% of world consumption of synthetic rubber and more than 26% of all rubber, natural or synthetic, in 2006 (ICIS 2008). Over 70% of styrene-butadiene rubber is consumed in the manufacture of tires and tire products; however, non-tire uses are growing with applications including conveyor belts, gaskets, hoses, floor tiles, footwear, and adhesives. This section provides a brief overview of the two main styrene-butadiene rubber production processes (emulsion process [Section 2.5.2.1] and solution process [Section 2.5.2.2]), followed by a discussion of exposure levels that have been found within the styrene-butadiene rubber industry (Section 2.5.2.3).

2.5.2.1 Emulsion process styrene-butadiene rubber production

The steps involved in synthetic rubber production include: (1) preparing the input materials to the required form, (2) mixing the input materials together to react, (3) stopping the reaction when the polymer chains have reached the appropriate length, (4)

recovering any unused material, and (5) extracting and cleaning the rubber product (IISRP 1973, Lattime 2000). Figures 2-6 and 2-7 provide process flow diagrams for the polymerization and finishing stages within styrene-butadiene rubber production. Styrene, butadiene, and other chemicals used in the production process are stored in tanks at the production facility (tank farm) until they are pumped to reaction vessels. Butadiene is a gas at normal temperature; however, it can be liquefied under pressure and is usually handled in this form in the production of styrene-butadiene rubber. The butadiene, styrene, water, emulsifier, and other materials are pumped into reaction vessels and vigorously stirred to produce an emulsion. Current emulsion process methods employ a cold production process whereby a combination of reducing and oxidizing agents are used as catalysts: these catalysts are added to the first reaction vessel with the styrene/butadiene mixture and polymerization begins immediately. Polymerization continues as the emulsion passes through a series of reaction vessels. It is then brought to a stop by the addition of a polymerization-inhibiting chemical called a shortstop. Typically dimethyldithiocarbamate (DMDTC) is used as the shortstop. At this stage, the rubber is in the form of minute rubber polymers suspended in the emulsion. The shortstopped material is transferred to large vessels referred to as blowdown tanks, then pumped into flash tanks where any unreacted butadiene is evaporated off, and then pumped to a stripping column where unreacted styrene is removed by steam distillation. At this point, the material is in the form of a relatively pure synthetic latex which is accumulated in large storage tanks. Roughly 10% of all styrene-butadiene rubber is sold in latex form for use as carpet backing, latex foam, and other products. While still in latex form, extender oil and antioxidants may be added if extended rubber is being produced. The latex is then passed into a tank where an acid brine is injected and the mixture is stirred. During this process, the rubber coagulates in the form of a fine crumb, which is then washed in fresh water, dewatered, and pressed into bales as a finished product.

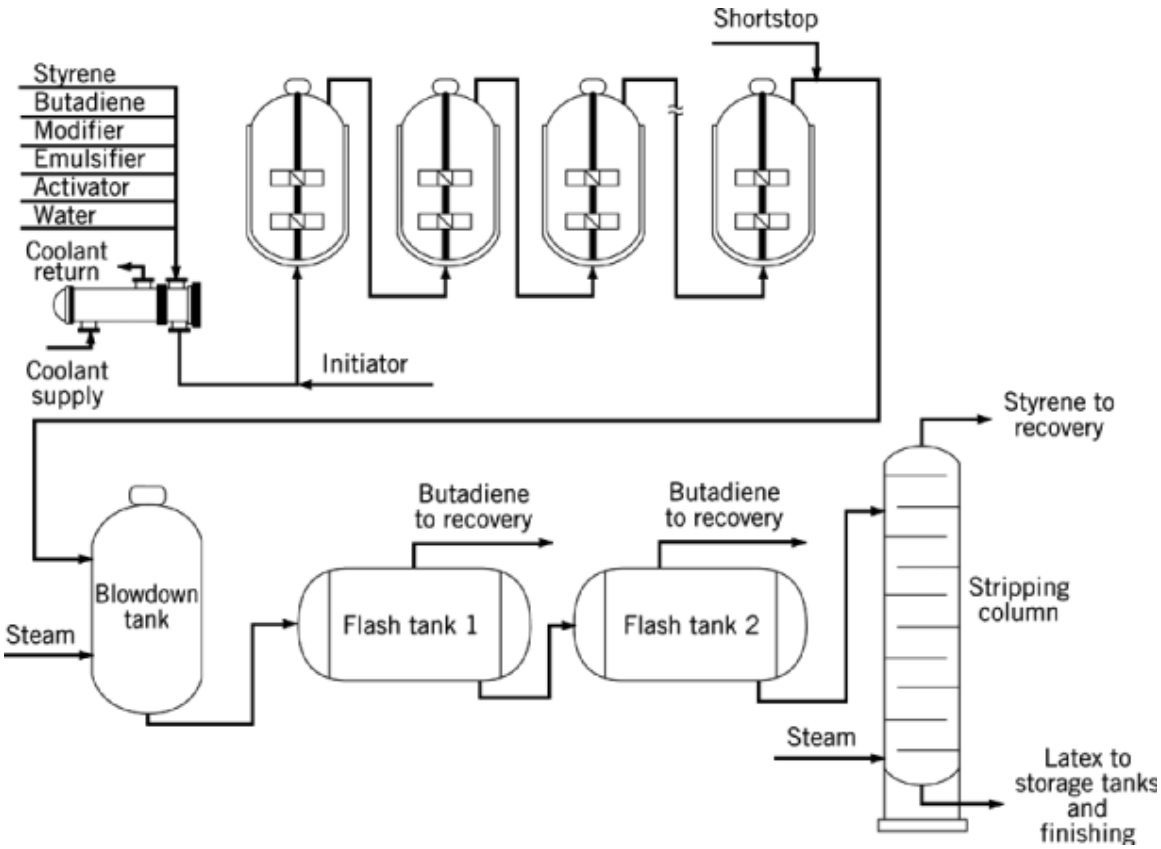


Figure 2-6. Typical continuous emulsion styrene-butadiene rubber polymerization process

Source: Lattime 2000

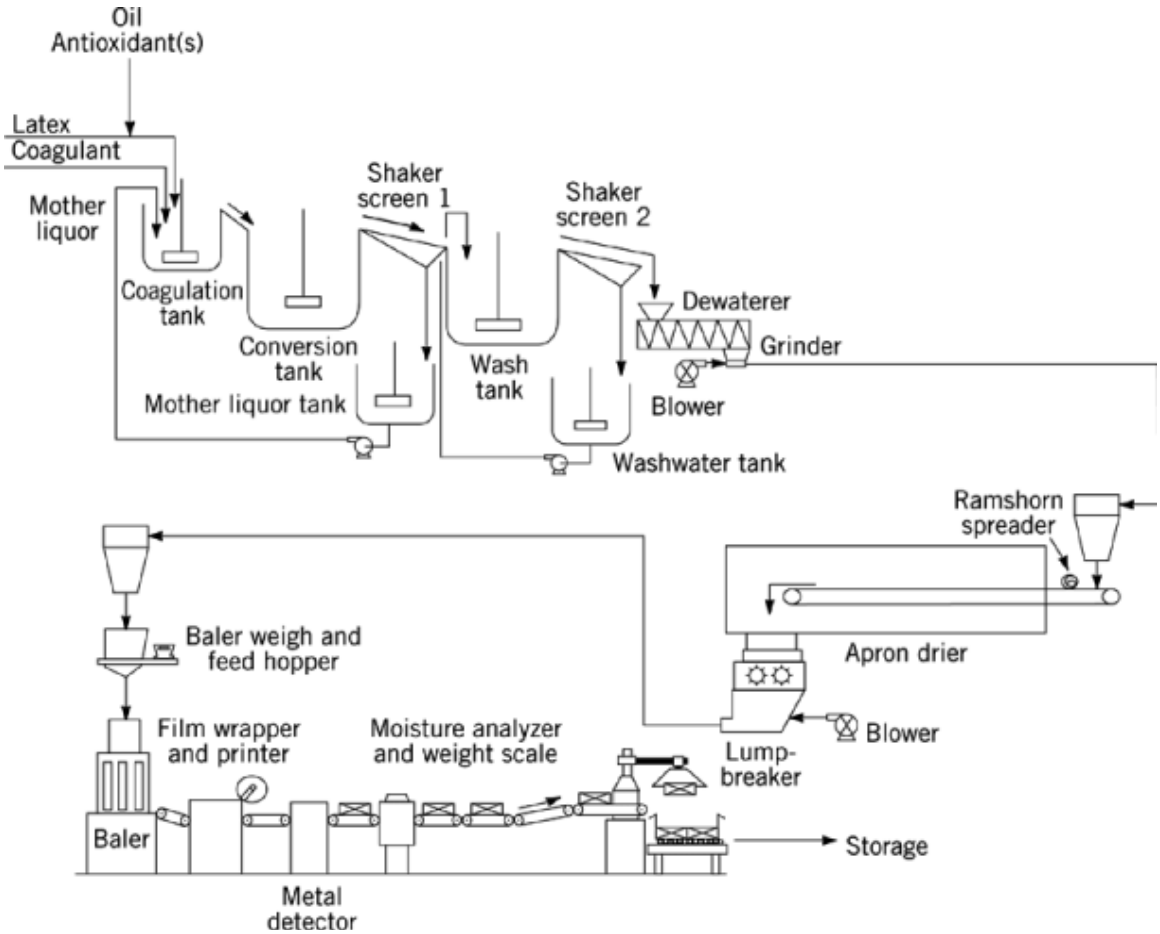


Figure 2-7. Typical emulsion styrene-butadiene rubber finishing process
Source: Lattime 2000

2.5.2.2 Solution process styrene-butadiene rubber production

The major difference between emulsion process, and solution process styrene-butadiene rubber production lies in the co-polymerization chemistry. In contrast to the emulsion process, where the feedstocks are suspended in a large proportion of water in the presence of an initiator, the solution styrene-butadiene rubber copolymerisation process proceeds in a hydrocarbon solution in the presence of an organometallic complex (Lattime 2000). Solution styrene-butadiene rubber involves termination-free, anionic polymerization initiated by alkyl lithium compounds, usually *n*-butyl lithium (NBL). The use of alkyl lithium compounds is due to the solubility of this class of organometallics in the hydrocarbon solvents (such as *n*-hexane or cyclohexane) that are used in the process. Solution styrene-butadiene rubber production allows great variation in producing different types of polymers. By adding certain chemicals, such as ethers, tertiary amines,

and phosphates, random distribution of the co-monomers is achieved, making it possible to control polymer composition, monomer distribution sequence, microstructure, molecular weight, molecular weight distribution, and polymer chain structure. Lattime (2000) noted that once the ability to control the randomization of solution styrene-butadiene rubber was better understood and established, it began to displace some of the market share of emulsion process styrene-butadiene rubber by allowing for more variation and fine tuning of the styrene-butadiene rubber properties. Figure 2-8 provides a process flow diagram for solution styrene-butadiene rubber production.

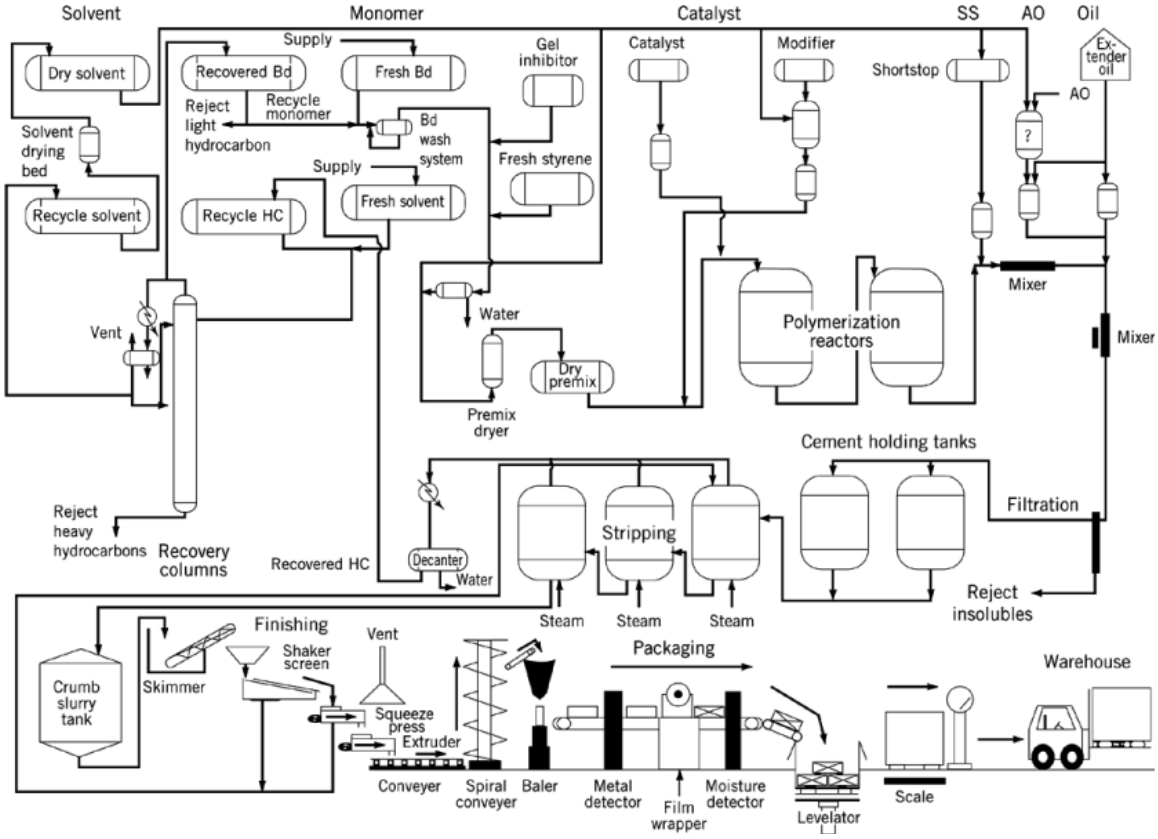


Figure 2-8. Solution styrene-butadiene rubber manufacture by continuous process
Bd=butadiene, HC=hydrocarbon, AO=antioxidant, and SS=shortstop.
Source: Lattime 2000

2.5.3 Styrene-butadiene rubber production exposure levels

Generally lower levels are seen in the styrene-butadiene rubber industry than the glass fiber–reinforced-plastics industry, although significant exposures to workers can still

occur. As can be seen in Table 2-14, mean levels reported for this industry generally are less than 15 ppm. IARC (2002) reported concentrations below 0.15 ppm for vulcanization and extrusion processes involving styrene-butadiene rubber, and exposure to end-users of styrene-butadiene rubber would likely be even lower. Exposure estimates from the series of cancer epidemiology studies of styrene-butadiene rubber production workers in North America (the United States and Canada) (see Section 3.2) are included in Table 2-14. The highest levels of exposure were reported for recovery operators, unskilled maintenance workers, and laboratory technicians (Macaluso *et al*. 2004). Macaluso *et al.* (1996) reported that mean styrene exposure levels declined from approximately 2 ppm in the 1940s and 1950s to 0.5 ppm or less in the 1990s; however, workers identified as recovery operators were frequently exposed to levels of 50 ppm or higher during the 1940s and 1950s.

In a mortality survey of workers engaged in the production of styrene-based products, including styrene-butadiene latex, Ott *et al.* (1980) noted that exposure concentrations were highest for workers involved in the initial phases of the production process (i.e., loading, operating, and cleaning polymerization reactors) (see Section 3.3). Other data presented by Ott *et al*. on the styrene monomer and polymer industry are in Table 2-15.

Mean concentrations from personal air samples taken in 1979 at a U.S. styrene-butadiene rubber production plant were 1.69 ppm for factory service workers and 13.7 ppm for tank farm workers (IARC 2002). It was noted that mean levels were below 1 ppm for other departments.

IARC (2002) reported styrene air concentrations of 61 to 146 ppb [0.06 to 0.15 ppm] in the curing area of the press room of a company that produced car tires. Area air samples taken in plants producing shoe soles, tire re-treading, and electrical cable insulation showed styrene levels from 2 to 500 $\mu g/m^3$ [0.0005 to 0.12 ppm] in vulcanization areas and from 0 to 20 $\mu g/m^3$ [0 to 0.005 ppm] in extrusion areas (IARC 2002). Table 2-14 summarizes workplace exposure levels for the styrene-butadiene industry.

Anttinen-Klemetti *et al.* (2006) assessed exposure to 1,3-butadiene and styrene in three plants manufacturing styrene-butadiene co-polymers in Finland. A total of 885 air

samples were collected from the breathing zone of 28 workers over four months. For styrene, 336 samples (38%) were below the limit of quantitation (0.007 ppm), 548 samples (62%) were between the limit of quantitation and 20 ppm [which is the Finnish TLV], and one sample (0.1%) exceeded 20 ppm [actual level not reported]. Mean styrene levels for the three plants were 0.024, 0.07, and 0.188 ppm.

Table 2-14. Summary of occupational styrene exposure levels in the styrene-butadiene rubber industry

Type of plant (Year measured)	Specific job/process/production area	Mean (range), ppm	Reference (Location)
Synthetic rubber production (1974–1977)	tank farm operator recovery operator finishing operator maintenance, skilled maintenance, unskilled laboratory technician all workers	0.7 (0.14–3.35)[a] 0.61 (0.12–4.4) 1.0 (0.0–3.7) 0.14 (0.02–0.8) 2.9 (0.11–12.3) 0.6 (0.09–2.86) 0.77 (0–12) (N = 214 total)	Crandall 1981 (NIOSH survey) as reported by Macaluso *et al.* 2004
Synthetic rubber (estimated exposures for comparison with NIOSH data from 1974–1977)	tank farm operator recovery operator finishing operator maintenance, skilled maintenance, unskilled laboratory technician all workers	1.7 (1–2.4)[a] 5.5 (2.9–8.5) 1.4 (1.0–1.7) 0.9 (0.6–1.2) 9.4 (5.4–14) 4.6 (3.5–6.9) 1.3 (1.2–1.4)	Macaluso *et al.* 2004
Styrene-butadiene rubber plant (NR)	across all production areas plant 1 plant 2	 0.94 (0.03–6.46) (N = 55) 1.99 (0.05–12.2) (N = 35)	Meinhardt *et al.* 1982 (USA)
Styrene-butadiene rubber plant (NR)	concentrations across five plants	3.53 (0.29–6.66)[b] (N = 3,649)	Matanoski *et al.* 1993 (USA and Canada)
Synthetic rubber industry (NR)	medians across 48–164 specific tasks/plant plant 1 plant 2 plant 4 plant 5 plant 7 plant 8	 3.0 2.6 2.7 2.7 3.0 3.0	Macaluso *et al.* 1996
Styrene-butadiene latex mfg. plants (1965) (1973)	high-exposure jobs during initial phases of production	4–22 (NR)[a] 3.6–7.3 (NR)	Ott *et al.* 1980 (USA)

Type of plant (Year measured)	Specific job/process/production area	Mean (range), ppm	Reference (Location)
Styrene-butadiene rubber plant (1979)	tank farm workers factory service workers other various departments	13.7 (NR) 1.69 (NR) (< 1.0) (N = 159 total)	IARC 2002 (USA)
Car tire production facility (NR)	curing area of press room	NR (0.06–0.15)	IARC 2002 (NR)
Shoe sole, tire retreading, and electrical cable insulation plants (NR)	extrusion process vulcanization processes	NR (0–0.005)[c] NR (0.0005–0.12)[c]	IARC 2002 (NR)
Styrene-butadiene latex production (1997)	not specified	0.024–0.188 (NR)[d] (N = 885; 336 samples below LOQ of 0.007 ppm)	Anttinen-Klemetti *et al.* 2006 (Finland)

LOQ = level of quantitation; NR = not reported.
[a] Numbers in parentheses represent 90% uncertainty interval.
[b] Levels presented are the reported mean value across 5 plants and the range of the mean values for the individual plants.
[c] Levels presented in $\mu g/m^3$ in source document.
[d] Range of mean styrene levels for three factories.

2.5.4 The styrene monomer and polymer industry

Polystyrene can be manufactured by either a batch polymerization or a continuous polymerization process (Ott *et al.* 1980). As reported by Ott *et al.* (1980), the earliest manufacturing process was the batch polymerization method at Dow Chemical, but that process was discontinued by 1951 with the possible exception of some experimental work on the method. In this method, the benzene-washed polymerization containers were filled with styrene monomer, sealed, and heated during the polymerization step. A batch process for suspension polymerization was described in the European Union Risk Assessment Report for styrene (EU 2002) in which styrene is dispersed in water in the presence of 0.01% to 0.05% suspending agent and a mixture of organic peroxides or other polymerization initiator. The reaction mix is heated until polymerization is substantially complete, and the resulting polymer beads are washed, dried, and pelletized.

The continuous polymerization process is described briefly in Section 2.2 (Production) and illustrated in Figure 2-9. As shown below, styrene monomer, which may be mixed

with a nonpolymerizable volatile diluent, is passed through a series of two or more reactors with heat exchange zones and agitators (EU 2002). The mixture resulting from this process contains approximately 85% styrene together with residual monomer and is transferred to a low-pressure, high-temperature devolatilization tower (labeled as "Separation Section" below) for removal and recycling of the unreacted monomer and diluent. The hot polystyrene product is cooled and cut into pellets.

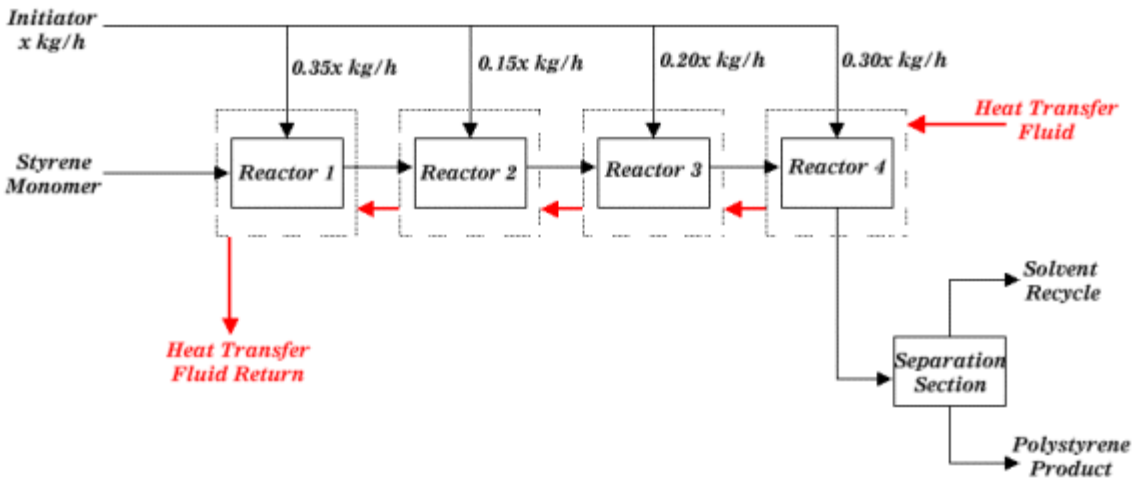


Figure 2-9. Polymerization of polystyrene by the continuous process
Source: Cheresources 2008a.

Styrene exposure levels in the styrene monomer and polymer production industries are generally much lower than levels in the reinforced-plastics industry, and levels in this industry have declined over the past several decades. Table 2-15 provides measured air levels that have been reported in the literature.

Nicholson *et al.* (1978) presented data collected in 1974 by NIOSH at a plant that produced styrene monomer and polystyrene (see Section 3.3). Styrene exposure levels generally ranged from 5 to 20 ppm in high-exposure areas and were below 1 ppm in low-exposure areas; however, it was noted that wide excursions from these values occurred at specific locations.

In the breathing zone of a U.S. plant producing ester-styrene co-polymers, styrene concentrations ranged from nondetectable (< 1 ppb) to 19.8 ppm with an average of about

0.6 ppm. It was noted that the highest concentrations occurred during styrene unloading operations (IARC 2002).

Some older studies, however, have reported styrene levels in excess of 20 ppm. IARC (2002) presented data from 8-hour personal air samples taken in 1978, 1979, and 1980 in U.S. workplaces where polystyrene and acrylonitrile-butadiene-styrene molding was performed. Styrene levels were 17 to 285 mg/m^3 [4.0 to 67 ppm] in 1978, 1.4 to 3.2 mg/m^3 [0.33 to 0.75 ppm] in 1979, and below the detection limit of 0.01 mg/m^3 [0.002 ppm] in 1980.

Other studies have shown styrene levels that varied mainly with the operations being performed. Based on five separate industrial hygiene surveys conducted between 1962 and 1976, Ott *et al.* (1980) reported that TWA exposure levels were below 10 ppm for all jobs in the styrene monomer production industry, including one where excursions were measured as high as 50 ppm during the drumming of styrene. In batch polymerization processes in 1942, styrene levels ranging from 5 to 88 ppm were measured during filling operations; however, subsequent continuous polymerization processes generally resulted in personal exposure measurements of 1 ppm or less. Residual styrene monomer concentrations ranging from less than 1 to 16 ppm have been reported in the vicinity of polystyrene compounding rolls (used for production of sheets of polystyrene) (Ott *et al.* 1980).

In a U.S. styrene production and polymerization plant, styrene levels were highest in the polymerization, manufacturing, and purification areas, where mean exposure levels ranged from 8 to 35 ppm (IARC 2002). For maintenance, laboratory, and packaging operations, styrene levels were less than 5 ppm. It was noted that urinary mandelic acid and blood styrene were not detectable in most samples from workers at the end of a shift.

Thiess and Friedheim (1978) presented styrene air concentrations from periodic air sampling for 1975 to 1976 in a styrene manufacturing plant and a polystyrene manufacturing plant, both in Germany (see Table 2-15). As part of the same study, worker exposures were assessed through air monitoring and assessment of urinary mandelic acid levels in three plants where polymers containing free styrene were

converted into finished or semi-finished articles. Of the 83 urine samples, 20 were below the 10 mg/L detection limit, 4 were greater than 500 mg/L and 5 were greater than 1,000 mg/L [concentrations estimated from a graph]. The authors noted that the facilities with higher styrene air concentrations had a correspondingly higher number of employees with high mandelic acid levels.

Table 2-15. Summary of occupational styrene exposure levels in the styrene monomer and polymer industry in the United States

Type of plant (year monitored)	Specific job/process/production area	Mean (range) (ppm)	Reference (Location)
Styrene monomer and polystyrene plant (1978)	low-exposure areas high-exposure areas	< 1.0[a] 5.0–20.0[a] [est. levels]	Nicholson *et al.* 1978 USA
Ester-styrene co-polymer production (NR)	not specified	0.6 (< 0.001–19.8) (N = 50)	IARC 2002 (USA)
Polystyrene and ABS molding facility (1978) (1979) (1980)	not specified	4–67[b,c] 0.33–0.75 < 0.002	IARC 2002 (NR)
Styrene monomer production (1962–1976)	not specified	< 10 (up to 50)	Ott *et al.* 1980 (NR)
Batch polymerization (1942)	filling operations	NR (5–88)	Ott *et al.* 1980 (NR)
Polystyrene production (NR)	compounding and rolling	NR (< 1–16)	Ott *et al.* 1980 (NR)
Styrene production and polymerization (NR)	polymerization, manufacturing, and purification areas maintenance, laboratory, and packaging operations	8–35 (NR) < 5 (NR)	IARC 2002 (USA)
Plant producing styrene monomer and polystyrene (1975–1976)	styrene monomer production polystyrene production	NR (< 0.01 to 6.84) (N = 60) NR (< 0.01 to 46.92) (N = 70)	Thiess and Friedheim 1978 (Germany)
3 production facilities where styrene polymers are converted into other products (1975–1976)	facility A facility B facility C	< 50–70 (NR) (N = 93) 50–300 (NR) (N = 68) 60–300 (NR) (N = 68)	Thiess and Friedheim 1978 (Germany)

ABS = acrylonitrile-butadiene-styrene; NR = not reported.
[a] Noted as "generally" at these levels although wide excursions were seen.
[b] Presented in units of mg/m^3 in source document.
[c] No additional information provided to ascertain if the data were a range of means or the full range of sampling points.

2.5.5 Other occupational exposures

Styrene can occur at low levels in a vast array of industries and occupations. This section provides information on exposure potential for a number of different industries, focusing on literature published since the most recent IARC review in 2002. Much lower levels were reported for occupational exposures outside of the industrial settings presented above (*i.e.*, levels in the low ppb) and this section again presents air levels in ppb rather than ppm.

Due to the potential VOC production from the irradiation of organic matter and the potential for direct uses and leaks of VOCs during the operation of nuclear power plants, Hsieh *et al.* (2006) investigated the composition and concentrations of a number of VOCs in air-conditioned office space and low-level waste repository sites of three nuclear power plants in Taiwan in 2000. Depending on the type of building being assessed, concentrations were presented only for the 10 or 20 most abundant chemicals. The average styrene level at one low-level waste building was 4.01 ppb by volume (ppbv). Styrene levels were lower in the administrative buildings of the three power plants (0.21, 0.65, and 1.07 ppbv). While the authors noted that concentrations of aromatics, chlorofluorocarbons, and chlorinated hydrocarbons were markedly higher in the low-level waste buildings compared with administrative buildings, there was no indication that styrene levels were higher.

Lee *et al.* (2006) used personal and area sampling to investigate levels of styrene and other pollutants in seven photocopy centers in Taiwan in 2002 and 2003. Concentrations across the seven facilities ranged from 0.5 to 107 $\mu g/m^3$ [0.0001 to 0.025 ppb]. Styrene exposure levels from copied paper have been estimated by the U.S. EPA (EPA 2008b). Based on the low air concentrations that were estimated [levels not provided], the U.S. EPA concluded that copied paper does not pose a health risk.

Styrene and 38 other air toxics were measured in worksite air of 11 companies in a petrochemical complex in Taiwan between 1997 and 1999 (Chan *et al.* 2006). The mean concentration was either 9.6 ppb or 13.3 ppb depending on how samples that were below the limit of detection were treated in the calculation of the mean.

In a study assessing indoor air quality in printing plants, indoor air was monitored for several VOCs in seven printing plants of varying sizes in Hong Kong (Leung *et al.* 2005). There were a total of 10 sampling points across the seven facilities, and styrene levels were below detection [0.1 ppb] for four sampling points, while 8-hour TWA values ranged from 1.4 to 7.1 ppb for the remaining 6 sampling points.

Using personal monitoring, Thorud *et al.* (2005) assessed exposure levels of styrene and several other VOCs during surface coating with acid-curing lacquers and paints in 27 Norwegian woodworking and furniture manufacturing facilities during the late 1990s. Styrene had a geometric mean level of 0.10 ppm [100 ppb] for nine samples and a range from 0.01 to 1.47 ppm [10 to 1,470 ppb].

In a study to estimate the level of protection that tollbooths afford workers, Sapkota *et al.* (2005) measured styrene air levels in indoor air and outdoor air of a Baltimore Harbor Tunnel tollbooth in the summer of 2001. For indoor air, the mean styrene concentration was 0.45 $\mu g/m^3$ [0.11 ppb] with a range of 0.05 to 1.19 $\mu g/m^3$ [0.01 to 0.28 ppb], and for outdoor levels the mean concentration was 0.61 $\mu g/m^3$ [0.14 ppb] with a range of 0.05 to 1.68 $\mu g/m^3$ [0.01 to 0.39 ppb].

In an assessment of occupational risks to workers at a hazardous waste incinerator in Turkey, Bakoğlu *et al.* (2004) measured levels of numerous pollutants, including styrene, at two sampling points in the vicinity of the incinerator. The sampling points chosen were those expected to be where maximum airborne exposures occurred. Single air samples for each sampling location were taken over 16- to 24-hour periods and contained styrene levels of 2.98 and 5.7 ppb.

Kim *et al.* (2003) measured styrene at 3 different locations in a factory producing PVC film and presented mean levels of 1.8 $\mu g/m^3$ [0.42 ppb] at two of the sampling locations and a level of 1.6 $\mu g/m^3$ [0.38 ppb] at the third location.

In two cooking-ware manufacturing companies where styrene-based resins were used, the 8-hour TWA concentrations of styrene ranged from 0.2 to 81 ppm and two short-duration samples were 142 and 186 ppm (IARC 2002). Area samples taken at a college sculpture

class where polyester resins were used contained styrene at concentrations from 0.8 to 1.2 ppm, and two breathing zone air samples contained 2.8 and 3.0 ppm. In a study of taxidermists who used polyester resins to prepare specimens, air concentrations of styrene ranged from 21 to 300 mg/m^3 [4.9 to 70 ppm]. Firefighters can be exposed to styrene during firefighting activities: IARC (2002) reported a level of 1.3 ppm during the knockdown phase of a fire. Styrene air levels exceeding 20 ppm have been reported during the manufacture of polyester paints, lacquers, and putties, and the application of polyester putties during cable splicing operations resulted in exposure levels ranging from 2 to 16 ppm. In a Japanese production plant where buttons were made from polyester resins, 8-hour TWA levels were 7.1 ppm with a maximum air level of 28 ppm.

2.6 Biological indices of exposure

Direct measures of exposure to styrene in humans have included unmetabolized styrene, which has been measured in expired air, blood, and urine (Guillemin and Berode 1988), adipose tissue (Engström 1978), and breast milk (Howard 1989). Although individuals may differ in their ability to metabolize styrene because of differences in metabolizing enzymes resulting from genetic polymorphisms (see Section 5.4.5), metabolites of styrene are widely used as biomarkers of exposure. These metabolites include Phase I intermediates and their conjugates (Phase II intermediates) of styrene glycol and styrene-7,8-oxide in blood; and the urinary biomarkers mandelic acid and phenylglyoxylic acid (IARC 2002), 4-vinylphenol (Manini *et al.* 2003), and phenylhydroxymercapturic acids (PHEMAs) from glutathione conjugation of styrene oxides (Ghittori *et al.* 1997). Finally, adducts of styrene formed through reaction of styrene-7,8-oxide with albumin, hemoglobin, and DNA also have been used as biomarkers of exposure. In contrast with measurements of styrene air concentrations to estimate exposure levels, the use of biological indices will account for exposures from all exposure routes (*i.e.*, inhalation, ingestion, and dermal exposure).

The biological indices of exposure for styrene listed here are described briefly below and the half-lives of styrene-7,8-oxide-DNA adducts are discussed in Section 5.4. In general, the half-lives in blood for styrene and its metabolites range from less than an hour to slightly greater than a day [due in part to a biphasic clearance with both a rapid and a

slow phase for each], while protein adducts have half-lives of one to three months, and DNA adducts have estimated half-lives ranging from 19 hours for the N^7 DNA adducts to 1,320 hours for the O^6 DNA adduct. The half-lives of styrene, its metabolites, and adducts are discussed further in Section 5.4.

[Since biological measures of exposure are a metric of actual exposure, they often are considered to be superior to measurements of environmental levels. However, there are a number of limitations to biological monitoring. Biological monitoring data are difficult to interpret without information on the kinetics of metabolism and clearance, and the intensity and duration of personal contact, and therefore, the data are often study-specific and not generalizable across the body of literature. Also, as noted above, metabolism and clearance parameters may vary across individuals due to genetic polymorphisms and, therefore, differences in biological levels across individuals may not reflect accurately their relative exposure levels. The assessment of metabolites can be complicated by the fact that often the metabolite being measured is not specific for the agent for which exposure is being assessed. For example, mandelic acid and phenylglyoxylic acid are not specific for styrene, but can be metabolically derived from other chemicals. Because biological measurements account for all exposure routes, exposures outside of the source of concern can inflate exposure estimates. For styrene, smoking and diet are potential sources of styrene exposure, and thus, smokers or people who get more styrene through their diet may appear to have higher occupational exposure levels when compared with non-smokers. Regardless of these issues, biological monitoring is still an important tool for assessing exposure, especially when used in concert with environmental levels.]

Styrene levels in blood and levels of the major styrene metabolites mandelic acid and phenylglyoxylic acid in urine are the most commonly used biological indices of exposure to styrene (IARC 2002). The American Conference of Governmental Industrial Hygienists (ACGIH) provides Biological Exposure Indices (BEIs) for mandelic acid plus phenylglyoxylic acid as the sum of free acid and conjugates in urine, and styrene in venous blood. These indices are designed to represent the levels of these determinants in specimens collected from healthy workers exposed to the ACGIH Threshold Limit Values (TLVs) (see Section 2.7.2 for styrene TLVs). The BEI indicates a marker

concentration below which nearly all workers should not experience adverse health effects. The mandelic acid/phenylglyoxylic acid BEI is 400 mg/g creatinine for an end of shift sample, and the BEI for styrene in venous blood is 0.2 mg/L for an end of shift sample (ACGIH 2007). Pekari *et al.* also examined *p*-hydroxymandelic acid, a minor metabolite of styrene, as a potential biomarker but concluded that while it might be of toxicological interest, it is not suitable for monitoring. Storage of samples after collection might affect urinary mandelic acid and phenylglyoxylic acid levels; therefore, Eitaki *et al.* (2008) examined their stability under different storage conditions. They recommended that urine samples be analyzed on the day of collection; however, if that is not possible, the urine samples should be stored for no longer than 4 days at a temperature of 4°C or lower.

Pekari *et al.* (1993) evaluated urinary mandelic acid, phenylglyoxylic acid, and styrene as biomarkers of exposure to styrene and concluded that the sum of the urinary metabolites (mandelic acid plus phenylglyoxylic acid) in specimens was preferable to the use of either metabolite alone. The authors noted a close linear relationship between airborne styrene levels and urinary concentrations of mandelic acid, phenylglyoxylic acid, and styrene in workers exposed through the lungs, but not in workers exposed mainly through the skin. For mandelic acid plus phenylglyoxylic acid, correlation coefficients were 0.85 for urinary measurements taken after the work shift and 0.81 for measurements taken the next morning. Pekari *et al.* noted that styrene monomer levels in urine were also related to airborne styrene levels ($r = 0.89$), and that in principal, styrene levels in urine could be used to assess exposure. They further noted, however, that the literature is mixed on the quantitative relationships between styrene in urine and airborne levels.

Similar to the results reported by Pekari *et al.*, Ong *et al*. (1994) reported good correlations between styrene levels in air and end-of-shift, creatinine-corrected urinary levels of mandelic acid ($r = 0.83$) or phenylglyoxylic acid ($r = 0.84$), but a better correlation between styrene air levels and end-of-shift creatinine-corrected levels mandelic acid and phenylglyoxylic acid combined ($r = 0.86$). For next-morning urinary collection, correlation coefficients fell to 0.47 for mandelic acid, 0.61 for phenylglyoxylic acid, and 0.65 for mandelic acid plus phenylglyoxylic acid. The best correlation,

however, was between styrene air levels and styrene blood levels (r = 0.87). Styrene in exhaled breath taken immediately after the work shift also showed good correlation with airborne styrene levels (r = 0.76), and the authors concluded that styrene in breath could be a useful indicator for low-level styrene exposure as the method is specific, non-invasive, and rapid. They further noted for biological monitoring of styrene exposure, exhaled styrene and blood levels of styrene are preferred by some because mandelic acid and phenylglyoxylic acid are not specific for styrene, but can be metabolically derived from other chemicals such as ethylbenzene, phenylglycol, as well as a few common drugs, and that alcohol consumption can decrease mandelic acid levels. Contributions from dermal exposure to styrene were not assessed in this study; however, as noted above in Section 2.5.1, Dutkiewicz and Tyras (1968) noted that urinary mandelic acid does not provide a reliable index of absorption where there is simultaneous skin and lung exposure.

Elia *et al*. (1980) found an excellent correlation (r = 0.96) between log styrene air concentrations and log creatinine-corrected urinary mandelic acid, either alone or in combination with phenylglyoxylic acid. Ikeda *et al*. (1982) reported that the best correlation in styrene-exposed workers (r = 0.88) was found between styrene in air and combined measurements of mandelic acid and phenylglyoxylic acid corrected for creatinine. Neither Elia *et al*. nor Ikeda *et al*. assessed the contribution of dermal styrene exposure.

Other indicators of exposure that have been used include measurements of styrene in urine and styrene-7,8-oxide in blood (HSDB 2008a). Mixed data have been reported on the effectiveness of styrene levels in urine as they relate to exposure levels. Pezzagno *et al*. (1985) reported a linear relationship and correlation coefficients for TWA styrene levels in air and styrene in urine of 0.88 (for exposed workers) and 0.93 (for experimental volunteers), while Ong *et al*. (1994) reported a “poor correlation” (r = 0.24) between air and urinary styrene levels. Tornero-Velez *et al*. (2001) determined styrene and styrene-7,8-oxide in human blood and reported detection limits of 2.5 μg/L for styrene and 0.05 μg/L for styrene-7,8-oxide. The authors reported a linear relationship between levels of styrene in blood and the corresponding air concentrations. Linear regression of logged

values yielded the following relationship: ln[blood styrene (mg/L)] = -4.35 + 0.97 ln[air styrene (ppm)] (N = 35, r = 0.89). The authors noted that a styrene exposure level of 50 ppm resulted in a level of 0.57 mg/L styrene in blood at the end of a work shift. Levels of styrene-7,8-oxide in the blood were significantly correlated with air levels of both styrene and styrene-7,8-oxide. For styrene-7,8-oxide in blood, linear regression of logged values yielded the following relationship: ln[blood SO (μg/L)] = -3.23 + 0.415 ln[air styrene (ppm)] (N = 27, r = 0.73). The contribution of dermal styrene exposure was not assessed in these studies.

The conjugated urinary metabolites of 4-vinylphenol, a metabolite of styrene, also have been studied for use as biomarkers of exposure to styrene. 4-Vinylphenol was found to be significantly correlated both with airborne styrene (r = 0.607, P = 0.001) and the sum of mandelic acid and phenylglyoxylic acid (r = 0.903, P = 0.001) in end-of-shift samples (Manini *et al*. 2003). Manini *et al*. reported that while the 4-vinylphenol conjugates represented only about 0.5% to 1% of the total excretion of styrene metabolites, 4-vinylphenol is the only styrene metabolite, other than styrene-7,8-oxide, not shared with ethylbenzene, and is therefore considered to be a highly specific marker for styrene exposure. Manini *et al.*, however, reported a measurable background level of 4-vinylphenol for both controls and workers occupationally exposed to styrene; this background level was highly correlated with smoking, and the authors theorized that it was possibly also from dietary intake. The authors recommended the use of 4-vinylphenol as a biomarker for styrene exposure only for ambient concentrations greater than 1 ppm. The contribution from dermal styrene exposure was not assessed in this study.

The use of PHEMAs as biomarkers of exposure to styrene has been limited, but Ghittori *et al.* (1997) proposed this potential biomarker because the molecules could provide information on the internal exposure to the *R*- and *S*-enantiomers of styrene-7,8-oxide, which have been reported to differ in their toxicity (see Sections 5.1 and 5.2). The *R*- and *S*-enantiomers of styrene oxide can be conjugated with glutathione to form both *R*- and *S*-diastereoisomers of specific mercapturic acids, *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)-L-cysteine (M1) and *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)-L-cysteine (M2). Linear

relationships were found between air concentrations of styrene and concentrations of the metabolites mandelic acid, phenylglyoxylic acid, and M2 corrected for creatinine, and urinary styrene not corrected for creatinine. The excretion of mercapturic acids exhibited a significant correlation with styrene air concentration. The M2 mercapturic acid showed a better correlation ($r = 0.56$) with respect to M1-*R* ($r = 0.41$) and M1-*S* ($r = 0.36$). The authors noted that the results of this analysis suggest that large inter-individual differences may occur in the metabolism of styrene to mercapturic acids in humans; the M1-*S* to M1-*R* ratio varying between 7.78 and 41.05. [The contribution of dermal exposure was not assessed.]

In a review of mercapturic acids as biomarkers of exposure, Haufroid and Lison (2005) reported that while excretion of PHEMAs has been shown to be significant, this correlation is modest when compared with the very good correlation seen with mandelic acid and phenylglyoxylic acid.

Negri *et al.* (2006) examined the effects of different storage methods on the stability of PHEMAs and reported that the metabolites were stable for 1 week at 4ºC and with repeated freezing and thawing; however, because of an unexplained increase in the PHEMA levels for samples that were not kept frozen, they recommended that samples should be frozen as soon as possible after collection and thawed only one time immediately before the analysis.

The measurement of styrene-induced DNA adducts has been reported (Vodicka *et al.* 2002a, Vodicka *et al.* 2003, Vodicka *et al.* 2002b) (see Section 5.4), and these adducts have been shown to correlate significantly with measures of styrene exposure, including styrene in workplace air, styrene in exhaled air, styrene in blood, and urinary mandelic acid (see Section 5.4.4, DNA adducts). Vodicka *et al*. (1993) detected 4.7 DNA adducts/10^8 nucleotides among 10 hand-lamination workers, and 0.3 adducts/10^8 nucleotides in 8 controls. Vodicka *et al.* (2002a, 2002b) reported significant linear relationships between styrene exposure and DNA adducts of styrene, *i.e.*, the N^2- and O^6-guanines, with approximately six-fold higher levels of O^6-guanine DNA adducts in hand-lamination workers as compared with controls. The levels of O^6-styrene–guanine DNA

adducts were significantly correlated with styrene workplace air concentration ($r = 0.588$, $P < 0.001$), duration of employment ($r = 0.479$, $P = 0.002$), and exposure coefficient (workplace air concentration multiplied by years of employment) ($r = 0.659$, $P < 0.001$). Vodicka *et al.* (2003) reported that O^6-styrene-7,8-oxide–guanine DNA adducts were significantly higher in exposed subjects as compared with controls and were significantly correlated with workplace styrene air concentration ($r = 0.73$, $P < 0.001$) and cumulative exposure ($r = 0.659$, $P = 0.001$). The limit of detection for the DNA adducts was 0.4 adducts per 10^9 nucleotides [0.04 adducts per 10^8 nucleotides] (Vodicka *et al.* 2003). (Biomarkers of effect (such as DNA repair and toxic endpoints) are discussed in Section 5.)

Adducts of styrene-7,8-oxide to the *N*-terminal valine in hemoglobin and to cysteine residues in albumin and hemoglobin also have been used as biomarkers of exposure to styrene (Brenner *et al.* 1991, Christakopoulos *et al.* 1993, Fustinoni *et al.* 1998, IARC 2002, Liu *et al.* 2001, Yeowell-O'Connell *et al.* 1996, Yuan *et al.* 2007) that can be used to estimate exposure over longer periods of one to three months (Fustinoni *et al.* 1998). Fustinoni *et al.* compared levels of styrene-7,8-oxide adducts of albumin and hemoglobin with the urinary markers of mandelic acid and phenylglyoxylic acid among workers exposed to styrene in the reinforced-plastics industry and in unexposed subjects. They found high levels of albumin and hemoglobin adducts of styrene-7,8-oxide in unexposed controls that were not significantly different from those of the exposed workers. The authors concluded that cigarette smoking is a source of background levels of styrene-7,8-oxide–protein adducts and they suggested that hemoglobin adducts of styrene-7,8-oxide can be detected above background levels only when high-level exposure to styrene exists, which they considered to be 100 mg/m^3 [23.5 ppm]. Vodicka *et al.* (2003) reported that *N*-terminal valine adduct levels were significantly higher in exposed subjects as compared with controls and were significantly correlated with workplace styrene air concentration ($r = 0.779$, $P < 0.001$) and cumulative styrene exposure ($r = 0.657$, $P = 0.006$). Yeowell-O'Connell *et al.* (1996) found no exposure-related increase in hemoglobin adducts; however, albumin adducts were found to increase with exposure to styrene or styrene-7,8-oxide (the latter being more important). High levels were also found in people without occupational exposure, suggesting to the authors that styrene-

7,8-oxide is either produced endogenously or exposure was occurring from other sources *(i.e.*, dietary or other environmental exposures). Significant correlations were found for the styrene-7,8-oxide–albumin adduct 2-phenylethanol versus styrene air levels (P = 0.017) and styrene-7,8-oxide air levels (P = 0.01). These studies did not evaluate the contribution of dermal styrene exposure.

2.7 Regulations and guidelines

2.7.1 Regulations

Department of Homeland Security

46 CFR 150 and 151 detail procedures for shipping styrene monomer and for shipping styrene monomer and various styrene co-polymers with incompatible mixtures

Department Of Transportation (DOT)

Considered a hazardous material, and special requirements have been set for marking, labeling, and transporting this material

Environmental Protection Agency (EPA)

Clean Air Act

National Emission Standards for Hazardous Air Pollutants: Listed as a hazardous air pollutant

New Source Performance Standards: Synthetic Organic Chemical Manufacturing Industry (SOCMI) facilities that meet the definition of a new source and produce styrene are subject to provisions for the control of VOC emissions

Control of Emissions of Hazardous Air Pollutants from Mobile Sources: Listed as a mobile source air toxic

Clean Water Act

Styrene has been designated a hazardous substance with a reportable quantity (RQ) of 1,000 lb

Comprehensive Environmental Response, Compensation, and Liability Act

Reportable quantity (RQ) = 1,000 lb

Emergency Planning and Community Right-To-Know Act

Toxics Release Inventory: Listed substance subject to reporting requirements

Safe Drinking Water Act

Maximum contaminant level (MCL) = 0.1 mg/L

Food and Drug Administration (FDA)

Maximum permissible level in bottled water = 0.1 mg/L

The food additive poly (2-vinylpyridine-co-styrene) may be safely used as nutrient protectant in feed for beef cattle and dairy cattle and replacement dairy heifers with residual styrene levels not to exceed 200 ppb

Polystyrene basic polymers used as components of articles intended for use in contact with food shall contain not more than 1 weight percent of total residual styrene monomer (0.5 weight percent on certain fatty foods)

Rubber-modified polystyrene basic polymers used as components of articles intended for use in contact with food shall contain not more than 0.5 weight percent of total residual styrene monomer

Styrene-maleic anhydride co-polymers may be used as articles or as components of articles intended for use in contact with food provided that conditions detailed in the regulation are met, including a maximum residual styrene monomer of 0.3% by weight

Styrene-acrylic co-polymers may be used as components of the food-contact surface of paper and paperboard provided that certain conditions are met, including residual styrene monomer levels in the polymer not exceeding 0.1% by weight

Occupational Safety and Health Administration (OSHA)

Acceptable peak exposure = 600 ppm (5-minute maximum peak in any 3 hours)

Ceiling concentration = 200 ppm

Permissible exposure limit (PEL) = 100 ppm

2.7.2 Guidelines

American Conference of Governmental Industrial Hygienists (ACGIH)

Threshold limit value – short-term exposure limit (TLV-STEL) = 40 ppm

Threshold limit value – time-weighted average limit (TLV-TWA) = 20 ppm

Biological exposure indices

Mandelic acid plus phenylglyoxylic acid in urine, end of shift = 400 mg/g creatinine

Styrene in venous blood, end of shift = 0.2 mg/L

National Institute for Occupational Safety and Health (NIOSH)

Immediately dangerous to life and health limit (IDLH) = 700 ppm

Short-term exposure limit (STEL) = 100 ppm

Recommended exposure limit (REL) = 50 ppm

2.8 Summary

The primary use of styrene is in the manufacture of polystyrene, which is used extensively in the manufacture of plastic packaging, thermal insulation in building construction and refrigeration equipment, and disposable cups and containers. Styrene also is used in styrene-butadiene rubber and other polymers and resins that are used to manufacture boats, shower stalls, tires, automotive parts, and many other products. U.S. production of styrene has risen steadily over the past 70 years, with 11.4 billion pounds produced in 2006 (domestic production capacity for 2006 was estimated at 13.7 billion pounds). Styrene and styrene metabolites in blood and urine, and styrene-7,8-oxide–DNA adducts and styrene-7,8-oxide–hemoglobin adducts are generally accepted biological indices of exposure to styrene. The primary source of exposure to the general public is inhalation of indoor air; however, exposure can also occur from inhalation of outdoor air, ingestion of food and water, and potentially from skin contact. Tobacco smoke also can be a major source of styrene exposure for both active smokers and individuals exposed to environmental tobacco smoke. Outdoor and indoor air levels (including air levels in most other occupational settings) are generally below 1 ppb [0.001 ppm]; although higher levels have been reported. Workers in certain occupations, including the reinforced-plastics, styrene-butadiene, and styrene monomer and polymer industries, may be exposed to higher levels of styrene than the general public. Air levels in the reinforced-plastics industry are generally lower than 100 ppm [although much higher levels have frequently been measured] while levels in the styrene-butadiene industry and the styrene monomer and polymer industries have rarely been reported to exceed 20 ppm. Numerous Federal agencies have established regulations for styrene including the Department of Homeland Security, DOT, EPA, FDA, and OSHA, and both ACGIH and NIOSH have established guidelines to limit occupational exposure to styrene.

This Page Intentionally Left Blank

3 Human Cancer Studies

Three IARC working groups reviewed human studies on the carcinogenicity of styrene in 1979, 1994, and 2002. The 1979 and the 1994 working groups characterized the evidence available to them at the time on carcinogenicity in humans as "inadequate" (IARC 1979, 1994a). The 2002 working group upgraded the human evidence to "limited" (IARC 2002).

The 1994 IARC evaluation (IARC 1994a) was based on 19 epidemiologic studies (Bond *et al.* 1992, Coggon *et al.* 1987, Flodin *et al.* 1986, Frentzel-Beyme *et al.* 1978, Härkönen *et al.* 1984, Hodgson and Jones 1985, Kogevinas *et al.* 1994a, Kogevinas *et al.* 1994b, Kolstad *et al.* 1994, Matanoski *et al.* 1993, Matanoski *et al.* 1990, McMichael *et al.* 1976a, Meinhardt *et al.* 1982, Okun *et al.* 1985, Ott *et al.* 1980, Santos-Burgoa *et al.* 1992, Siemiatycki 1991, Wong 1990, Wong *et al.* 1994) and 3 case reports (Block 1976, Lemen and Young 1976, Nicholson *et al.* 1978). The 2002 IARC evaluation added 13 epidemiologic studies not included in the 1994 evaluation (Anttila *et al.* 1998, Delzell *et al.* 2001, Delzell *et al.* 1996, Dumas *et al.* 2000, Gérin *et al.* 1998, Kogevinas *et al.* 1993, Kolstad *et al.* 1995, Kolstad *et al.* 1993, Loughlin *et al.* 1999, Macaluso *et al.* 1996, McMichael *et al.* 1976b, Sathiakumar *et al.* 1998, Sielken and Valdez-Flores 2001). Cohen *et al.* (2002)[1] included 4 additional epidemiologic studies not reviewed by IARC (Cantor *et al.* 1995, Matanoski *et al.* 1997, Meinhardt *et al.* 1978, Parent *et al.* 2000). This background document reviews the epidemiologic studies (or latest update) previously reviewed by IARC and Cohen *et al.* and additional epidemiologic studies (Coyle *et al.* 2005, Graff *et al.* 2005 (also reported in Delzell *et al* 2006), Guenel *et al.* 2002, Kolstad *et al.* 1996, Ruder *et al.* 2004, Sathiakumar *et al.* 2005, Scélo *et al.* 2004, Seidler *et al.* 2007), as well as 5 studies that characterized styrene exposure (Crandall 1981, Jensen *et al.* 1990, Kolstad *et al.* 2005, Macaluso *et al.* 2004, Thiess and Friedheim 1978) and were used for several of the epidemiologic studies. One paper published in 2002 (Magnavita *et al.* 2002) was not included in this review because it was a case report for a single individual who worked as a boat builder. A population-based study of cancer

[1] The expert panel evaluation conducted by the Harvard Center for Risk Analysis and funded by the Styrene Information and Research Center (SIRC).

among persons with potential occupational exposure to styrene in comparison with other working men and women, published in 2007 in German (with an English abstract), was also identified but not reviewed here because only limited details of the study were reported in the English abstract. (Note that the abstract reported that no significant increases in all cancers combined or specific cancers (not specified) were observed for men or women in styrene processing industries, although there were small numbers of potentially exposed women.)

In accordance with the IARC evaluation and Cohen *et al.* (2002), this review is organized by the three major industrial settings where workers are exposed to styrene – the reinforced-plastics industry (Section 3.1), the styrene-butadiene rubber industry (Section 3.2), and the styrene monomer and polymer industry (Section 3.3) – because exposure conditions differ significantly among these industries. A fourth category includes studies conducted in the general population or other industrial settings (Section 3.4). Section 3.5 describes the available case-control and ecological studies. Section 3.6 discusses strengths and limitations of the literature, and Section 3.7 summarizes previous evaluations by IARC (1994a, 2002) and Cohen *et al.* (2002). Section 3.8 summarizes the findings for selected cancer sites. Section 3.9 provides an overall summary for this section.

Tables 3-1 and 3-4 to 3-7 present study characteristics and findings for each individual study. Tables 3-2 and 3-3 provide specific findings from the largest study of styrene-butadiene rubber workers (Delzell *et al.* 2006). In addition, Table 3-8 summarizes the findings for all cancer sites for all 12 independent cohorts reviewed. Table 3-9 presents the pooled results for selected cancers (which appear to have the most consistently increased risks based on the tabulations in Table 3-8 obtained from studies of workers in the reinforced-plastics industry, and Table 3-10 presents the pooled results for those same selected cancers among workers in high-styrene–exposure groups (laminators and others) in the reinforced-plastics industry.

3.1 The reinforced-plastics industry

As noted in Section 2.6.1, the highest occupational exposures to styrene, with respect to the number of employees and exposure levels, occur in the fabrication of objects such as boats, car and truck parts, tanks, tubs, and shower stalls from reinforced plastics (IARC 2002). Exposures in this industry have been in the range of several hundred parts per million in the past, but reported levels have declined over the past several decades. Workers in the reinforced-plastics industry may also be exposed to other chemicals, including acetone and other solvents; organic peroxides; cross-linking agents such as methyl methacrylate; chlorinated hydrocarbons such as dichloromethane; hydroquinone; oxidation products such as styrene-7,8-oxide; dusts and fibers (such as glass fibers, silica, asbestos) from filler and reinforcement materials; foaming agents such as isocyanates; and cobalt salts and amines used as accelerators (EPA 1997b, IARC 2002).

Cancer mortality or incidence has been studied in the following four populations of reinforced-plastics workers: (1) in Washington state in the United States (Okun *et al.* 1985, Ruder *et al.* 2004), (2) in 30 manufacturing plants in unspecified U.S. locations (Wong 1990, 1994), (3) in Denmark (Kolstad *et al.* 1995, Kolstad *et al.* 1993, Kolstad *et al.* 1994, Kolstad *et al.* 1996) and (4) in Europe (Denmark [a subset of the workers from the studies by Kolstad *et al.*], Finland, Italy, Norway, the United Kingdom, and Sweden) (Kogevinas *et al.* 1994a, 1993). Results from the U.K. subset of the European population were also reported separately by Coggon *et al.* (1987). The Danish and the European populations were partly overlapping, as 13,682 Danish male workers were included among the 36,610 male workers in the Danish studies reported by Kolstad *et al.* (1995, 1994). The two U.S. studies did not overlap. An overview of the individual studies is presented in Table 3-1.

3.1.1 Washington state

Okun *et al.* (1985) reported on cancer mortality among 5,201 workers (82% men) employed for at least one day in two reinforced-plastics boat-building facilities in Washington state between 1959 and 1978. Ruder *et al.* (2004) extended follow-up through 1998. Vital status of each subject was determined using data from the Social Security Administration, Internal Revenue Service, Department of Motor Vehicles, and

National Death Index. Cause of death was obtained from death certificates. Standardized mortality ratio (SMR) analyses compared observed deaths classified by the underlying cause of death with expected numbers computed from state and national rates. Of workers classified as highly exposed (see below), 74% worked less than 1 year, and 1% worked more than 10 years. A total of 135,707 person-years were accumulated, and the average follow-up was 26 years.

Exposure was assessed using industrial hygiene surveys that classified the jobs and departments according to the level of styrene exposure. According to this assessment, 2,060 employees (40%) had ever worked in fibrous glass or lamination departments; these constituted a well-defined high-exposure group. Full-shift average styrene exposure levels within these departments was 42.5 ppm (range 7.3 to 84.7 ppm) at Plant A and 71.7 ppm at Plant B (range 14.5 to 183 ppm) in 1978 to 1979 (Crandall 1981). A total of 3,141 employees worked with boat assembly, in administration, and in general plant-wide departments with lower styrene exposure levels; these workers were classified as having low exposure and were assigned an exposure level of 5 ppm by the authors; no measurements were reported.

For the total cohort, overall cancer mortality was significantly elevated in comparison with Washington state reference rates (SMR = 1.17, 95% CI = 1.02 to 1.33, 233 observed deaths). Statistically significant increases in mortality were also seen for cancer of the esophagus (SMR = 2.30, 95% CI = 1.19 to 4.02, 12 observed deaths), prostate (SMR = 1.71, 95% CI = 1.09 to 2.54, 24 observed deaths), and other and unspecified sites (ICD-9 codes 194 to 199, SMR = 1.68, 95% CI = 1.01 to 2.62, 19 observed deaths).

Among highly exposed workers, a statistically nonsignificant increase in overall cancer mortality was observed (SMR = 1.26, 95% CI = 0.96 to 1.63, 58 observed deaths), as well as statistically nonsignificant increases in mortality due to cancer of the esophagus (SMR = 1.85, 95% CI = 0.22 to 6.67, 2 observed deaths), stomach (SMR = 1.55, 95% CI = 0.19 to 5.61, 2 observed deaths), intestine except rectum (SMR = 1.55, 95% CI = 0.50 to 3.63, 5 observed deaths), pancreas (SMR = 1.88, 95% CI = 0.51 to 4.81, 4 observed deaths), lung (SMR = 1.29, 95% CI = 0.76 to 2.04, 18 observed deaths), ovary (SMR =

2.32, 95% CI = 0.28 to 8.38, 2 observed deaths), prostate (SMR = 2.06, 95% CI = 0.43 to 6.04, 3 observed deaths), kidney (SMR = 3.60, 95% CI = 0.98 to 9.20, 4 observed deaths), bladder (SMR = 3.17, 95% CI = 0.38 to 11.5, 2 observed deaths), and brain (SMR = 1.28, 95% CI = 0.26 to 3.75, 3 observed deaths), and Hodgkin's disease (SMR = 1.78, 95% CI = 0.05 to 9.89, 1 observed death). Site-specific mortalities were generally comparable for the low-exposure group except for cancers of the urinary organs, which were higher in the high-exposure group.

In general, gender-specific SMRs were not calculated; however, the authors noted that there was a statistically nonsignificant increase in lung cancer mortality among white females (SMR = 1.82, 95% CI = 0.78 to 3.59, 8 observed deaths), and among white females with high exposure to styrene (SMR = 2.11, 95% CI = 0.77 to 4.60, 6 observed deaths).

Among workers employed for at least one year (N = 1,678; 580 high exposure, and 1,098 low exposure), statistically nonsignificant increases in SMRs were observed in high-exposure departments compared with low-exposure departments, for cancer of the esophagus, intestine (not including rectum), kidney, and bladder. [This analysis was limited by small numbers of expected and observed cancer deaths in the high-exposure subcohort of workers employed for more than one year (all cancer deaths, 20 observed and ~22 expected deaths).] A statistically nonsignificant increase in overall mortality was found among workers employed for less than 1 year (short-term workers). The authors also stated that for urinary cancer, there was a trend towards increasing mortality with increasing duration of employment in the high-exposure departments, and also with increasing levels (terciles) of cumulative exposure.

The authors stated that the study was limited by lack of information on lifestyle choices, previous or subsequent employment, exposure to other occupational agents, and job information after 1978. Cumulative exposure estimates were not job specific and did not include any exposures between 1978 and when the plant closed (1989 for Plant B and 1993 for Plant A). They noted that the lack of job information after 1978 meant that cumulative exposure and duration of exposure are underestimates and would bias results

towards the null hypothesis. The authors also stated that the work-history records did not include specific job titles and that the exposures varied widely with the high-exposure departments.

3.1.2 United Kingdom

Coggon *et al.* (1987) studied 7,949 workers (6,638 men and 1,311 women) at eight reinforced-plastics companies in the United Kingdom. All employees, regardless of duration of exposure, were included between 1947 and 1984 (inclusion periods differed among the companies) and followed through 1984. Follow-up was incomplete for 3%. For one company, sufficient employment data were identified from personnel records for only 61.9% of the employees, and results for these workers were presented separately from the detailed analyses. Vital status was traced through the National Health Service Cancer Register, and National Insurance Index. Cause of death was obtained from death certificates. Mortality was compared with expected values computed from national mortality rates. Gender-specific SMRs were not calculated. Durations of employment were less than 1 year for 51% of the workers and 10 years or more for 8%. From personnel records, workers were classified into four categories: hand laminators (high exposure) (44%), regular bystander exposure (7%), occasional bystander exposure (17%), or background exposure (32%). The authors estimated that hand laminators were exposed to styrene at levels of 40 to 100 ppm 8-hour TWA, based on measurements conducted at the companies since 1975; however, no styrene exposure measurements were presented.

Among all workers at the seven companies with almost complete data, a statistically significant decrease in the SMR for overall cancer mortality was observed (SMR = 0.80 (95% CI = 0.69 to 0.93, 167 observed deaths). The SMR did not differ statistically from unity for any specific cancer, but statistically nonsignificant increases (> 10%) in SMRs were observed for larynx (SMR = 1.16, 95% CI = 0.14 to 4.18, 2 observed deaths), lung (SMR = 1.12, 95% CI = 0.89 to 1.39, 83 observed deaths), melanoma (SMR = 1.19, 95% CI = 0.14 to 4.30, 2 observed deaths), non-melanoma skin cancer (SMR = 3.57, 95% CI = 0.43 to 12.90, 2 observed deaths), and cancer of the ovary (SMR = 1.49, 95% CI = 0.41 to 3.81, 4 observed deaths). Lung cancer mortality was highest in individuals with moderate and high exposure, and individuals exposed from 1 to 9 years, but the exposure-

response relationship was not consistent. Mortality of other cancers was not consistently related to first year of exposure, duration of exposure, or latency for any type of cancer; however, the numbers of deaths were small. Among hand laminators (the well-defined high-exposure category), mortality was increased (statistically nonsignificant) for cancer of the large intestine, lung, cervix, ovary, and prostate. Kogevinas *et al*. included this population in the European study, with follow-up extended through 1990 (Kogevinas *et al.* 1994a, 1993).

The authors stated that the study had limited power to detect cancers with long latency (only 5 cancer deaths were expected among workers exposed at least 12 months with a latency period of 20 years).

3.1.3 United States

Wong *et al.* reported on cancer mortality among 15,826 workers (75.6% men) at 30 U.S. reinforced-plastics plants between 1948 and 1977 (Wong 1990) and later extended follow-up through 1989 (Wong *et al.* 1994). Employees who had worked for at least 6 months in an area with potential exposure to styrene were included in the study. However, not all workers actually worked in activities that entailed direct and significant exposure to styrene (Wong 1990). The duration of employment was less than 1 year for 24% of the workers and 5 years or more for 27%. Vital status information was obtained from the participating plants, the Social Security Administration, the National Death Index, the National Center for Health Statistics, and a commercial retail credit bureau. Cause of death was determined from death certificates. Vital status was unknown for 547 workers at the end of follow-up, and death certificates were not identified for 42; loss to follow-up was thus 3.7%. Standard SMR analyses were conducted, based on expected values computed from national death rates for whites (no information on race was available for the study population), as well as internal Cox regression analyses. Gender-specific risk estimates were not calculated. Odds ratios (ORs) for respiratory cancer mortality were computed by the Mantel-Haenszel procedure among 40 cases and 102 controls nested within the study population. This analysis included information on smoking obtained from 78% of cases and 61% of controls (Wong 1990).

A job exposure matrix was constructed for each plant from styrene measurements and process descriptions collected circa 1980. Only 43% of the total study population had direct exposure to styrene, according to information obtained for the 102 controls included in the nested case-control study (Wong 1990). The estimated styrene TWA values by job were 60 ppm (5 to 120 ppm) for spray and hand lay-up; 20 to 45 ppm for sheet molding, gel coating and winding; and 2 to 7 ppm for office, injection molding, field service, finish and assembly, store and ship, and preform production (Wong *et al.* 1994). The worker population was then classified into six processing categories based on exposures to styrene and other substances: open-mold processing, mixing and closed-mold processing, finish and assembly, plant office and support, maintenance and preparation, and supervisors and professionals. Cumulative exposure to styrene was estimated, taking account of job changes and duration of employment (Wong *et al.* 1994).

For the total study population, the SMR for all cancer was 1.16 (95% CI = 1.05 to 1.27, 425 observed deaths) (Wong *et al.* 1994). Statistically significantly increased SMR values were also seen for cancer of the esophagus (SMR = 1.92, 95% CI = 1.05 to 3.22, 14 observed deaths), lung (SMR = 1.41, 95% CI = 1.20 to 1.64, 162 observed deaths), cervix (SMR = 2.84, 95% CI = 1.36 to 5.21, 10 observed deaths), and other female genital organs (SMR = 2.02, 95% CI = 1.07 to 3.45, 13 observed deaths). Decreased risks were seen for all lymphatic and hematopoietic malignancies (SMR = 0.82, 95% CI = 0.56 to 1.17, 31 observed deaths) and among the subcategories (lymphosarcoma, Hodgkin's disease, leukemia, or cancer of all other lymphopoietic tissue). A statistically nonsignificant increase in mortality from pancreatic cancer was observed (SMR = 1.13, 95% CI = 0.68 to 1.77, 19 observed deaths), while mortality from laryngeal cancer was as expected.

The category with the expected highest styrene exposure levels was workers employed in open-mold processing. Among those working for more than two years in this category [the only results presented for this category], statistically nonsignificant increases in mortality were seen for cancer of the esophagus, stomach, uterus, cervix, kidney, lymphosarcoma, and all other lymphopoietic tissue. Mortality for pancreatic cancer, lung cancer, Hodgkin's disease, and leukemia was decreased. These findings were based on

few observed cases. Among workers with > 2 years employment in the other 5 work categories, statistically significant increases in mortality were observed for cancer of the biliary tract and liver among office and support workers (SMR = 4.56, $P < 0.05$, 4 observed deaths), and for bronchus, trachea, and lung cancer among maintenance and support workers (SMR = 1.49, $P < 0.05$, 30 observed deaths).

Statistically nonsignificant increases in SMRs [CIs not reported] were observed for all long-term workers (≥ 10 years) for cancer of the esophagus (SMR = 2.13, 4 observed deaths), and cervix (SMR = 4.04, 2 observed deaths), lymphosarcoma (SMR = 1.86, 2 deaths), and cancer of all other lymphopoietic tissue (SMR = 1.32, 4 observed deaths), as well as for several other cancers, but this analysis did not take exposure level into consideration. Cox regression analyses (internal analysis) of cumulative styrene exposure or duration of styrene exposure showed no indications of exposure-response relationships for cancer of the esophagus, lung, uterus, other female genital organs, kidney, or all lymphopoietic tissue, non-Hodgkin's lymphoma (NHL), multiple myeloma, or leukemia (Wong *et al.* 1994). In addition, no trends were seen in SMR analyses of duration of employment, duration of styrene exposure, or cumulative styrene exposure. However, lung cancer mortality increased with latency; statistically significantly increased SMRs were seen for workers with latencies of 10 to 19 years or at least 20 years. The nested case-control study (15 cases and 44 matched deceased controls) showed no increased risk of lung cancer among workers with direct exposure to styrene (Wong 1990).

3.1.4 Denmark

Kolstad *et al.* (1995, 1994) studied the incidence of cancer among 36,610 male workers at 386 Danish reinforced-plastics companies and a reference population of 14,293 male industrial workers at 84 companies with no styrene exposure. The method of exposure classification of workers in the Kolstad cohort was based on data obtained from two independent dealers (who identified the companies from a list of 552 likely producers of reinforced plastics) rather than information obtained from employers. The decision to do so was based partly on indications that the employers' exposure assessments were not independent of health outcomes for some companies. The two independent dealers agreed on all but 4 of 328 companies that they could both classify (kappa = 0.94) (80 companies

remained unknown to one or both of the dealers). There was agreement with the employers in 281 of 302 companies known to both. In addition, there was good agreement between employers and dealers on the proportion of all employees in a company engaged in the production of reinforced plastics. For 287 companies (12,862 workers), the estimate was 50% to 100%, and for 99 companies (23,748 workers), the estimate was 1% to 49%. [Nevertheless, the posthoc decision to rely solely on the dealers' estimates of exposure, together with a lack of exposure measurements (except in a small sample of the companies included in the study in a separate survey) represents a methodological limitation of this study.]

Female workers were included in an early overview of this population but were omitted from subsequent studies because the majority were not involved in the production of reinforced plastics (Kolstad *et al.* 1993). The population was followed from 1970 to 1989 (Kolstad *et al.* 1994) or 1990 (Kolstad *et al.* 1995), and loss to follow-up was 2%. Cancer cases were identified in the national cancer registry, and standardized incidence ratios (SIRs) were computed from the national reference rates. In internal analyses, Poisson regression models were used to estimate incidence rate ratios (IRRs). A total of 618,900 person-years were accumulated, and the average follow-up was 10.9 years. No information was available on individual indicators of exposure such as task or job title, but time and duration of employment were reported in a national pension scheme for the period 1964 to 1988. The duration of employment was less than 1 year for 60% of the workers and 10 years or more for 3%.

Measurements of styrene exposure levels in the industry were available back to the early 1960s, based on 2,473 personal air samples collected by the work inspection service. About 90% of the samples were taken during lamination procedures (Jensen *et al.* 1990). The mean period-specific styrene exposure levels were 180 ppm (1964 to 70), 88 ppm (1971 to 75), and 43 ppm (1976 to 88), and an estimated 43% of the study population were laminators (Kolstad *et al.* 1994).

No SIR for overall cancer incidence was reported for all workers (at the 386 reinforced plastic companies), but the incidence of all solid cancers was as expected (SIR = 0.99,

95% CI = 0.93 to 1.05, 1,134 observed cases) (Kolstad *et al.* 1995), and a statistically nonsignificant increase in the incidence of all lymphohematopoietic malignancies was observed (SIR = 1.20, 95% CI = 0.98 to 1.44, 112 observed cases) (Kolstad *et al.* 1994). Analyses of specific cancers revealed no statistically significantly increased SIRs (Kolstad *et al.* 1995, 1994). Incidences were nonsignificantly increased for cancer of the pancreas (SIR = 1.20, 95% CI = 0.86 to 1.63, 41 observed cases), nasal cavities (SIR = 1.84, 95% CI = 0.74 to 3.80, 7 observed cases), lung (SIR = 1.12, 95% CI = 0.98 to 1.26, 248 observed cases), pleura (SIR = 1.78, 95% CI = 0.85 to 3.28, 10 observed cases), external male genital organs (SIR = 1.60, 95% CI = 0.64 to 3.30, 7 observed cases), and bladder (SIR = 1.16, 95% CI = 0.96 to 1.39, 117 observed cases) (Kolstad *et al.* 1995), NHL (SIR = 1.33, 95% CI = 0.96 to 1.80, 42 observed cases), and leukemia (SIR = 1.22, 95% CI = 0.88 to 1.65, 42 observed cases) (Kolstad *et al.* 1994).

The risks of cancers with elevated SIRs were further evaluated by an internal analysis using workers not exposed to styrene as controls. The category with the highest potential styrene exposure was workers at companies with 50% to 100% laminators. Among these workers, there was a statistically significant excess of pancreatic cancer (IRR = 2.2, 95% CI = 1.1 to 4.5, 17 observed cases) (Kolstad *et al.* 1995). The risk was higher in long-term workers (≥ 1 year) than in short-term workers and among workers with earlier first years of employment (1970 or before) than later; however, latency had no influence on IRR values. The risk of lung cancer was not increased (IRR = 1.0, 95% CI = 0.7 to 1.3, 72 cases) among workers with higher exposure potential, and lower risk was seen in long-term workers. Analyses by first year of employment, length of employment, and latency revealed no consistent findings for cancer of the nasal cavities, pleura, external male genital organs, or urinary bladder, NHL or the other lymphohematopoietic malignancies except for leukemia (Kolstad *et al.* 1994, 1995).

The risk of leukemia was non-significantly increased with the probability of exposure to styrene (SIR = 1.38, 95% CI = 0.75 to 2.32, 14 observed cases for high probable exposure) and significantly increased among workers with earlier date of first employment (SIR = 1.54, 95% CI = 1.04 to 2.19, 30 observed cases for employment during the 1960's), and with latency (SIR = 1.57, 95% CI = 1.07 to 2.22, 32 observed

cases for at least 10 years since first employment). However, no excess of leukemia was apparent among those employed for 1 year or more (Kolstad *et al*. 1994).

In a case-control study nested within the cohort, Kolstad *et al.* (1996) studied the risk of myeloid leukemia with clonal chromosome aberrations, based on 12 cases (out of 34 cases of myeloid leukemia) and 57 non-exposed controls selected from the study population. Exposure classification relied on company-level assessments, as in the previous studies (Kolstad *et al.* 1995, Kolstad *et al.* 1994). A statistically nonsignificant odds ratio of 2.5 (95% CI = 0.2 to 25.0) was observed for reinforced-plastics workers (i.e., for any styrene exposure); the risk was higher among workers employed for less than 1 year (OR = 5.9, 95% CI = 0.5 to 74.3, 8 exposed cases) than workers employed longer than 1 year (OR = 1.1, 95% CI = 0.1 to 15.3, 3 exposed cases. [However, the risk estimates were imprecise.]

3.1.5 Denmark, Finland, Norway, Italy, Sweden, and the United Kingdom.

Kogevinas *et al.* (1994a, 1993) reported on cancer mortality among 40,688 employees at 660 companies in Denmark (15,867), Finland (2,085), Norway (2,035), Italy (7,256), Sweden (3,667), and the United Kingdom (9,778). Cancer mortality among 7,971 of the U.K. workers was previously reported by Coggon *et al.* (1987) but with a shorter follow-up. The international study included the male (13,682) workers that were also included in the cancer incidence studies of the Danish population (Kolstad *et al.* 1995, Kolstad *et al.* 1994) and the the female (2,185) workers that were not included in those studies from 287 Danish plants where reinforced plastics were the main products produced. The follow-up periods started between 1945 (United Kingdom) and 1970 (Denmark) and ended between 1987 (Sweden) and 1991 (Norway). The duration of employment was less than 2 years for 60% of the workers and 10 years or more for 9%. Loss to follow-up was 1.4%, and the average follow-up was 13 years. In SMR analyses, cancer mortality was compared with expected mortality computed from national reference rates. In internal analyses, Poisson regression models were used to compare exposure-specific cancer rates (rate ratios) and conduct trend analyses.

From job titles recorded on individual payroll records, the population was categorized as laminators (N = 10,629), workers with unspecified tasks (19,408), other exposed workers

with bystander exposure (5,406), workers not exposed to styrene (4,044), and workers with unknown job titles (1,201). The 15,867 Danish workers were categorized as workers with unspecified tasks, because no job titles were available. An exposure matrix was constructed from 16,500 personal styrene measurements obtained between 1955 and 1990 and from 18,500 measurements of styrene metabolites in urine conducted in the 1980s. Styrene exposure levels averaged across country, period, and job were linked with the individual workers, and cumulative styrene exposure was estimated from additional information on duration of exposure. All Danish workers and other workers classified as having unspecified tasks were assigned a styrene exposure level that was the average value for the calendar period and branch of the industry (boats vs. other). Styrene exposure levels recorded among laminators declined from about 200 ppm before 1965 to below 80 ppm in the 1980s.

Among the total study population, overall cancer mortality was statistically significantly lower than in the European reference population (SMR = 0.87, 95% CI = 0.81 to 0.94, 686 observed deaths). This was also the case for cancer of the buccal cavity and pharynx (SMR = 0.33, 95% CI = 0.11 to 0.77, 5 observed deaths), rectum (SMR = 0.62, 95% CI = 0.38 to 0.95, 21 observed deaths), breast (SMR = 0.52, 95% CI = 0.28 to 0.89, 13 observed deaths), and brain (SMR = 0.62, 95% CI = 0.37 to 0.98, 18 observed deaths). No site-specific SMR values were statistically significantly above unity, but excesses were seen for small intestine (SMR = 1.50, 95% CI = 0.31 to 4.38, 3 observed deaths), larynx (SMR = 1.11, 95% CI = 0.53 to 2.05, 10 observed deaths), ovary (SMR = 1.40, 95% CI = 0.70 to 2.51, 11 observed deaths) and myeloid leukemia (SMR = 1.10, 95% CI = 0.63 to 1.79, 16 observed deaths) (Kogevinas *et al.* 1994a).

The worker category with the best-documented high-level styrene exposure was laminators. Laminators showed no statistically significant elevated mortality from cancer at any site, but statistically nonsignificant increases in mortality were observed for cancer of the esophagus (SMR = 1.82, 95% CI = 0.87 to 3.34, 10 observed deaths), small intestine (SMR = 2.27, 95% CI = 0.06 to 12.66, 1 death), pancreas (SMR = 1.48, 95% CI = 0.76 to 2.58, 12 observed deaths), larynx (SMR = 1.55, 95% CI = 0.32 to 4.52, 3 deaths), ovary (SMR = 2.61, 95% CI = 0.71 to 6.69, 4 observed deaths), and thyroid

(SMR = 2.27, 95% CI = 0.06 to 12.66, 1 observed death), NHL (SMR = 1.40, 95% CI = 0.56 to 2.88, 7 observed deaths), and Hodgkin's disease (SMR = 1.33, 95% CI = 0.27 to 3.88, 3 observed deaths) (Kogevinas *et al.* 1994a).

Among workers classified as having unspecified tasks, overall cancer mortality was greater than expected (SMR = 1.06, 95% CI = 1.00 to 1.12, 1,167 observed deaths), but no statistically significant increases were seen for any of the specified cancers (Kogevinas *et al.* 1994a).

Lower SMRs were observed for long-term workers (≥ 2 years) for all lymphohematopoietic malignancies, Hodgkin's disease, multiple myeloma, and leukemia, but not for NHL (< 2 years: SMR = 0.60, 95% CI = 0.19 to 1.40, 5 observed deaths; ≥ 2 years: 1.05, 95% CI = 0.42 to 2.17, 7 observed deaths). More than 20 years after first exposure, a statistically nonsignificant increase in mortality for NHL in long-term workers was observed (SMR = 2.21, 95% CI = 0.45 to 6.45, 3 observed deaths). Among workers exposed for at least 2 years, mortality was non-significantly elevated for all lymphohematopoietic malignancies (SMR = 1.73, 95% CI = 0.70 to 3.57, 7 observed deaths) and leukemia (SMR = 1.94, 95% CI = 0.40 to 5.66, 3 deaths) in workers with at least 20 years latency, but not in those with latency of 10 to 19 years (Kogevinas *et al.* 1994a).

In internal analyses, the relative risk increased with increasing latency for all lymphohematopoietic malignancies ($P = 0.012$), leukemia ($P = 0.094$), and malignant lymphoma (NHL and Hodgkin's disease, $P = 0.072$). Similarly, the rate ratio increased with increasing average styrene exposure for all lymphohematopoietic cancers (P for linear trend = 0.019) and for malignant lymphoma ($P = 0.052$), though not for leukemia ($P = 0.47$). A trend for increased risk of pancreatic cancer with increasing cumulative styrene exposure was of borderline significance ($P = 0.07$), but no such trend was seen for all cancer, cancer of esophagus, lung, or kidney, all lymphohematopoietic malignancies, leukemia, or malignant lymphoma (Kogevinas *et al.* 1994a).

Table 3-1. Epidemiologic studies of cancer risk following styrene exposure in the reinforced-plastics industry, 1985–2004 (results of the most recent follow-up[a])

Study	Study design & follow-up	Study population and methods	Exposure Assessment Levels	Effects SMR (95% CI), no. of observed deaths[b]	Comments
Okun *et al.* 1985 Ruder *et al.* 2004 U.S.	historical cohort 1959–98 avg. 26 yr 135,707 person-years	5,201 workers (4,520 men, 681 women) at 2 reinforced-plastics plants employed ≥ 1 day, 1959–78 74% worked < 1 yr and 1% > 10 yr SMRs based on state and national rates; effects reported here based on state rates	I*ndustry hygiene surveys* Workers employed in fibrous glass or lamination departments (40% of population) classified as highly exposed. *Exposure Levels* High-exposure workers Average TWA (ppm) 1978–79 (Crandall 1981) Plant A: 42.5 Plant B: 71.7 Low-exposure workers 5 ppm (assigned not measured)	*Total cohort* Cancers with excess mortality (significant) overall 1.17 (1.02–1.33), 233 esophagus 2.30 (1.19–4.02), 12 prostate 1.71 (1.09–2.54), 24 unspecified 1.68 (1.01–2.62), 19 *High-exposure workers* Cancers with non-significant excess mortality overall 1.26 (0.96–1.63), 58 esophagus 1.85 (0.22–6.67), 2 stomach 1.55 (0.19–5.61), 2 intestine[c] 1.55 (0.50–3.63), 5 pancreas 1.88 (0.51–4.81), 4 lung 1.29 (0.76–2.04), 18 ovary 2.32 (0.28–8.38), 2 prostate 2.06 (0.43–6.04), 3 kidney 3.60 (0.98–9.20), 4 bladder 3.17 (0.38–11.5), 2 brain 1.28 (0.26–3.75), 3 Hodgkin's 1.78 (0.05–9.89), 1 *SMR for lung cancer no longer elevated in high exposed workers exposed for>1 yr* Statistically significant elevated SMRs were also observed for cancers of the esophagus, prostate and unspecified sites in the low exposed workers Urinary tract cancer *Trend towards increasing SMR with increasing*	Job information not available after 1978 No information on lifestyle factors or other environmental exposures

Study	Study design & follow-up	Study population and methods	Exposure Assessment Levels	Effects SMR (95% CI), no. of observed deaths[b]	Comments
				duration of exposure, and level (tercile) of exposure	
Coggon *et al.* 1987 U.K.	historical cohort 1947–84	7,949 (6,638 men and 1,311 women) workers employed in 8 reinforced-plastics companies 1947–84 (differing periods for each company) 51% worked < 1 yr and 8% worked ≥ 10 yr 979 workers excluded from exposure-related analyses SMRs based on national rates	From personnel records, workers were classified as hand laminators (high exposure) (44%), regular bystander exposure (7%), occasional bystander exposure (17%), or background exposure (32%) Authors estimated TWA styrene exposure levels for hand laminators at 40–100 ppm	*Total cohort (7 of the 8 companies)*[d] all cancer *0.*80 (0.69–0.93), 167 <u>Cancers with non-significant excess mortality (> 10%)</u> larynx 1.16 (0.14–4.18), 2 lung 1.12 (0.89–1.39), 83 melanoma 1.19 (0.14-4.30), 2 non-melanoma skin cancer 3.57 (0.43–12.9), 2 ovary 1.49 (0.41–3.81), 4 Lung cancer mortality was highest among individuals with moderate and high exposure, and individuals exposed from 1 to 9 yr, but the exposure response relationship was not consistent. Mortality of other cancers was not consistently related to first year of exposure, duration of exposure, or latency for any type of cancer *Laminators* <u>Cancers with non-significant excess mortality</u> large intestine 1.40, 5 lung 1.20, 25 cervix 1.96, 1 ovary 2.82, 2 prostate 1.20, 2	Low statistical power No data on smoking Included in the European multinational study by Kogevinas *et al.* (1994a)
Wong 1990 U.S.	historical cohort 1948–77 nested case-control study	*Historical cohort* 15,908 workers employed ≥ 6 mo in 30 reinforced-plastics companies in work	*Cohort study* Work histories were obtained from employment records. An industrial hygiene survey, which	*Cohort study: Repiratory cancer* total cohort 1.16, 34 No clear trend of increasing mortality with increasing duration of employment or increase in exposure (potential maximum TWA or average	Young cohort; 46% worked for less than 2 years Four cases identified in the

Study	Study design & follow-up	Study population and methods	Exposure Assessment Levels	Effects SMR (95% CI), no. of observed deaths[b]	Comments
	of respiratory cancer	areas with exposure to styrene 1948–77; 499 deaths were observed SMRs based on national rates *Nested case-control study* *Cases:* 40 respiratory cancer deaths; 44 deaths occurred in the cohort (including deaths after the observation period), but eligible controls could not be found for 4 deaths *Controls:* 102 matched controls; deceased members of cohort, maximum of 3 per case, matched for plant, age at death (within 5 yr), year of death (within 5 yr), sex, and race ORs calculated by Mantel-Haenszel, and logistic regression methods	contained current or past TWA and peak range exposure values, was used to consolidate record job titles (from personnel records) into study job titles. This information was incorporated into a job dictionary and used to classify individuals into exposure groups. *Case-control study* More detailed work history (than for the cohort analysis), exposure of each job segment, exposures from employment outside the plastics industry, and smoking history were obtained from employment records, medical and insurance records, and some interviews with next of kin or co-workers.	TWA) *Subgroups with excess respiratory cancers* Respiratory cancer mortality increased non-significantly with increasing latency. Among workers with ≥ 20 years latency; significant SMRs for lung cancer were observed in women (total across all durations of exposure) and men who worked 2–5 years. Higher SMRs were observed in workers in hot process than cold process (See later update [Wong *et al.* 1994] for findings on cancer at other sites) *Case-control study of respiratory cancer* Mantel-Haenszel OR, cases/controls, P styrene (direct) exposure 0.63, 15/44, 0.29 Logistic regression Only smoking showed an association in a multivariate analysis that included direct exposure to styrene, duration of exposure, type of exposure (hot or cold process), smoking, and interaction terms.	cohort were not used in the case-control analysis Potential exposure to styrene is higher for cold process than hot process
Wong *et al.*	historical cohort	Same cohort as Wong 1990, except 15,826	Company-specific JEMs constructed from	*Total cohort (1,628 deaths)*	Small proportion of the total study

Study	Study design & follow-up	Study population and methods	Exposure Assessment Levels	Effects SMR (95% CI), no. of observed deaths[b]	Comments
1994 U.S.	1948–89 avg. 19.5 yr 307,972 person-years	(75.6 % men) (identified 30 with duplicate records and 52 who had worked less than 6 months) 24% worked < 1 yr and 27% ≥ 5 yr SMRs based on national rates Cox proportional hazard models including age, sex, exposure duration, and cumulative exposure used for internal analysis	measurements and production characteristics, linked with workers by job and department reported on employment records The worker population was classified into six process categories based on exposures to styrene and other substances: open-mold processing, mixing and closed-mold processing, finishing operations, plant supports, maintenance and preparation, and supervisory and professional Approximately 12% of the workers were engaged in open-mold processing, with estimated average TWAs of 20–60 ppm Other worker categories exposed at average ≤ 5 ppm TWA	<u>Cancers with significant excess mortality</u> all cancer 1.16 (1.05–1.27), 425 esophagus 1.92 (1.05–3.22), 14 cervix 2.84 (1.36–5.21), 10 other female genital organs 2.02 (1.07–3.45), 13 lung 1.41 (1.20–1.64), 162 No exposure-response relationship (cumulative exposure or duration) observed for any cancer <u>Lung cancer</u> *Latency (years since first employment)* < 10 1.07, 23, $P > 0.05$ 10–19 1.46, 70, $P < 0.01$ ≥ 20 1.51, 69, $P < 0.01$ <u>Internal analysis (stepwise regression and multivariate); entire cohort</u> Cumulative exposure or duration of exposure was not associated with increased mortality from cancers of the esophagus, lung, female genital organs uterus, kidney, lymphohematopoietic tissue, NHL, multiple myeloma, or leukemia *High-exposure workers (open mold processing category) with > 2 years of exposure* A statistically nonsignificant excess in mortality was observed for cancer of the esophagus, stomach, uterus, cervix, kidney, lymphosarcoma, and cancer of all other lymphopoietic tissues. SMRs based on small numbers of observed deaths	population was exposed to high styrene levels Cox regression models of cumulative styrene exposure may have been over-controlled by the inclusion of duration of employment SMRs for high-exposure workers based on small numbers of observed and expected deaths
Kolstad *et al.*	historical	36,610 workers at 386	No data on individual	*Total cohort (Kolstad* et al. *1994 LH, 1995, solid*	Imprecise

Study	Study design & follow-up	Study population and methods	Exposure Assessment Levels	Effects SMR (95% CI), no. of observed deaths[b]	Comments
1995, Kolstad *et al.* 1993, Kolstad *et al.* 1994 Denmark	cohort 1970–89 avg. 10.9 yr 618,900 person-years	reinforced-plastics companies employed > 1 day 1964–88 60% worked < 1 yr, 3% worked ≥ 10 yr SIRs based on national rates 14,293 workers in 84 companies not producing reinforced plastics were used as controls in the internal analysis Incidence RRs were calculated in the internal analysis using Poisson regression models that included the following variables: exposure probability (none, low, high), age, year of first employment, duration of employment and time since first employment	exposure or job titles was available; information on duration of employment was obtained from a national pension fund Classification of exposure was based on percent of workforce producing reinforced plastics Probable high exposure: ≥ 50% of the workforce producing plastics: 12,862 employees Possible low exposure: < 50% of the workforce producing plastics: 23,748 employees An estimated 43% of the study population were laminators Historical personal air samples (N = 2,473) showed average styrene levels declining from 180 ppm (1964–70) to 43 ppm (1976–88) (Jensen *et al.* 1990)	*tumors)* all solid cancers 0.99 (0.93–1.05), 1,134 Cancers with non-significantly elevated SIRs pancreas 1.20 (0.86–1.63), 41 nasal cavities 1.84 (0.74–3.80), 7 lung 1.12 (0.98–1.26), 248 pleura 1.78 (0.85–3.28), 10 external male genital organs 1.60 (0.64–3.30), 7 bladder 1.16 (0.96–1.39), 117 LH 1.20 (0.98–1.44), 112 NHL 1.33 (0.96–1.80), 42 leukemia 1.22 (0.88–1.65), 42 Leukemia, by employment variables (Kolstad *et al.* 1994) *Probable exposure* high 1.38 (0.75–2.32), 14 low 1.15 (0.77–1.67), 28 *Year of first employment* 1964–70 1.54 (1.04–2.19), 30 1971–75 1.00 (0.46–1.90), 9 1976–88 0.51 (0.11–1.50), 3 *Years since first employment* < 10 0.71 (0.34–1.31), 10 ≥ 10 1.57 (1.07–2.22), 32 *Years of employment (≥ 10 years latency)* < 1 2.34 (1.43–3.61), 20 ≥ 1 1.01 (0.52–1.77), 12 Internal analysis (probable high exposure)	measures of exposure duration Few long-term workers Short duration of follow-up The workers at companies with ≥ 50% styrene-exposed workers were included in the European study by Kogevinas *et al.* (Kogevinas *et al.* 1994a, Kogevinas *et al.* 1993)

Study	Study design & follow-up	Study population and methods	Exposure Assessment Levels	Effects SMR (95% CI), no. of observed deaths[b]	Comments
				(Kolstad *et al.* 1995) IRR (95% CI), no. of observed cases *Pancreatic cancer* overall 2.2 (1.1–4.5), 17 *Years of exposure* < 1 3.1 (0.9–10.3), 9 ≥ 1 3.4 (1.0–11.4), 8 *Year of first employment* 1970 or before 2.0 (0.6–6.9), 10 after 1970 1.4 (0.4–4.7), 7 *Other cancers with elevated SIRs* No consistent findings in internal analyses by first year of employment, employment duration, and latency	
Kolstad *et al.* 1996 Denmark	nested case-control study of myeloid leukemia	*Cohort:* Kolstad *et al.* 1994, 1995 *Cases*: 12 myeloid leukemia patients with clonal chromosome aberrations (chromosome analysis was available on 19 of the myeloid leukemia cases in the cohort) *Controls:* 57 randomly selected employees without styrene exposure and matched to cases by age (3 per	See Kolstad *et al*. 1994, 1995	OR (95% CI) for myeloid leukemia with clonal chromosome aberrations *Any exposure* 2.5 (0.2–25.0) *Probability of exposure* low 3.0 (0.3–32.2) high 1.6 (0.1–22.0) *Years of exposure* < 1 5.9 (0.5–74.3) ≥ 1 1.1 (0.1–15.3) *Years since first exposure* < 10 no exposed cases ≥ 10 3.7 (0.4–40.3) *Year of first employment*	Small numbers of exposed cases

Study	Study design & follow-up	Study population and methods	Exposure Assessment Levels	Effects SMR (95% CI), no. of observed deaths[b]	Comments
		case) ORs calculated by conditional and unconditional regression that included probability of styrene exposure (none, low, high), year of first employment, time since first employment, age, and year of diagnosis		1970 or before 5.9 (0.6–57.8) after 1970 2.3 (0.2–26.2)	
Kogevinas *et al.* 1994a, Kogevinas *et al.* 1993 Europe	historical cohort follow-up varied by country 1945–91 avg. 13 yr 539,479 person-years	40,688 workers (85% men) at 660 reinforced-plastics plants in Denmark (39%), Finland (5%), Norway (5%), Italy (18%), Sweden (9%), and U.K. (24%) 60% worked < 2 yr, 9% worked ≥ 10 yr SMRs based on national rates Poisson regression models used to compare exposure-specific cancer rates (RR) and conduct trend analyses for exposure within the	Job titles were used to assign workers to the following exposure categories: laminators (26%) unspecified tasks (48%) other exposed jobs (13%) non-styrene exposed (10%) unknown job titles (3%) Exposure matrix was constructed from 16,500 personal styrene measurements obtained 1955–90 and 18,500 measurements of urinary metabolites in the 1980s Cumulative styrene exposure and average exposure estimated for each subject from job	*Total cohort* *Cancers with non-significant excess mortaltiy (> 10%)* small intestine 1.50 (0.31–4.38), 3 larynx 1.11 (0.53–2.05), 10 ovary 1.40 (0.70 – 2.51), 11 myeloid leukemia 1.10 (0.63 – 1.79), 16 *Cancers with significantly decreased mortality* all cancer 0.87 (0.81–0.94), 686 buccal cavity and pharynx 0.33 (0.11–0.77), 5 rectum 0.62 (0.38–0.95), 21 breast 0.52 (0.28–0.89), 13 brain 0.62 (0.37–0.98), 18 *Laminators* *Cancers with non-significant excess mortality* small intestine 2.27 (0.06–12.66), 1 pancreas 1.48 (0.76–2.58), 12 larynx 1.55 (0.32–4.52), 3	Imprecise measures of cumulative styrene exposure and short duration of follow-up limit power to detect an effect Study population included the Danish workers (39%) (Kolstad *et al.* 1994, 1995)

Study	Study design & follow-up	Study population and methods	Exposure Assessment Levels	Effects SMR (95% CI), no. of observed deaths[b]	Comments
		population	records and exposure levels averaged across country, period, and job Exposure levels among laminators declined from ~200 ppm before 1965 to < 80 ppm in the 1980s	ovary 2.61 (0.71–6.69), 4 thyroid 2.27 (0.06–12.66), 1 NHL 1.40 (0.56–2.88), 7 Hodgkin's disease 1.33 (0.27–3.88), 3 *Poisson regression exposure models for cancer* Test for trend in RRs: *P*-value *Average exposure* LH 0.019 leukemia 0.47 malignant lymphoma 0.052 *Time since first exposure* LH 0.012 leukemia 0.094 malignant lymphoma 0.072 *Cumulative exposure* no increase in RR with increasing exposure for any LH cancer type *Pancreas* RR increased with increasing cumulative exposure ($P = 0.068$) *Esophagus, or kidney* RR increased (non-significantly) with cumulative exposure (slightly for esophagus) *Lung* No increase in RR with increasing cumulative exposure	

CI = confidence interval, IRR = incidence rate ratio, LH = lymphohematopoietic cancer, OR = odds ratio, RR = rate ratio, SIR= standard incidence ratio, SMR = standard mortality ratio.

[a]The table contains results from the latest update of a study population. Separate entries are made for related studies if there were major differences between publications, such as differences in the study design (e.g., nested case control and cohort) or population composition. The results of the earlier publication for the

8-company U.S. cohort (Wong *et al.* 1990) for respiratory cancer are also presented in addition to the later publication (Wong *et al.* 1994) since the excess of respiratory cancer was the basis for the nested case-control study.
[b]Unless otherwise stated.
[c]Not including rectum.
[d]Records and follow-up from one company were incomplete, so the analysis for that company was reported separately.

3.2 The styrene-butadiene rubber industry

Generally lower styrene exposure levels are seen in the styrene-butadiene rubber industry than the glass fiber–reinforced plastics industry, although significant exposures to workers can still occur (see Section 2.6). Mean levels reported for this industry are generally less than 15 ppm for synthesis of styrene-butadiene latex, and concentrations below 0.15 ppm have been reported for vulcanization and extrusion processes involving styrene-butadiene rubber. Exposure to end-users (such as rubber tire manufacturers) would likely be even lower. Workers in the styrene-butadiene industry can be exposed to 1,3-butadiene and DMDTC, in addition to styrene (Delzell *et al.* 2001, Macaluso *et al.* 2004). This section does not include studies on end-users except for McMichael *et al.* (1976a) because it provides specific estimates for workers in a plant producing styrene butadiene (primarily) and other rubbers.

McMichael *et al.* (1976a) studied cancer mortality at a rubber tire manufacturing plant in the United States. Meinhardt *et al*. (1982, 1978) and Lemen *et al*. (1990) initially studied 2,756 workers at two styrene-butadiene rubber plants (forming one complex) in Texas. Matanoski and coworkers (Matanoski *et al.* 1997, Matanoski *et al.* 1993, Matanoski *et al.* 1990, Matanoski and Schwartz 1987, Santos-Burgoa *et al.* 1992) studied workers (from 12,110 to 13,686, depending on study)[2] in eight other styrene-butadiene rubber plants (seven U.S. plants and one Canadian plant). Later, Delzell and colleagues (Delzell *et al.* 1996, 2001, 2006, Macaluso *et al.* 2004, 1996; Sathiakumar *et al*.1998, 2005, and Graff *et al.* 2005) studied 13,130 to 16,610 styrene-butadiene rubber industry workers from the same plants studied by Meinhardt *et al*. and seven of the plants studied by Matanoski, Santos-Burgoa, and coworkers (Delzell *et al.* 2001, Delzell *et al.* 1996). Delzell *et al.*, Macaluso *et al*. (1996, 2004), Sathiakumar *et al.* (1998, 2005), and Graff *et al.* (2005) did not have access to information from the previous studies by Meinhardt *et al.*, Matanoski *et al.*, and Santos-Burgoa *et al*. that allowed identification of individual subjects and a formal evaluation of the overlap between the populations. The study populations were established by different procedures and exclusion criteria, which may partly explain the

[2] Number of workers varied among the publications.

lack of complete consistency in the number of study subjects across the populations. An overview of the studies is presented in Table 3-4

3.2.1 United States: McMichael et al.

McMichael *et al.* (1976a) studied 6,678 male workers at a rubber tire manufacturing plant. A small fraction of the total cancer cases (2% with 5 or more years of employment and 3% with 2 or more years of employment) was observed among workers engaged in the production of styrene-butadiene and other synthetic rubbers; however, the proportion of the total cohort exposed to synthetic rubbers was not given. The total population was followed from 1964 to 1972, deaths were identified, and work histories were compared between cases and an age-stratified random sample of all workers (22%) in internal analyses. Work histories were extracted from personnel records and grouped into 16 major work areas. One of these areas was work in the synthetic plant where styrene-butadiene rubber was produced. For workers with at least 5 years employment in the synthetic plant, significantly increased risk ratios were observed for stomach cancer (RR = 2.2 , 99.9% CI = 1.4 to 4.3, number of deaths not stated), lymphohematopoietic cancer (RR = 6.2, 99.9% CI = 4.1 to 12.5), and lymphatic leukemia (RR = 3.9, 99.9% CI = 2.6 to 8.0). No other significant associations between work in synthetic rubber production and other selected cancers were observed. [Note: exposure ratios and/or risk ratios were reported by the authors only for certain selected cancer sites (see Table 3-9). It appears that if an exposure ratio greater than 1 was observed, an age-standardized risk ratio (in comparison with all workers in the cohort) was then calculated and reported.]

3.2.2 United States: Meinhardt et al.

Meinhardt *et al.* studied the mortality of 2,756 styrene-butadiene rubber workers at two plants in Texas, prompted by the deaths of 2 workers from leukemia (Meinhardt *et al.* 1982, 1978). White male workers with at least six months of non-management or non-administrative employment were included in the study. The population was followed from 1943 through 1976, and 3% were lost to follow-up. SMR analyses compared observed deaths with expected values computed from the national rates. The average length of employment was about 10 years. A total of 53,929 person-years were accumulated, and the average follow-up was 19 years. Average TWA styrene exposure

levels based on samples from all areas of the production facilities were 0.94 ppm (0.03 to 6.46) and 1.99 ppm (0.05 to 12.3) for the two plants.

Among all workers, 56 cancer deaths were recorded [SMR = 0.72, 95% CI = 0.54 to 0.93]. Statistically nonsignificant increases in mortality were seen for all lymphohematopoietic malignancies [SMR = 1.32, 95% CI = 0.66 to 2.37, 11 deaths], NHL 1.65 [95% CI = 0.45 to 4.27, 4 observed deaths], and leukemia 1.73 [95% CI = 0.63 to 3.73, 6 observed deaths]. [The bracketed SMRs for both plants combined were calculated using the data provided in the publication for each plant.] Because most leukemia mortality in one of the plants was among workers who had started work before the end of 1945, a separate analysis was conducted for all 600 workers who started work before the end of 1945; this analysis showed a statistically significant increase in mortality for all lymphohematopoietic malignancies (SMR = 2.12, $P < 0.05$, 9 observed deaths). No subanalyses were conducted by level of estimated styrene exposure.

Lemen *et al*. (1990) reported on a further follow-up of this cohort through December 31, 1982 at one plant (A) and December 31, 1981 for a second plant (B), yielding a total of 43,341 person-years at risk of death and 390 observed deaths, 77 from cancers, at plant A and 26,314 person-years and 148 observed deaths, 29 from cancers, at plant B. In the subcohort exposed to the batch production process used between 1943 and 1945, a total of 19,582 person-years and 291 observed deaths, 61 from cancers, were available for analysis. No SMRs for cancers were reported in this follow-up report. However, the authors noted that mortality for cancers of the trachea, bronchus, and lung had increased (from 16 to 34 deaths) and that the only other increases in SMRs were observed for lymphosarcoma and reticuloma (3 deaths in the first analysis and 5 deaths in the follow-up; these 2 additional deaths occurred in the subcohort exposed to the batch process, as did the one additional lymphohematopoietic death in this follow-up.

3.2.3 United States and Canada: Matanoski, Santos-Burgoa, and coworkers

Matanoski and coworkers established a cohort of male workers employed for more than 1 year in seven U.S. styrene-butadiene rubber plants and for more than 10 years in one Canadian styrene-butadiene rubber plant between 1943 and 1976 (Matanoski *et al.* 1997,

Matanoski *et al.* 1993, Matanoski *et al.* 1990, Matanoski and Schwartz 1987, Santos-Burgoa *et al.* 1992). The number of workers included in the different publications differed slightly, from 12,110 (Matanoski *et al.* 1997, Matanoski *et al.* 1993, Matanoski *et al.* 1990), to 13,686 (Santos-Burgoa *et al.* 1992), or 13,920 (Matanoski and Schwartz 1987). The population was initially followed to 1979 (Matanoski and Schwartz 1987) and was updated through 1982 (Matanoski *et al.* 1990). Loss to follow-up was 3%, and 251,431 person-years were accumulated; the average follow-up was about 21 years. Mortality was compared with national rates, and SMR values were calculated (Matanoski *et al.* 1990, Matanoski and Schwartz 1987). In internal analyses of lymphohematopoietic malignancies, odds ratios were estimated by Mantel-Haenszel methods and by conditional and unconditional regression analysis (Matanoski *et al.* 1997, Santos-Burgoa *et al.* 1992). Initial internal analyses relied on 59 cases and 193 controls individually matched by plant (and other variables) (Santos-Burgoa *et al.* 1992). Subsequent analyses included 58 cases and replaced the original controls with 1,242 controls sampled without individual matching (Matanoski *et al.* 1997). This was done to avoid over-matching, because measurements indicated that styrene exposure levels differed between plants.

A panel of experts constructed a job-exposure matrix for internal analyses (Santos-Burgoa *et al.* 1992). From the job titles listed in the personnel records, cases and controls were classified as exposed or not exposed to styrene and butadiene and were assigned a relative exposure rank (0 to 10, 10 representing the highest exposure). Cumulative exposure was calculated from duration of employment in each job, and cases and controls were classified as having a cumulative exposure score above or below the geometric mean value.

Later, five of the eight plants provided 3,649 measurements of styrene in work-room air taken between 1978 and 1983 (Matanoski *et al.* 1993). The average styrene level for all plants was 3.53 ppm (SD = 14.32) and varied between 0.29 ppm and 6.66 ppm across the plants. Styrene levels were averaged across jobs and plants, and average cumulative styrene exposure levels were estimated for cases and controls from information about plant, job title, and number of months exposed (Matanoski *et al.* 1997).

Among all workers at the eight plants, overall cancer mortality was less than expected (SMR = 0.85, 95% CI = 0.78 to 0.93, 518 observed deaths). Statistically nonsignificant increases in mortality were observed for Hodgkin's disease (SMR = 1.20, 95% CI = 0.52 to 2.37, 8 observed deaths) and for other lymphatic malignancies (SMR = 1.11, 95% CI = 0.64 to 1.77, 15 observed deaths). No increased mortality was reported for the other lymphohematopoietic cancers, including leukemia (Matanoski *et al.* 1990). Mortality from all lymphopoietic [lymphohematopoietic] malignancies was not related to duration of employment. SMR values were also presented separately for white and black production workers, maintenance workers, utility workers, and other workers, but it was not clear whether styrene exposure differed among these categories.

Among workers categorized as having a cumulative styrene exposure score above average, the internal matched analyses presented by Santos-Burgoa *et al.* (1992) showed statistically nonsignificant increases in the odds ratios for leukemia (OR = 3.13, 95% CI = 0.84 to 11.2, 26 cases, 84 controls), lymphosarcoma (OR = 1.33, 95% CI = 0.11 to 16.6, 6 cases, 23 controls), and other lymphatic malignancies (mainly myeloma) (OR = 1.35, 95% CI = 0.25 to 7.40, 18 cases, 56 controls), but a non-significant decrease in the OR for Hodgkin's disease (OR = 0.40, 95% CI = 0.05 to 3.25, 8 cases, 29 controls). Comparable ORs were seen in unmatched analyses of the same dataset. In matched models that controlled for exposure to butadiene, no increased risk was apparent for leukemia (OR = 1.06, 95% CI = 0.23 to 4.95), or other lymphatic malignancies (OR = 0.94, 95% CI = 0.16 to 5.53); the OR for all lymphohematopoietic malignancies was 1.29 (95% CI = 0.53 to 3.15). There was no indication of positive interaction between exposure to styrene and butadiene for all lymphohematopoietic malignancies; however, no results were obtained for leukemia, because the model did not converge.

Matanoski *et al.* (1997) and colleagues presented updated analyses that relied on non-matched controls and measurement-derived estimates of styrene exposure. Analyses were based on average or cumulative styrene exposure levels (calculated across all exposed years). Using a step-down unconditional logistic regression with age, age at first hire, race, year of hire before 1950, and both styrene and butadiene in the initial model, a time-weighted working lifetime average styrene exposure level of 1 ppm increased the ORs for

myeloma (OR = 3.04, 95% CI = 1.33 to 6.96, 10 deaths), lymphomas (OR = 2.67, 95% CI = 1.22 to 5.84, 12 deaths), and all lymphohematopoietic malignancies (OR = 2.20, 95% CI = 1.46 to 3.33, 58 deaths), but not for leukemia (no estimate was provided for leukemia). [Note that styrene alone remained in the final model for each of these cancers.] Also, final models for leukemia and Hodgkin's disease (which were not associated with styrene exposure) included only exposure to butadiene. With respect to cumulative exposure, using the same initial variables, the mortality for leukemia increased statistically significantly by increasing cumulative styrene exposure ($P = 0.006$) in a final model in which both butadiene exposure and duration of employment remained. Styrene alone was also significantly associated with myeloma ($P = 0.023$) and with all lymphohematopoietic cancers ($P = 0.000$ [P-value as reported in the paper]) in a final model in which styrene and duration of employment remained. [Final models for Hodgkin's disease included butadiene exposure and duration. Note that the ICD codes for lymphomas (200 & 202) are the same as non-Hodgkin's lymphoma.]

3.2.4 United States and Canada: Delzell, Sathiakumar, Macaluso, Graff

Delzell *et al.* (1996) established a cohort of all 17,964 male workers employed for at least one year between 1943 and 1990 at eight U.S. styrene-butadiene rubber plants (two of these plants were organized as one company complex) and one Canadian styrene-butadiene rubber plant. (The start date for some of the plants varied between 1950 and 1965.) The population included workers at seven of the eight plants previously studied by Matanoski *et al.* (1993, 1990) and Santos-Burgoa *et al.* (1992) and at the two-plant complex studied by Meinhardt *et al.* (1982). The companies employed an estimated total of 25,500 workers. [Note that the Delzell cohort exanded the cohort to include more recent employees with start dates up to 1990, whereas the earlier cohorts followed workers employed from 1943 to 1976. Adding workers with lower exposures, shorter latency, and duration worked might reduce apparent risk.]

One series of studies reporting findings (SMRs) for multiple cancers sites (Sathiakumar *et al.* 1998, Sathiakumar *et al.* 2005) or specifically for lymphopoietic cancers (Delzell *et al.* 1996) for the entire cohort, and for subcohorts based on work areas (lymphohematopoietic cancer only) whereas a second series of studies reported findings

(RR) based on quantitative estimates of exposure to styrene (and butadiene or DMDTC) and mortality from lymphohematopoietic cancers (Delzell *et al.* 2001, Graff *et al.* 2005, Macaluso *et al.* 1996). A full report of the latest update (Graff *et al.* 2005, Satiakumar *et al.* 2005) of the cohort was published by Delzell *et al.* in 2006. This report contains details of some analyses that were not included in the individual papers.

3.2.4.1 Study population

The first study was conducted among workers employed for at least one year between 1943 and 1990 and followed up until 1991 (Delzell *et al.* 1996, Sathiakumar *et al.* 1998); at that time, 25% had died (3,976), 70% were presumed alive, and 5% were lost to follow-up. Mortality analysis was conducted on 15,649 subjects and excluded 2,315 Canadian workers who had not worked in styrene-butadiene rubber or other related operations or who had worked in unspecified areas of one of the plants. Delzell *et al.* 2006 reported that the "2,312" Candadian workers had worked in butyl rubber production or styrene production or were unspecified. Follow-up was later extended to 1998 for the cohort of 17,924 workers, and included 6,327 observed deaths while vital status was unknown for 570 (3%) (Delzell *et al.* 2006, Sathiakumar *et al.* 2005). The authors stated that 40 workers from the orignal cohort were excluded because they did not meet study criteria (employment length or gender), or were duplicates, [but they did not discuss the 2,315 non-styrene-butadiene rubber workers excluded from the first study]. Analyses based on work areas were limited to workers in styrene-butadiene rubber operations (N = 15,612).

Different subsets of the cohort were used in the series of studies of quantitative estimates of styrene exposure and lymphohematopoietic cancer mortality (primarily leukemia) (Delzell *et al.* 2001, Delzell *et al.* 2006, Graff *et al.* 2005, Macaluso *et al.* 1996). The analyses reported by Macaluso *et al.* (1996) and Delzell *et al.* (2001) were based on the 1991 follow-up, and analyses reported by Graff *et al*. and Delzell *et al.* 2006 were based on the 1998 follow-up. The study by Macaluso *et al.* (1996) (which evaluated cumulative exposure to styrene, butadiene and mortality from leukemia) excluded 1,354 workers (of the 17,964 member cohort) at two plants for whom quantitative estimates of exposure could not be established, and the analyses were based on 16,610 workers. Delzell e*t al.*

(2001) further excluded twelve workers with duplicate records and 3,468 workers who died before reaching 40 years of age or 10 years latency, because no leukemia deaths occurred in these groups; this left 13,130 workers. This study also used a revised exposure assessment (see below) and evaluated leukemia mortality and quantitative exposure to styrene, butadiene, and DMDTC. The study reported by Graff *et al.* (2005) and Delzell *et al.* (2006) stated that their analysis was on 16,579 workers for whom quantitative estimates of exposure could be established (which excluded 25 workers (from Macaluso *et al*. 2006) who were determined to have duplicate records or did not meet study criteria such as employment length or gender).

Vital status was established for U.S. workers via plant records, the National Death Index and DMV records, and in Canada, through plant personnel and benefit records and record linkage with the Canadian Mortality Database. [Note that Matanoski *et al.* (1990) reported that they relied on company pension and insurance records to identify deaths among employees who worked 10 years or more or reached age 45 during employment because of the high cost of a death search through Statistics Canada for the workers.]

3.2.4.2 Exposure assessment and job classification

Personnel records were reviewed, and 308 work-area groups or job groups with similar tasks and exposure potential were identified. The groups were further combined into five main process groups and seven process subgroups: (1) rubber/butadiene production (37%): polymerization, coagulation, finishing; (2) maintenance (24%): shop, field; (3) labor (15%): production, maintenance; (4) laboratories (9%); and (5) other operations (15%) (Sathiakumar *et al.* 1998). Macaluso *et al.* (1996) constructed a plant-specific, job-exposure matrix from industrial hygiene monitoring surveys, archival material, walk-through surveys, meetings with plant officials, and interviews with workers. Eight-hour TWA exposure levels for styrene, butadiene, and benzene were estimated for each year between 1943 and 1992 for each of the work areas or job groups by air dispersion models (Macaluso *et al.* 1996). Cumulative exposure was computed, taking into account the extents and durations of different tasks. As of the end of the 1991 follow-up, 83% of the cohort were considered to have been exposed to styrene, with a median cumulative

exposure of 7.4 ppm-years, and 75% to butadiene, with a cumulative exposure of 11.2 ppm-years (Macaluso *et al.* 1996).

Delzell *et al.* (2001) later characterized this exposure estimation process as controversial, and they revised the exposure estimates and added estimates of exposure to DMDTC, as further described by Macaluso *et al.* (2004). The authors did not substantiate this critique, but others have cited them as characterizing the original estimates as uncertain and not completely validated (Sielken and Valdez-Flores 2001). [The revised exposure estimates used by Macaluso *et al.* (2004) represented an improvement over the original estimates because of detailed industrial hygiene and chemical engineering reviews of the processes, job activities and work area, and historical changes. A senior industrial hygienist, who had extensive experience within the industry and with the methodology for estimating historical exposures, guided the work, which included identification of new tasks, additional information on the operations, modification of some of the assumptions needed to estimate exposure, and verification that all of the assumptions were reasonable. Information on use of personal protective equipment was obtained through interviews with long-term employees.] According to the original exposure assessment, estimated TWA styrene exposure levels for active workers declined from 1.8 ppm in the 1940s to 0.1 ppm in the early 1990s (Macaluso *et al.* 2004), partly because of decreasing exposure levels and partly because of decreasing styrene exposure prevalence. The revised styrene TWA exposure estimates were about twice as high as the original estimates reported in Macaluso *et al.* 1996 and declined from about 2 ppm during 1940 to 1970 to about 0.5 ppm in the late 1980s. The revised assessment estimated a median cumulative exposure of 17 to 18 ppm-years (Delzell *et al.* 2001, Macaluso *et al.* 2004) for the 85% of the workers who were exposed to styrene. The cumulative styrene exposure estimates were highly correlated with those for butadiene and DMDTC (Spearman rank correlations of 0.78 and 0.60, respectively). Note that 79% of workers were estimated to have been exposed to butadiene and 62% to DMDTC (Delzell *et al.* 2001). Exposure to DMDTC occurs primarily though dermal absorption, and cumulative estimated exposure was calculated as mg-years DMDTC/cm of skin.

3.2.4.3 SMR analyses

SMR analyses compared observed with expected deaths calculated from national mortality rates (Delzell *et al.* 2006, Delzell *et al.* 1996, Sathiakumar *et al.* 1998, Sathiakumar *et al.* 2005).

SMR analyses based on follow-up until 1991 showed that among all styrene-butadiene rubber workers, overall cancer mortality was less than expected (SMR = 0.93, 95% CI = 0.87 to 0.99, 950 observed deaths) (Sathiakumar *et al.* 1998). Significant deficits in mortality were also seen for cancer of the buccal cavity and pharynx (SMR = 0.50, 95% CI = 0.28 to 0.82, 15 observed deaths) and esophagus (SMR = 0.59, 95% CI = 0.35 to 0.93, 18 observed deaths). A statistically nonsignificant excess of mortality from leukemia was seen among all workers (SMR = 1.31, 95% CI = 0.97 to 1.74, 48 observed deaths), and significant excesses were seen among all workers ever employed hourly (SMR = 1.43, CI not reported, $P < 0.05$, 45 deaths) and among ever-hourly workers with employment duration of at least 10 years and latency of at least 20 years (SMR = 2.24, 95% CI = 1.49 to 3.23, $P < 0.05$, 28 deaths) (Sathiakumar *et al.* 1998). Increased leukemia mortality was also seen among workers in polymerization (SMR = 2.51, 95% CI = 1.40 to 4.14, 15 deaths), coagulation (SMR = 2.48, 95% CI = 1.00 to 5.11, 7 observed deaths), the maintenance subgroup of labor (SMR = 2.65, 95% CI = 1.41 to 4.53, 13 observed deaths), and laboratories (SMR = 4.31, 95% CI = 2.07 to 7.93, 10 observed deaths) (Delzell *et al.* 1996).

Repeated SMR analyses based on the extended follow-up until 1998 did not change this mortality pattern considerably (Sathiakumar *et al.* 2005). All cancer mortality (SMR = 0.92, 95% CI = 0.88 to 0.97, 1,608 observed deaths), and buccal cavity-pharynx cancer mortality (SMR = 0.47, 95% CI = 0.29 to 0.71, 22 observed deaths), still showed significant deficits, while this no longer was the case for esophageal cancer (SMR = 0.94, 95% CI = 0.68 to 1.26, 44 observed deaths). With respect to lymphohematopoietic cancers, non-significant excesses in mortality were observed for leukemia (SMR = 1.16, 95% CI = 0.91 to 1.47, 71 observed deaths) and Hodgkin's disease. All lymphohematopoietic malignancies, NHL, and multiple myeloma showed observed numbers of death close to the expected (Sathiakumar *et al.* 2005). Sub-analyses indicated

statistically nonsignificant increases in leukemia in workers ever employed hourly (SMR = 1.23, 95% CI = 0.94 to 1.57, 63 observed deaths), and significant excesses among those employed for at least 10 years and 20 to 29 years since hire (SMR = 2.58, 95% CI = 1.56 to 4.03, 19 observed deaths). No excess of leukemia was seen for 30 years or more after first employment (SMR = 1.02, 95% CI = 0.62 to 1.58, 20 observed deaths).

Several analyses of cell-type specific leukemias were conducted by Sathiakumar *et al*. (2005), Graff *et al.* (2005), and Delzell *et al.* (2006) including acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML) and other leukemias and NHL+CLL. A statistically significant increase in SMR for CML was observed among the ever-hourly employed workers (SMR = 2.00, 95% CI = 1.00 to 3.58, 11 observed deaths) (Sathiakumar *et al.* 2005). A statistically nonsignificant increase in mortality from CLL was observed (SMR = 1.71, 95% CI = 0.96 to 2.81, 15 observed deaths), while there was no increase in the numbers of AML (SMR = 0.97, 95% CI = 0.48 to 1.73, 11 observed deaths) or ALL (SMR = 0.51, 95% CI = 0.01 to 2.82, 1 observed death). Statistically significant increases were observed for AML among workers with < 20 years since hire and < 10 years employment (SMR = 4.78, 95% CI = 1.30 to 12.24, 4 deaths) and for CML (SMR = 6.55, 95% CI = 2.4 to 14.26, 6 deaths). Significant (or borderline) increases were also found for NHL+CLL among every-hourly workers (SMR = 1.30, 95% CI + 0.99 to 1.67, 60 observed deaths), workers with 20 to 29 years since hire and 10+ years employment (SMR = 1.90, 95% CI = 1.01 to 3.25, 13 observed deaths), and with 30+ years since hire and 10+ years employment (SMR = 1.49, 95% CI = 1.02 to 2.10, 32 observed deaths).

With respect to work area and job type, statistically significant excesses of all leukemias were observed for workers employed in polymerization (SMR = 2.04, 95% CI = 1.21 to 3.22, 18 observed deaths), coagulation (SMR = 2.31, 95% CI = 1.11 to 4.25, 10 observed deaths), maintenance labor (SMR = 2.03, 95% CI = 1.14 to 3.35, 15 observed deaths) and laboratories (SMR = 3.26, 95% CI = 1.78 to 5.46, 14 observed deaths) (which appear to be due primarily to increases in CLL in the same departments; the SMRs for CLL were 4.97 (95% CI = 2.15 to 9.80, 8 observed deaths) in polymerization; 6.07 (95% CI = 1.97

to 14.17, 5 observed deaths), for coagulation; 3.09 (95% CI = 0.84 to 7.92, 4 observed deaths) in maintenance labor; and 5.59 (95% CI = 1.52 to 14.31, 4 observed deaths) in laboratories. SMR also were increased for AML (SMR = 2.95, 95% CI = 0.96 to 6.88, 5 observed deaths) in maintenance labor, CML in laboratories (SMR = 5.22, 95% CI = 1.08 to 15.26, 3 observed deaths), and CLL among finishers (SMR = 3.44, 95% CI = 1.38 to 7.09, 7 observed deaths). (The authors noted that while workers were assigned to one department for these analyses, there was a considerable likelihood of overlap between various departments (Sathiakumar *et al.* 2005, Delzell *et al.* 2006). Significant increases were also observed for NHL+CLL among workers in polymerization (SMR = 2.18, 95% CI = 1.31 to 3.41, 19 observed deaths) and finishing (SMR = 1.91, 95% CI = 1.21 to 2.86, 23 observed deaths).

Graff *et al.* (2005) and Delzell *et al.* (2006) also reported SMRs, adjusted for age, race, and calendar year, for all leukemias, CLL, AML, CML, and other leukemias, NHL, and multiple myeloma (MM), by cumulative level of styrene exposure. Statistically significant increases in SMRs were observed in the two highest categories of styrene exposure for all leukemias: SMR = 1.87 (95% CI = 1.02 to 3.13, 14 observed deaths) for 31.8 to < 61.1 ppm-years, and SMR = 1.91 (95% CI = 1.09 to 3.10, 16 observed deaths) for 61.1+ ppm-years. Statistically significant increases also were seen for the highest category of exposure only for NHL (SMR = 1.97, 95% CI = 1.08 to 3.31, 14 observed deaths), CLL (SMR = 3.10, 95% CI = 1.01 to 7.24, 5 observed deaths), and for the highest category for NHL+CLL (SMR = 2.29, 95% CI =1.36 to 3.62, 18 observed deaths).

3.2.4.4 Internal analyses of leukemia and other lymphohematopoietic cancers

Among the 15,649 workers studied by Delzell *et al*. (1996), 48 deaths with leukemia as the underlying diagnosis had been identified as of the end of follow-up in 1991. [This analysis excluded the 2,315 non-styrene-butadiene rubber workers.] Macaluso *et al.* (1996) included 58 leukemia deaths (7 with leukemia as a contributory diagnosis and 51 with leukemia as an underlying diagnosis on the death certificate) identified among 16,610 workers followed up to 1991. In a later analysis, Delzell *et al*. (2001) added 1 decedent with myelodysplasia as the underlying cause of death and a medical record that

indicated leukemia. In the follow-up of 17,924 men to 1998, Sathiakumar *et al.* (2005) identified a total of 162 lymphohematopoietic cancers: 53 NHL, 12 Hodgkin's disease, 71 leukemia and 26 multiple myeloma based on the underlying cause of death. In the analysis of 16,579 workers followed to 1998 for whom quantitative exposure estimates were available, Graff *et al.* (2005) identified 81 deaths from leukemia, 58 from NHL, 27 from multiple myeloma, and 13 from Hodgkin's disease, with these diagnoses as the underlying or contributing cause of death and confirmed by medical records, if available. (Note: Delzell *et al.* 2006 stated that the death certificate diagnoses and ICD codes (e.g., 71 leukemias) were used for the external analysis to avoid information bias).Relative risks were computed by Poisson regression models (Delzell *et al.* 2001, Delzell *et al.* 2006, Graff *et al.* 2005, Macaluso *et al.* 1996) or the Mantel-Haenszel method (Macaluso *et al.* 1996).

Internal analyses of lymphohematopoietic cancers used several approaches, resulting in a large number of statistical analyses. Relative risks were calculated for quartiles of cumulative (total ppm-years) of styrene exposure and ppm-years of exposure due to peaks above 50 ppm or below 50 ppm using single-chemical (styrene), two-chemical (styrene + butadiene or styrene + DMDTC), or three-chemical (styrene + butadiene + DMDTC) models. Models were adjusted for age and time since hire. In addition, analyses were also conducted using cross-categories of different levels of cumulative styrene or butadiene exposure.

Macaluso *et al*. (1996) presented rate ratios (relative risks) for leukemia mortality by cumulative exposure to styrene based on the original exposure assessment and adjusted for exposure to butadiene. Although SMRs for leukemia in the cohort tended to increase with increasing cumulative styrene exposure, the internal analyses that controlled for butadiene exposure showed no significant trend (Macaluso *et al.* 1996). The findings of the internal analysis were as follows: 0 ppm-year (reference group), RR = 1; 1 to 4 ppm-years, RR = 0.9; 5 to 9 ppm-years, RR = 5.4; 10 to 39 ppm-years, RR = 3.4; ≥ 40 ppm-years, RR = 2.7 (*P* for linear trend = 0.14). The authors also evaluated the association between benzene and leukemia and reported a weak association between increasing levels of cumulative exposure to benzene and leukemia mortality rates; that association

was eliminated when they controlled for exposure to butadiene and styrene. The authors concluded that no association existed between benzene and leukemia and excluded benzene from their other analyses.

Delzell *et al*. (2001) presented RRs for leukemia mortality by cumulative exposure to styrene based on the revised exposure assessment. As mentioned previously, the analysis excluded workers who died before reaching 40 years of age or 10 years latency because no deaths from leukemia occurred before this age and length of employment. Controlling for age and time since hire, Delzell *et al.* also found an increasing trend of leukemia deaths with cumulative styrene exposure. The findings were as follows: 0 ppm-years (reference group), RR = 1.0; > 0 to < 20.6 ppm-years, RR = 1.2 (95% CI = 0.5 to 3.3); 20.6 to < 60.4 ppm-years, RR = 2.3 (95% CI = 0.9 to 6.2); ≥ 60.4 ppm-years, RR = 3.2 (95% CI = 1.2 to 8.8). This trend was reduced when butadiene exposure was introduced to the model; the findings were as follows: 0 ppm-years (reference group), RR = 1.0; > 0 to < 20.6 ppm-years, RR = 1.1 (95% CI = 0.3 to 4.0); 20.6 to < 60.4 ppm-years, RR = 1.6 (95% CI = 0.4 to 6.4); ≥ 60.4 ppm-years, RR = 1.8 (95% CI = 0.4 to 7.3). If analyses furthermore included DMDTC exposure, no increasing trend was seen; the findings were as follows: 0 ppm-years, RR = 1.0; > 0 to < 20.6 ppm-years, RR = 0.6 (95% CI = 0.1 to 2.5); 20.6 to < 60.4 ppm-years, RR = 0.8 (95% CI = 0.2 to 3.7); ≥ 60.4 ppm-years, RR = 0.8 (95% CI = 0.2 to 3.8).

Among workers with a cumulative butadiene exposure below 20 ppm-years (the worker category with the lowest butadiene exposure according to the original exposure assessment), the risk of leukemia increased with increasing cumulative exposure to styrene. The findings were as follows: 0.1 to 9 ppm-years (reference), RR = 1.0; 10 to 39 ppm-years, RR = 1.7 (95% CI = 0.5 to 6.0); ≥ 40 ppm-years, RR = 7.0 (95% CI = 2.2 to 22) (Macaluso *et al.* 1996). No such trend was seen for strata with higher levels of cumulative butadiene exposure. Two deaths from leukemia occurred among styrene-exposed workers with no exposure to butadiene, but no formal risk assessment was conducted for this category of workers. No deaths from leukemia occurred among workers exposed to butadiene but not to styrene. No trend with cumulative styrene exposure was seen among the workers with the lowest cumulative butadiene exposure

(< 38.7 ppm-years) according to the revised exposure estimates, but this category included only 4 workers with styrene exposure above the reference category (Delzell *et al.* 2001). On the other hand, among workers classified with the highest cumulative butadiene exposure (≥ 287.3 ppm-years), the risk of leukemia increased with cumulative styrene exposure; the findings were as follows: 10.4 to 40.5 ppm-years, RR = 2.6 (95% CI = 0.7 to 9.2, 3 observed deaths); ≥ 40.6 ppm-years, RR = 4.1 (95% CI = 2.0 to 8.4, 18 observed deaths). No deaths from leukemia occurred in the reference category (< 10.4 ppm-years).

Graff *et al.* (2005) repeated these analyses based on the revised exposure assessment by (Delzell *et al.* 2001) and the 81 leukemia deaths observed up to the end of follow-up in 1998 (Graff *et al.* 2005). In addition, Graff *et al.* analyzed these leukemias by subtype: CLL (25 deaths); AML (including myelogenous and monocytic leukemias) (26 deaths); CML (16 deaths), and other leukemias (14 deaths). Graff *et al.* also analyzed findings for other lymphohematopoietic cancers, including NHL (58 deaths), multiple myeloma (27 deaths), and Hodgkin's disease (13 deaths). Detailed descriptions of the methods and full models included in these analyses were reported in Delzell *et al.* 2006. Findings for the single-chemical, two-chemical and three-chemical models and leukemia, NHL and NHL+CLL are present in Tables 3-2 and 3-3. [Note that no trend analyses were performed for most of these models.]

With respect to cumulative exposure to styrene and all leukemias combined, the single-chemical (styrene) model, adjusted for age and years since hire, showed an increased risk with increasing exposure; however, the only statistically significant risk estimate was for the highest quartile of exposure (see Table 3-2). When butadiene was added to this model, the exposure response was attenuated (all non-significant), and when both butadiene and DMDTC were included in the model with styrene RRs were less than one. The risk of leukemia increased with increasing exposure to butadiene in single-chemical, two-chemical and three-chemical models although was somewhat attenuated after adjusting for styrene and/or DMDTC. Signficant risk estimates for leukemia were also observed for DMDTC, which were somewhat attenuated in two- and three-chemical

models but still remained significant. When these analyses were repeated incorporating a ten-year exposure lag, the results were similar (Delzell *et al.* 2006).

These analyses were also repeated using total styrene peaks (> 50 ppm) and total butadiene peaks (> 100 ppm). The single-chemical (styrene) model showed an increasing trend with exposure compared with zero exposure (See Table 3-4). Intermediate and three-chemical models also showed an increasing trend with styrene exposure, but in each model the level of risk was somewhat attenuated (Graff *et al.* 2005, Delzell *et al.* 2006).

Table 3-2. Risk of leukemia with cumulative and peak exposure[a] to styrene, butadiene, and DMDTC[b]

Styrene exposure	No. of cases/person years	Styrene only RR (95% CI)	Styrene + butadiene RR (95% CI)	Styrene + DMDTC RR (95% CI)	Styrene + butadiene + DMDTC RR (95% CI)
Cumulative exposure, ppm-years					
0	7/77,460	1.0	1.0	1.0	1.0
> 0 to < 8.3	18/177,551	1.3 (0.6–3.2)	1.2 (0.4–3.7)	0.7 (0.3–2.0)	0.6 (0.2–2.2)
8.3 to < 31.8	19/132,311	1.6 (0.7–3.9)	1.4 (0.4–4.5)	0.7 (0.3–2.1)	0.7 (0.2–2.5)
31.8 to < 61.1	18/55,797	3.0 (1.2–7.1)	1.9 (0. 6–6.5)	1.2 (0.4–3.5)	0.8 (0.2–3.1)
61.1 +	19/57,056	2.7 (1.1–6.4)	1.3 (0.4–4.3)	1.0 (0.3–2.9)	0.5 (0.1–2.0)
Number of styrene peaks					
0	14/202,225	1.0	1.0	1.0	1.0
> 0 to < 58	16/151,484	1.5 (0.7–3.0)	1.1 (0.4–2.9)	1.1 (0.9–3.7)	1.0 (0.4–2.7)
58 to < 170	17/53,266	3.6 (1.8–7.3)	2.6 (1.0–7.0)	2.3 (1.0–4.5)	2.0 (0.7–5.6)
170 to < 699	17/40,653	4.6 (2.3–9.4)	3.3 (1.2–8.9)	3.3 (1.4–6.6)	2.9 (1.0–8.2)
699 +	17/52,545	4.2 (2.0–8.6)	2.8 (1.0–7.8)	3.0 (1.4–6.4)	2.4 (0.8–6.9)

Source: Delzell *et al.* 2006.
DMDTC = dimethyldithiocarbamate.
[a]Peak exposure was defined as total peaks > 50 ppm for styrene, as total peaks > 100 ppm for butadiene, and as cumulative exposure to DMDTC expressed in mg/cm-years (similar to analysis above).
[b]Models were adjusted for age and years since hire.

Of special interest was Graff *et al.*'s analysis of leukemia mortality by cross-classified cumulative exposure to styrene and butadiene. Styrene and butadiene exposure were each categorized into three levels (low = no exposure plus the first quartile of exposure; medium = the second and third quartiles of exposure; and high = the fourth quartile of

exposure). In the low-butadiene–exposure stratum (< 33.7 ppm-years), the RR for leukemia mortality was 1.6 (95% CI = 0.7 to 3.9, 7 observed deaths) for the medium-styrene–exposure group (8.3 to < 61.1 ppm-years), compared with the low-styrene–exposure group (> 0 to < 8.3 ppm-years. No deaths occurred in the high-styrene–exposure group (≥ 61.1 ppm-years).

The four categories defined by medium and high exposure to styrene (≥ 8.3 ppm-years) and butadiene (≥ 33.7 ppm-years) had RRs between 1.6 and 3.5. The rate ratio for leukemia mortality was 3.3 (95% CI = 1.6 to 6.7, 13 observed deaths) for the combined high-styrene (≥ 61.1 ppm-years) and high-butadiene (≥ 425.0 ppm-years) exposure category. In the middle- and high-butadiene exposure categories, the RR did not increase with increasing styrene exposure. No increased risk of leukemia mortality was apparent for workers exposed to butadiene above low levels (≥ 33.7 ppm-years) when styrene exposure was low (< 8.3 ppm-years, RR = 1.2, 95% CI = 0.4 to 3.1, 5 observed deaths). The marginal RR for styrene adjusted for butadiene was 1.5 (95% CI = 0.8 to 2.8) for 8.3 to < 61.1 ppm-years exposure, and 1.4 (95% CI = 0.6 to 3.0) for the 61.1+ ppm-years category, and the test for trend was not significant with $P = 0.65$. [A discrepancy exists between the person-years cited for this analysis of low-exposure styrene workers between Graff *et al*. 2005 (155,011) and Delzell *et al.* 2006 (255,011), but no other differences in the figures were identified.]

Graff *et al.* (2005) also presented separate relative risks for cumulative exposure to styrene and CLL, AML, CML, and other leukemias. These models, using terciles of exposure to styrene, butadiene, and DMDTC, were restricted to workers 40 years or older and with at least 20 years of employment, and were adjusted for age and time since hire. While increasing relative risks were observed for all subtypes of leukemia, except for AML, in single-chemical models these risks were attenuated in three-chemical models. At each level of styrene exposure, there were no significantly increased risks of these subtypes of leukemia in either single- or three-chemical models, but the number of deaths in each stratum was small (data for intermediate two-chemical models were not shown in either report).

Single-chemical, two-chemical, and three-chemical models were also reported for cumulative exposure to styrene, butadiene, and DMDTC for the other lymphohematopoietic cancers (NHL and multiple myeloma). No results were reported for Hodgkin's disease, but the deaths were few (13) (Graff *et al.* 2005, Delzell *et al.* 2006). Similar quartiles of cumulative exposure for the three chemicals were used as in the leukemia analyses. No increased risk was suggested by the results for multiple myeloma. Delzell *et al.* (2006) also presented data for CLL and NHL deaths combined, since CLL and small B-cell NHL represent the same B-cell cancers. (Note that in 8 cases, a diagnosis of both CLL and NHL was recorded, so a total of 75 rather than 83 deaths was used in this analysis. Similar findings (patterns) were obtained for both NHL and NHL+CLL (see Table 3-2). RRs were adjusted for age and time since hire, and the models were restricted to workers 40 or more years of age). The risks of NHL+CLL increased with increasing styrene exposure; the response appeared to be weaker for NHL alone. (The only significant RR was for the highest category of styrene exposure and NHL+CLL). When butadiene was added to the model (styrene + butadiene), the RRs for both NHL and NHL+CLL were increased. The RRs in the intermediate model with styrene and DMDTC in the model were somewhat reduced. When all three chemicals were added to the model, the RRs were slightly attenuated compared with styrene alone, and none were statistically significant. [No trend analyses were performed, but this would have likely increased the power to detect risks associated with styrene exposure.]

Cumulative exposure to butadiene did not appear to be risk factor for NHL or NHL+CLL. The risk of NHL or NHL+CLL was marginally increased at the two highest doses of butadiene exposure, but were reduced to less than or equal to one after controlling for styrene. RRs for DMDTC were non-significantly elevated in some exposure categories, but no clear exposure-response relationships were observed.]

Table 3-3. Cumulative exposure to styrene, butdadiene and DMDTC and risk of NHL and NHL+CLL.

Styrene exposure ppm-years	No. of cases/person years	Styrene only RR (95% CI)	Styrene + butadiene RR (95% CI)	Styrene + DMDTC RR (95% CI)	Styrene + butadiene + DMDTC RR(95% CI)
NHL[a]					
0	6/53,165	1.0	1.0	1.0	1.0
> 0 to < 8.3	16/106,811	1.4 (0.5–3.6)	1.7 (0.5–5.6)	1.2 (0.4–3.2)	1.4 (0.4–4.9)
8.3 to < 31.8	11/88,810	1.1 (0.4–2.9)	1.8 (0.5–6.3)	0.9 (0.3–2.6)	1.3 (0.3–5.2)
31.8 to < 61.1	9/42,612	1.5 (0.5–4.2)	2.3 (0.6–8.7)	1.2 (0.4–3.8)	1.7 (0.4–7.0)
61.1 +	16/47,008	2.3 (0.9–5.9)	3.2 (0.9–11.2)	1.8 (0.6–5.5)	2.3 (0.6–9.2)
NHL+CLL[a,b]					
0	6/53,165	1.0	1.0	1.0	1.0
> 0 to < 8.3	20/106,811	1.7 (0.7–4.4)	2.2 (0.7–6.8)	1.4 (0.5–3.8)	1.9 (0.6–6.1)
8.3 to < 31.8	15/88,810	1.5 (0.6–3.8)	2.2 (0.7–7.1)	1.1 (0.4–3.1)	1.7 (0.5–6.2)
31.8 to < 61.1	13/42,612	2.2 (0.8–5.7)	2.7 (0.8–9.2)	1.5 (0.5–4.5)	2.0 (0.5–7.5)
61.1 +	21/47,008	3.0 (1.2–7.5)	3.1 (0.9–10.3)	2.0 (0.7–5.8)	2.2 (0.6–8.2)

Source: Delzell *et al*. 2006.
CLL = chronic lymphocytic leukemia, DMDTC = dimethyldithiocarbamate, NHL = non-Hodgkin's lymphoma.
[a]8 deaths had double diagnosis of CLL and NHL.
[b]Models were restricted to 40+ years of age and were adjusted for age and year since hire.

Table 3-4. Epidemiologic studies of cancer risk following styrene exposure in the styrene-butadiene rubber industry, 1976–2005

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths)[a]	Comments
McMichael *et al.* 1976a U.S.	Internal case-control comparison of a cohort of rubber workers 1964–72	*Cohort* 6,678 male workers at a rubber tire plant that produced styrene-butadiene rubber (SBR) and other rubbers *Case-control comparison* Sample population: 22% of the study population (age-stratified random sample) Exposure (work group) age-adjusted risks calculated for each type of cancer or disease for workers exposed at least 2 yr and at least 5 yr, 1940–60	Work histories, obtained from personnel records, were used to assign workers to 16 major work groups; one group was a synthetic plant producing SBR and other synthetic rubbers (2%–3% of the sample population)	RR (99.9% CI) *Workers producing SBR and other synthetic rubbers with at least 5 years of exposure (significant findings)* LH 6.2 (4.1–12.5) lymphatic leukemia 3.9 (2.6–8.0) Stomach cancer 2.2 (1.4 – 4.3) *Number of deaths for all 16 work areas* (deaths specifically for the synthetic plant not reported) LH = 51 lymphatic leukemia = 14	SBR was the most prevalent rubber produced in the synthetic plant, but neoprene, nitrile, and ethylene-propylene-diene were also produced
Meinhardt *et al.* 1982, Meinhardt *et al.* 1978 U.S.	historical cohort 1943–76 avg. 19 yr 53,929 person-years	2,756 white male workers with ≥ 6 months of non-management or non-administrative employment in 2 SBR plants Avg. employment ~10 yr SMRs based on national rates	Average TWAs based on samples from all areas of production facilities were 0.94 ppm (0.03–6.46) and 1.99 ppm (0.05–12.3) in 2 plants Study population was not subclassified according to styrene exposure	*Total cohort* all cancer 0.72 (0.54–0.93), 56 <u>Cancers with non-significant excess mortality</u> LH 1.32 (0.66–2.37), 11 NHL 1.65 (0.45–4.27), 4 leukemia 1.73 (0.63–3.73), 6	Study initiated in response to leukemia deaths of 2 workers Study population included in studies by Delzell *et al.*

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths)[a]	Comments
Lemen *et al.* 1990	follow-up to end of 1981 and 1982; 69,655 person years	As above		Additional cancer deaths (N = 50): increases reported for lung, trachea, and bronchus (27), lymphosarcoma and reticulosarcoma (2), and other LH (2), including 1 leukemia and aleukemia death. No other cancer deaths reported, and no SMRs reported.	
Matanoski *et al.* 1997, Matanoski *et al.* 1993, Matanoski *et al.* 1990, Matanoski and Schwartz 1987 U.S. and Canada	historical cohort 1943–82 avg. 21 yr 251,431 person-years	12,110–13,920[b] male workers employed > 1 yr in 7 U.S. SBR plants and > 10 yr in 1 Canadian SBR plant, 1943–76 SMRs based on national rates	Exposure assessed from job titles; work areas obtained from personnel records Job descriptions and tasks information obtained from plant	*Total cohort* all cancer 0.85 (0.78–0.93), 518 Cancers with non-significant excess mortality Hodgkin's disease 1.20 (0.52–2.37), 8 other lymphatic 1.11 (0.64–1.77), 15 *Production workers* Cancers with excess mortality (non-significant and significant) kidney 1.53 (0.50–3.57), 5 LH cancer 1.46 (0.88–2.27), 19 Hodgkin's disease 1.20 (0.15–4.35), 2 leukemia 1.34 (0.53–2.76), 7 other lymphatic 2.60 (1.19–4.94), 9	Most of the study population included in studies by Delzell *et al.*
Santos-Burgoa *et al.* 1992 U.S. and Canada	nested case-control study of LH malignancies	*Cohort:* Matanoski and Schwartz 1987, Matanoski *et al.* 1990 *Cases:* 59 workers who died of LH malignancies *Controls:* 193 workers from cohort who were alive or had died of non-cancer causes; matched to cases by	JEM created by experts based on job titles and descriptions Workers classified according to ranks for relative exposure to styrene and 1,3-butadiene Cumulative exposure calculated based on exposure	OR (95% CI) *Cumulative styrene exposure > average* Matched analysis LH 1.91 (0.91–4.02) leukemia 3.13 (0.84–11.2) other lymphatic 1.35 (0.25–7.40) lymphosarcoma 1.33 (0.11–16.6) Hodgkin's disease 0.40 (0.05–3.25)	Styrene exposure ranks correlated poorly with measurements of styrene Styrene exposure and butadiene exposure were positively

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths)[a]	Comments
		plant, age, year of hire, employment duration, and survival ORs calculated by unconditional and conditional logistic regression (for matched subjects)	duration	Hodgkin's disease 0.40 (0.05–3.25) Unmatched analysis LH 1.89 (0.87–4.09) leukemia 4.26 (1.02–17.8) other lymphoma 2.42 (0.05–11.6) lymphosarcoma 1.39 (0.13–15.3) Hodgkin's disease 0.75 (0.14–3.92) In models that controlled for butadiene exposure, risk of leukemia was not increased No indication of a positive interaction between styrene and butadiene for all LH	correlated
Matanoski *et al.* 1997	nested case-control study of LH malignancies	*Cohort:* Matanoski and Schwartz 1987, Matanoski *et al.* 1990 *Cases:* 58 workers who died of LH cancer (starting with the same 59 cases as Santos-Burgoa *et al.*) *Controls:* 1,242 workers from cohort selected to represent distribution across plants and with similar age distribution to cases ORs calculated with unconditional regression models (controls, N = 1,242); multivariate models included birth year, hire date, and employment duration	Mean styrene exposure for all plants = 3.53 ppm (SD = 14.32), based on 3,649 measurements (1978–83) (Matanoski *et al.* 1993) Plant means = 0.29–6.66 ppm Styrene levels averaged across jobs and plants Average and cumulative exposure calculated from plant info., job title, and exposure duration	OR (95% CI), no. of observed deaths Logistic regression analysis controlling for butadiene exposure risk *Increase of 1 ppm in TWA styrene exposure (significant associations)* LH 2.20 (1.46–3.33), 58 lymphoma 2.67 (1.22–5.84), 12 lymphosarcoma 3.88 (1.57–9.59), 7 myeloma 3.04 (1.33–6.96), 10 *Cumulative exposure to styrene* Increasing risks with increasing exposure LH $P = 0.000$[c] leukemia $P = 0.006$ myeloma $P = 0.023$	See above

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths)[a]	Comments
		Analysis done to avoid overmatching on exposure (i.e., by plant), because exposure levels differed between plants.			
Delzell *et al.* 1996, Sathiakumar *et al.* 1998 Sathiakumar *et al.* 2005, extended follow-up Delzell *et al.* 2006 U.S. and Canada	historical cohort 1943 to 1/1/1991 follow-up through 1/1/1992 avg. 25 yr 386,172 person-years extended follow-up: 1944–1998 avg. 30 yr 540,586 person-years	Original cohort established by Delzell and colleagues 17,964 male workers employed ≥ 1 yr in 8 SBR plants, starting 1943–65 (depending on plant), followed through 1991 40 workers excluded from the extended follow up Mortality analysis: 15,649 workers (excluding those who had not worked in SBR or related activities) 44% worked ≥ 10 yr: avg. 7.8 yr SMRs based on national rates	Workers categorized from job title and department into 308 job groups organized into 5 main process groups: production (37%), maintenance (24%), labor (15%), laboratories (9%), and other operations (15%) Workers in the subgroups polymerization (production) and maintenance (labor) had high exposure to both styrene and butadiene Laboratory workers had high exposure to butadiene and low to moderate exposure to styrene Workers in the subgroup coagulation (production) had low to moderate exposure to styrene but only background exposure to butadiene *Extended follow-up based on identical exposure characterization as the initial*	*Follow-up 1943–91:* <u>Cancers with significantly decreased mortality (total cohort)</u> all cancer 0.93 (0.87–0.99), 950 buccal cavity & pharynx 0.50 (0.28–0.82), 15 esophagus 0.59 (0.35–0.93), 18 <u>Leukemia</u> total cohort 1.31 (0.97–1.74), 48 ever hourly 1.43 (1.04–1.91), 45 ever hourly (employed ≥ 10 yr, latency > 20 yr 2.24 (1.49–3.23), 28 production (job groups): polymerization 2.51 (1.40–4.14), 15 coagulation 2.48 (1.00–5.11), 7 labor (job groups): maintenance 2.65 (1.41–4.53), 13 laboratories 4.31 (2.07–7.93), 10 <u>Other cancers with significant excess mortality in certain subgroups:</u> large intestine: black hourly workers with ≥ 10 yr worked and ≥ 20 yr latency lung: maintenance job group *Follow-up 1944-98:* <u>Cancers with significantly decreased</u>	Includes workers from Meinhardt *et al.* 1982 (2[e] plants) and Matanoski *et al.* 1990 (7 of 8 plants) Mortality analysis of this cohort (or subpopulations) also published by Macaluso *et al.* 1996, Delzell *et al.* 2001, and Graff *et al.* 2005.

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths)[a]	Comments
			studies.	<u>mortality (total cohort)</u> all cancer 0.92 (0.88–0.97), 1,608 buccal cavity & pharynx 0.47 (0.29–0.71), 22 <u>Leukemia</u> total cohort 1.16 (0.91–1.47), 71 ever hourly 1.23 (0.94–1.57), 63 ever hourly (employed ≥ 10 yr, latency 20–29 yr 2.58 (1.56–4.03), 19 production (job groups) polymerization 2.04 (1.21–3.22), 18 coagulation 2.31 (1.11–4.25), 10 labor (job groups) maintenance 2.03 (1.14–3.35), 15[f] laboratories 3.26 (1.78–5.46), 14 <u>Cell-type specific leukemia (ever hourly employed)</u> CML 2.00 (1.00–3.58), 11 CLL 1.71 (0.96–2.81), 15 Significant associations also seen for CLL in polymerization, coagulation, finishing, and laboratories, for CML in laboratories and borderline significance seen for AML in maintenance labor <u>NHL+CLL (significant or borderline significant increased SMRs)</u> ever hourly 1.30 (0.99–1.67) ever hourly (employed ≥ 10 yr) by latency yr 20–29 1.90 (1.01–3.25) ≥ 30 1.49 (1.02–2.10)	

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths)[a]	Comments
				Job groups production (total) 1.73 (1.19–2.44) polymerization 2.18 (1.31–3.41) finishing 1.91 (1.21–2.86)	
Macaluso *et al.* 1996	historical cohort 1943–92 418,846 person years	Cohort established by Delzell *et al.* 1996 *Mortality analysis* 16,610 workers at 6 of 8 plants (with specific work histories), including workers involved in SBR-unrelated activities *External analysis* SMRs based on national rates 51 deaths from leukemia *Internal analysis* RRs that adjusted for multiple exposures were computed by the Mantel-Haenszel method or by Poisson regression models 58 deaths from leukemia, including 7 defined by contributory cause of death	*Plant-specific JEMs:* exposure values estimated from process descriptions and surveys; TWA values linked to workers by job group Estimated median cumulative styrene exposure was 7.4 ppm-years for 83% of the workers Average styrene exposure decreased from 1.8 ppm in the 1940s to 0.1 ppm in the early 1990s	*Leukemia* SMR (external analysis) and RR (adjusted for race, age, and cumulative exposure to butadiene) for cumulative exposure to styrene *ppm-years* *SMR* *RR* 0 0.89 1.0 < 5 0.63 0.9 5–9 1.61 5.4 10–39 1.36 3.4 ≥ 40 2.35 2.7 *Internal analysis* test for trend in RR, $P = 0.14$	Quantitative exposure estimates based on experts' judgment of complex data sources; no thorough validation Exposure estimates significantly higher than documented by measurements Exposures highly correlated; impossible to disentangle separate styrene or butadiene effects

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths)[a]	Comments
Delzell *et al.* 2001	historical cohort 1943–91	Cohort established by Delzell *et al.* 1996 Mortality analysis 13,130 workers from 6 of 8 plants (similar to Macaluso *et al.* 1996), but also excluding 3,468 workers who died or were lost to follow-up before age 40 or 10 years latency and 12 workers with duplicate records RRs calculated with Poisson regression models for exposure to single and multiple agents; models included age and latency 59 deaths from leukemia (1 from myelodysplasia with medical records indicating acute unspecified leukemia, in addition to 58 deaths identified by Maculuso *et al.* 1996	Exposure estimates by Macaluso *et al*. 1996 were revised (original estimates were noted as being controversial), and exposure to DMDTC was estimated Estimated median cumulative styrene exposure for exposed workers (85%) was 17–18 ppm-years	RR (95% CI), no. of observed deaths *Leukemia* *Styrene exposure (ppm-years)* Styrene only 0 — 1.0, 5 > 0–< 20.6 — 1.2 (0.5–3.3), 18 20.6–< 60.4 — 2.3 (0.9–6.2), 18 ≥ 60.4 — 3.2 (1.2–8.8), 18 Styrene + butadiene 0 — 1.0 > 0–< 20.6 — 1.1 (0.3–4.0) 20.6–< 60.4 — 1.6 (0.4–6.4) ≥ 60.4 — 1.8 (0.4–7.3) Styrene + butadiene + DMDTC 0 — 1.0 > 0–< 20.6 — 0.6 (0.1–2.5) 20.6–< 60.4 — 0.8 (0.2–3.7) ≥ 60.4 — 0.8 (0.2–3.8)	Revised exposure assessment gave styrene exposure levels twice as high as originally reported See also comments for Macaluso *et al*. 1996
Graff *et al.* 2005; Delzell *et al.* 2006	historical cohort 1943–98 500,174 person-years	Cohort established by Delzell *et al.* 1996 *Mortality analysis:* 16,579[g] workers at 6 of 8 plants (similar to Maculuso *et al.* 1996) RR calculated by Poisson	Revised exposure estimates by Macaluso *et al*. 2004 were used Individuals assigned to four quartiles of cumulative exposure to styrene (ppm-years):	*Internal RR analyses:* RR (95% CI), no. of deaths Models evaluating RR for 4 categories of cumulative styrene exposure: (1) styrene only, (2) styrene + butadiene, (3) styrene + DMDTC and (4) styrene + butadiene + DMDTC (adjusted for age and time since	All 3 exposures were correlated Spearman rank correlation with styrene exposure:

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths)[a]	Comments
		regression models for exposure to single agents and exposure to multiple agents; models included age, latency, period of employment (year range), and race SMRs based on U.S. or Ontario rates for expected numbers of LH cancer deaths 81 deaths from leukemia, 58 from NHL, 27 from multiple myeloma, and 13 from Hodgkin's disease	0–< 8.3 8.3–< 31.8 31.8–< 61.1 ≥ 61.1 Three exposure categories were created for 1) cross-classified combined analyses; 2) RR models of CLL, AML, CML, and other leukemias, and 3) SMR analyses of AML, CML, CLL and NHL+CLL (all other analyses used quartiles): *low:* no exposure + 1st quartile *medium:* 2nd + 3rd quartiles *high:* 4th quartile	hire) – See data in Table 3-2 and 3-3 for all leukemia, NHL and NHL+CLL. CLL alone by terciles of styrene exposure showed RRs of 1.0 (reference), 1.7 and 2.6 for styrene alone, and RRs of 1.0 (reference), 1.2 and 0.9 for 3-chemical model (all non-significant) No statistically significant increases in RR were reported for CLL, AML, CML, other leukemias, or multiple myleloma (single- and 3-chemical models RR for all myeloid neoplasms combined was significantly increased for styrene levels 31.8 – < 61.1 ppm-years in single- (2.6, 1.2–5.5, 13 deaths) but not 3-chemical models. Analysis of cross-classified categories of butadiene and styrene; reference group = low styrene, low butadiene <u>RR for leukemia and styrene exposure</u> Low-butadiene exposure: medium 1.6 (0.7–3.9) 7 high no deaths Medium-butadiene exposure low 1.2 (0.4–3.1), 5 medium 1.6 (0.9–2.9), 25 high 1.6 (0.6–4.0), 6 High-butadiene exposure low no deaths medium 3.5 (1.3–9.3), 5	Correlation of styrene exposure with butadiene = 0.7 and with DMDTC = 0.63

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths)[a]	Comments
				high 3.3 (1.6–6.7), 13 RR for styrene (ppm-year exposure) adjusted for BD 8.3 – < 61.1 1.5 (0.8-2.8) 61.1+ 1.4 (0.6-3.0) Test for trend: $P = 0.65$ *External analysis* SMRs for all leukemias and NHL increased with increasing cumulative exposure to styrene; significant at the 3rd and 4th quartiles for leukemia (1.87, 1.02–3.13, 14 deaths; 1.91, 1.09–3.10, 16 deaths, respectively) and 4th quartile for NHL (1.97, 1.08–3.31, 14 deaths), CLL (3.10, 1.01–7.24, 5 deaths) and NHL+CLL (2.29, 1.36–3.62, 18 deaths)	

AML = acute myelogenous leukemia; BD = butadiene; CI = confidence interval; CLL = chronic lymphocytic leukemia; CML = chronic myelogenous leukemia; DMDTC = dimethyldithiocarbamate; IRR = incidence rate ratio; LH = lymphohematopoietic cancer; MM = multiple myeloma; NHL = non-Hodgkin's lymphoma; OR = odds ratio; RR = relative risk or rate; SIR = standard incidence ratio; SMR = standard mortality ratio.
[a]Unless otherwise stated.
[b] Number of workers varied among the publications.
[c]*P*-value as reported in paper.
[d]Delzell *et al.* (Delzell *et al.* 1996) reported mortality from LH cancer, whereas Sathiakumar *et al*. (1998) reported mortality from all cancer.
[e]Delzell *et al.* (1996) referred to these two plants as one facility.
[f]Reported in abstract of Sathiakumar *et al.* (2005) as SMR = 326, 95% CI = 178 to 456, which is the result for "Laboratories" in the same analysis reported in the body of the paper.
[g]Combines the results for 16 men who had worked in different plants and had separate records and eliminates 8 men who had worked < 1 year.

3.3 The styrene monomer and polymer industry

Styrene exposure levels in the styrene monomer and polymer production industries are generally much lower than levels in the reinforced-plastics industry, and are similar in magnitude to levels seen in the styrene-butadiene rubber production industry (see Section 2.6). Although levels exceeding 20 ppm have been reported in polymerization, manufacturing, and purification areas for this industry, the styrene levels in maintenance, laboratory, and packaging operations were generally less than 5 ppm. Workers in the styrene monomer industry can also be exposed to benzene, toluene, ethylbenzene, and other alkylbenzene compounds. In addition to benzene, toluene, and ethylbenzene, workers in polystyrene production can be exposed to various solvents such as 1,2-dichloroethane, carbon tetrachloride, ethyl chloride, methylene dichloride, and chlorobenzene. Workers could also be exposed to boron trifluoride which is the preferred initiator for the polymerization reaction (see Section 2.2). Cancer mortality for workers in the styrene monomer and polymer industry has been studied in workers in Germany by Frentzel-Beyme *et al.* (1978), in the United States by Ott *et al.* (1980) (with follow-up by Bond *et al.* (1992) and by Nicholson *et al.* (1978), and in England by Hodgson and Jones (1985). Table 3-5 provides an overview of the studies conducted in the styrene monomer and polymer industry.

3.3.1 Germany

Frentzel-Beyme *et al.* (1978) studied 1,960 workers (sex not reported) engaged in the manufacture of styrene and styrene polymer for more than 1 month during the period 1931 to 1976. The population was identified from plant records and followed from 1956 through 1976. Percentage follow-up was much lower for non-German employees (29%), many of whom returned to their home countries, compared with German employees (93%). Observed numbers of cancer deaths were compared with the expected numbers based on regional mortality rates. A total of 20,138 person-years were accumulated, and the average follow-up was 10.3 years. Styrene exposure levels were generally below 1 ppm according to measurements conducted in 1975 and 1976 (Thiess and Friedheim 1978). Levels up to 6.84 ppm (styrene production) and 46.92 ppm (polystyrene production) occasionally were recorded. No subclassification of workers was done that allowed any assessment of cancer mortality by indicators of styrene exposure level. Only

12 deaths due to malignant tumors were observed compared with 18.5 expected. A statistically nonsignificant increase in mortality from pancreatic cancer was observed (2 observed deaths vs. 0.7 expected; $P = 0.16$), and mortality from lung cancer was decreased (3 observed deaths vs. 5.4 expected; *P* value not reported). Non-significant increases in mortality from rectal, peritoneal, and splenic cancer were also observed, but these increases were based on only one observed case for each site.

3.3.2 United States- multi-plant

Ott *et al.* (1980) studied 2,904 male workers employed for at least 1 year in the production or research units of a company that produced styrene monomer, styrene-butadiene latex, and styrene-based products at several U.S. locations. The workers were identified from annual census lists for 1937 to 1970 and followed from 1940 through 1976. Bond *et al.* (1992) extended follow-up to 1986 and only 0.4% were lost to follow-up. Vital status and cause of death were assessed using the company mortality surveillance system. Mortality was compared with expected numbers calculated from national rates and other worker populations within the company. A total of 90,000 person-years were accumulated, and average follow-up was 31 years. Industrial hygienists assigned all manufacturing jobs (categorized into 57 groups with common exposures) an exposure intensity with respect to five chemical exposures: (1) styrene and ethylbenzene (1 to 4 ppm, or ≥ 5ppm), (2) benzene, alkylbenzene compounds (≥ 1 ppm), (3) styrene, ethylbenzene, and acrylonitrile in equal concentrations (1 to 4 ppm, or ≥ 5 ppm), (4) extrusion fumes, and (5) colorants (indirect and direct exposure).

For the total study population, overall cancer mortality was significantly decreased (SMR = 0.81, 95% CI =0. 69 to 0.95, 162 observed deaths) (Bond *et al.* 1992). Increased (but statistically nonsignificant) SMRs were seen for all lymphatic and hematopoietic malignancies (SMR = 1.44, 95% CI = 0.95 to 2.08, 28 observed deaths), Hodgkin's disease (SMR = 2.22, 95% CI = 0.71 to 5.18, 5 observed deaths), NHL (SMR = 1.17, 95% CI = 0.47 to 2.40, 7 observed deaths), multiple myeloma (SMR = 1.84, 95% CI = 0.74 to 3.80, 7 observed deaths), and leukemia (SMR = 1.18, 95% CI = 0.54 to 2.24, 9 observed deaths). Among workers exposed to styrene and ethylbenzene, there were 16 deaths due to lymphohematopoietic malignancies, compared with 8.1 expected [SMR =

1.98], and mortality was slightly higher in workers exposed at least 1 year (< 1 year, 4 observed vs. 2.6 expected [SMR = 1.54]; ≥ 1 year, 12 observed vs. 5.5 expected [SMR = 2.2]) and in workers exposed to lower styrene levels (< 5 ppm, 12 observed vs. 5.1 expected [SMR = 2.4]; ≥ 5 ppm, 4 observed vs. 3.0 expected [SMR = 1.3]). A statistically significant increased risk was found for an analysis that allowed for a 15-year latency period (SMR = 1.60, 95% CI = 1.02 to 2.38, 24 observed deaths), but there was no significant trend of increasing risk with increasing time since first exposure.

3.3.3 United States- single plant

Nicholson *et al.* (1978) identified 560 male workers at a plant manufacturing styrene monomer and polystyrene employed for at least 5 years as of 1960 according to the local union's seniority list. The population was followed through 1975. Cause of death (N = 83) was determined by death certificate; autopsy results were available for 18 cases and other clinical information was available for 13 cases, and mortality was compared with that of the general U.S. population.

NIOSH conducted a health hazard evaluation in 1974 in the plant that showed styrene exposure levels of below 1 ppm in low-exposure areas (service and utilities) and 5 to 20 ppm in high-exposure areas (styrene production, polystyrene polymerization and extrusion, development, and special products and maintenance). Crude styrene monomer is produced from ethylbenzene and iron oxide, and styrene is purified by the removal of unreacted ethylbenzene, benzene, toluene, and xylene. In addition to polystyrene production, the plant also produced butadiene-styrene latex. The authors stated that some individuals might have experienced high exposure to benzene during the period of 1943 to 1962.

A total of 17 workers died of any cancer (21.01 expected [SMR = 0.81]). Observed vs. expected deaths were 6 vs. 6.99 [SMR = 0.86] for lung cancer, 1 vs. 0.79 [SMR = 1.27] for leukemia, and 1 vs. 1.25 [SMR = 0.80] for lymphoma. In addition to the leukemia that was the cause of death, a second individual who died from coronary disease also had a leukemia at the time of death. The authors also reported on a review of 361 randomly selected death certificates of individuals employed for at least 6 months (who were not included in the cohort because they did not have 5 years of experience by 1960). The

death certificates were obtained from either union records or as part of a company-initiated study on its progress to NIOSH. An additional 5 leukemias and 4 lymphomas were identified; however, information on work histories or exposures was not available.

3.3.4 United Kingdom

Hodgson and Jones (1985) studied 622 male workers employed for at least 1 year from 1945 to 1974 in a plant where styrene monomer was produced, polymerized, and processed. The workers were followed through 1978, and a total of 8,654 person years were accumulated, with an average follow-up of 13 years. An additional 3,072 male manual workers who had no exposure to styrene but had worked at least one year at the site were identified as a reference group. The lymphomas were confirmed by histological assessment by three pathologists. SMRs were computed from national mortality rates, and standard registration ratios (i.e., SIRs) were computed from regional cancer incidence rates. No measurements of styrene exposure were available, but the authors stated that styrene exposure levels were in general well below 100 ppm. Workers were also exposed to acrylonitrile, and there was potential exposure to benzene, dyestuffs, and ethylene oxide. For the total cohort, the SMRs were 0.90 for all cancer (10 observed vs. 10.9 expected), [1.19] for lung cancer (5 vs. 4.2), and [5.36] for lymphoma (3 vs. 0.56, P = 0.02); no deaths from leukemia were observed (0 vs. 0.3). No excess of deaths from lymphoma or leukemia was observed in the unexposed cohort. The SIRs were 2.50 for all lymphohematopoietic malignancies (4 vs. 1.6, P = 0.079), 3.75 for lymphoma (3 vs. 0.8, P = 0.047), and [1.67] for leukemia (1 vs. 0.6). An increased incidence of larynx cancer was also reported (3 observed vs. 0.5 expected; P values not given); however no deaths from larynx cancer were reported. The authors stated that laryngeal cancer is often amenable to treatment.

Table 3-5. Cohort studies of cancer risk following styrene exposure in the styrene monomer and polymer industry, 1978–1992

Study	Population, follow-up, and methods	Exposure	Effects	Comments
Frentzel-Beyme *et al.* 1978 Germany	1,960 workers engaged in styrene or polystyrene manufacture > 1 mo 1931–76 1956–76; avg. 10.3 yr 20,138 person-years 12 deaths from cancer SMRs based on German city and administrative region	Date of first exposure and date of leaving plant obtained from plant records; safety precautions improved over time Styrene exposure levels generally < 1 ppm but higher levels (up to 47 ppm) were occasionally reported (Thiess and Friedheim 1978)	SMR[a] (95% CI), no. of observed deaths *Cancers with > 1 death* pancreas [2.77 (0.34–10.03)], 2 lung [0.55 (0.11–1.62)], 3	Incomplete follow-up for non-German workers Low statistical power
Ott *et al.* 1980 Bond *et al.* 1992 U.S.	2,904 male workers employed ≥ 1 yr in production or research units of 1 company (several locations) that produced styrene monomer, styrene-butadiene latex, and styrene-based products; workers identified from annual census lists, 1937–70 1940–86; avg. 31 yr 90,000 person-years SMRs based on national rates RRs calculated by Mantel-Haenszel methods for cohort studies, with unexposed industrial populations within the company as reference group	Industrial hygienists assigned all manufacturing jobs (categorized into 57 groups with common exposures) an exposure intensity with respect to 5 chemical exposures: (1) styrene and ethylbenzene (1–4 or ≥ 5 ppm) (2) benzene, alkylbenzene compounds (≥ 1 ppm) (3) styrene, ethylbenzene, and acrylonitrile in equal concentrations (1–4 or ≥ 5 ppm) (4) extrusion fumes (5) colorants (indirect and direct exposure)	SMR (95% CI), no. of deaths *Total cohort* all cancer 0.81 (0.69–0.95), 162 *Cancers with non-significant excess mortality* LH 1.44 (0.95–2.08), 28 Hodgkin's disease 2.22 (0.71–5.18), 5 multiple myeloma 1.84 (0.74–3.80), 7 NHL 1.17 (0.47–2.40), 7 leukemia 1.18 (0.54–2.24), 9 stomach 1.27 (0.64–2.28), 11 Non-significant increases in RRs observed for NHL, leukemia+aleukemia, and Hodgkin's disease, and a statistically significant increase in RR for multiple myeloma (RR = 2.45, 1.07–5.65, 7 deaths) when unexposed workers were the reference group (RR for stomach cancer not reported) *Chemical grouping (obs. vs. exp.), LH cancer* styrene & ethylbenzene 16 vs. 8.1	Complex mixture of exposures

Study	Population, follow-up, and methods	Exposure	Effects	Comments
Nicholson *et al.* 1978 U.S.	560 male workers at a styrene monomer and polymer plant employed ≥ 5 yr as of 1960 according to the local union's seniority list 1960–75 Expected numbers of deaths based on national rates	Departments categorized into high and low exposure based on air concentrations, worker descriptions, and body burdens of metabolites measured in clinical study *Air measurements in 1974* low exposure: < 1 ppm high exposure: 5–20 ppm	Observed vs. expected deaths *Total cohort* all cancer 17 vs. 21.01 lung 6 vs. 6.99 leukemia 1 vs. 0.79 lymphoma 1 vs. 1.25 *High and low exposure areas* Data not given for specific cancers, because of small numbers	Small numbers of exposed cases Complex mixture of exposures, including ethylbenzene, toluene, xylene, and benzene
Hodgson and Jones 1985 U.K.	622 male manual workers engaged in production of styrene monomer, polymerization, manufacture of finished products, or working in laboratory ≥ 1 yr at 1 site, 1945–74 *Mortality:* 1945–78, avg. 13 yr 8,654 person-years *Incidence:* 1962–81 SMRs based on national rates SIRs based on regional rates	No styrene exposure measurements available; however, authors stated that styrene exposure levels were generally well below 100 ppm	SMR (obs. vs. exp. deaths) all cancer 0.90 (10 vs. 10.9) lung [1.19] (5 vs. 4.2) lymphoma [5.36] (3 vs. 0.56)* leukemia – (0 vs. 0.3) SRR (obs. vs. exp. cases) LH 2.50 (4 vs. 1.6) lymphoma 3.75 (3 vs. 0.8)* leukemia 1.67 (1 vs. 0.6) larynx 6.0 (3 vs. 0.5), $P = 0.041$	Small numbers of exposed cases Mixed exposures

CI = confidence interval, LHC = lymphohematopoietic cancer, NHL = non-Hodgkin's lymphoma, RR = relative risk or rate, SMR = standard mortality ratio, SRR = standard registration ratio.
* P (one sided) < 0.05
[a]Calculated from the original data using expected deaths from the Rhinehessia-Palatinate region.

3.4 Other cohort studies

Other cohort studies of styrene exposure are summarized in Table 3.5.

3.4.1 Styrene-exposed workers (biomarker study)

Anttila *et al.* identified 2,580 workers (distribution by sex was not reported for the styrene cohort) biomonitored for styrene exposure through measurement of mandelic acid in post-shift urinary samples (Anttila *et al.* 1998). They were followed from the first recorded measurement made between 1973 and 1983, through 1992. A total of 34,288 person-years were accumulated [with an average follow-up of 13.3 years]. The median mandelic acid level was 2.3 mmol/L [350 mg/L] (the authors noted that 2.9 mmol/L urinary mandelic acid corresponded roughly to 20 ppm). (Levels were higher in women than men, which the authors stated was probably due to the selection of the monitored task.) Cases of cancer were identified from the Finnish Cancer registry, and SIRs were computed from expected values estimated from cancer incidence rates in the general population. The overall cancer incidence was decreased (SIR = 0.80, 95% CI = 0.59 to 1.06, 48 observed cases), and the incidence of rectal cancer was significantly increased (SIR = 3.11, 95% CI = 1.14 to 6.77, 6 observed cases). Increased risks were indicated for cancer of stomach, liver, pancreas, and nervous system and Hodgkin's disease, but none of the findings were statistically significant. When the analysis was limited to workers followed for at least 10 years after first measurement, the SIR was 3.49 (95% CI = 0.72 to 10.2, 3 observed cases) for rectal cancer, 3.54 (95% CI = 0.09 to 19.7, 1 observed case) for liver cancer, 3.64 (95% CI = 0.75 to 10.6, 3 observed cases) for pancreatic cancer, and 3.11 (95% CI = 0.85 to 7.95, 4 observed cases) for cancer of the nervous system; no cases of lymphohematopoietic malignancy were observed. SIRs were not higher in the high-exposure group (based on lifetime mean urinary metabolite levels) compared with the low-exposure group, but the numbers of observed and expected cases were low. The authors did not provide sex-specific risk estimates but stated that there was no clear difference in the overall incidence pattern between styrene-exposed men and women.

3.4.2 Environmental exposure

Loughlin *et al.* (1999) evaluated the mortality from lymphatic and hematopoietic malignancies among 15,403 students (7,882 men and 7,521 women) attending a high

school adjacent to a styrene-butadiene rubber plant between 1963 and 1993 for at least three consecutive months during a school year. The population was identified from high school yearbooks, and school records (which do not allow recording of sex). Sex was assigned based on data from birth records (73%) of the population, yearbook pictures, and student's name. Data on name changes (such as married name) that occurred after high school were obtained from multiple searches of marriage databases (the first round used the "maiden name," and date of birth, and the second round used the "married name"). The population was followed through 1995, and vital status was obtained from the National Death Index, the Social Security Administration Death Master Files, and the Texas Department of Health death database. Cause of death was obtained from death certificates (matching on maiden or married name, date of birth and state of birth), and SMRs were based on expected numbers calculated from national death rates. A statistically nonsignificant increase in overall cancer mortality was observed for men (SMR = 1.22, 95% CI = 0.83 to 1.73, 31 deaths) but a significant decrease was observed for women (SMR = 0.52, 95% CI = 0.28 to 0.88, 13 deaths). The sex-specific SMRs were as follows (men vs. women): all lymphohematopoietic malignancies, 1.64 (95% CI = 0.85 to 2.87, 12 deaths) vs. 0.47 (95% CI = 0.06 to 1.70, 2 deaths); Hodgkin's disease, 1.46 (95% CI = 0.18 to 5.28, 2 deaths) vs. 1.20 (95% CI = 0.03 to 6.68, 1 death); and leukemia, 1.82 (95% CI = 0.67 to 3.96, 6 deaths) vs. 0.45 (95% CI = 0.01 to 2.48, 1 death). Among males, the SMR's for subtypes of lymphohematopoietic cancers were somewhat higher in those who attended school for 2 years or less compared with those who attended more than 3 years. [Note that only the SMR for leukemia+aleukemia among those attending high school for < 2 years was significantly elevated, SMR = 5.29, (95% CI = 1.09 to 15.46, 3 deaths)]. A significant excess of deaths from benign neoplasms (all of which were brain tumors) also was observed in men (SMR = 6.27, 95% CI = 2.04 to 14.63, 5 deaths); only one case was observed in females (SMR = 1.56, 95% CI = 0.04 to 8.71, 1 death).

Table 3-6. Other cohort studies evaluating cancer risk and exposure to styrene

Study	Population, follow-up, and methods	Exposure	Effects	Comments
Anttila *et al.* 1998 Finland	2,580 workers biologically monitored for styrene exposure, starting 1973–83 and followed through 1992 [avg. 13.3 yr] 34,288 person-years (styrene) SIRs based on national rates	Exposure assessed by measuring post-shift MA concentration in urine median = 2.3 mmol/L range = 0–47 mmol/L Median urinary MA level corresponds to a styrene concentration in air of about 20 ppm	SIR (95% CI), no. of observed cases *Total cohort* all cancer 0.80 (0.59–1.06), 48 *Cancers with significantly or nonsignificantly increased incidences* rectum 3.11 (1.14–6.77), 6 stomach 1.40 (0.45–3.26), 5 liver 1.63 (0.04–9.08), 1 pancreas 1.66 (0.34–4.85), 3 nervous system 1.61 (0.59–3.50), 6 Hodgkin's disease 1.89 (0.23–6.84), 2 *≥ 10 years after first measurement* *Cancers with increased incidences*[a] rectum 3.49 (0.72–10.2), 3 pancreas 3.64 (0.75–10.6), 3 nervous system 3.11 (0.85–7.95), 4	Well-characterized styrene exposure

Study	Population, follow-up, and methods	Exposure	Effects	Comments
Loughlin *et al.* 1999 U.S.	15,403 students (7,882 male, 7,521 female) attending a high school located near an SBR plant, 1963–93 1963–95 avg. 20.1 yr 310,254 person-years SMRs based on national rates	No exposure assessment Study prompted by potential exposure to plant emissions	SMR (95% CI), no. of observed deaths *Men* (241 deaths) *Cancers with excess mortality (significant or non-significant* all cancer 1.22 (0.83–1.73), 31 LH 1.64 (0.85–2.87), 12 Hodgkin's disease 1.46 (0.18–5.28), 2 leukemia 1.82 (0.67–3.96), 6 other lymphopoietic 2.05 (0.56–5.26), 4 peritoneum 1.26 (0.41–2.94), 5 respiratory 1.46 (0.47–3.40), 5 benign (brain) 6.27 (2.04–14.63), 5 lower mortality among long-term students (≥ 3 yr), except for Hodgkin's disease *Women* (97 deaths) all cancer 0.52 (0.28–0.88), 13 *Cancers with non-sigificant excess mortality* Hodgkin's disease 1.20 (0.03–6.68), 1 benign (brain) 1.56 (0.04–8.71), 1	Questionable completeness of study population Questionable identification of death certificates, especially among women

[a]Only cancers for which the SIR was higher after ≥ 10 years than 0–9 years of follow-up and there was > 1 exposed case.

3.5 Case-control and ecological studies

Four case-control studies and one ecological study in which potential exposure to styrene was analyzed, together with a series of case-control studies among a population in Montreal, Canada, are summarized briefly in the text that follows. Details of the study design, sample sizes, and findings are included in Table 3-7.

3.5.1 Lymphohematopoietic cancers

Flodin *et al.* (1986) conducted a clinic-based, case-control study in Sweden of 59 patients diagnosed with acute myeloid leukemia between 1977 and 1982 and 354 controls. A total of 354 population controls was selected, 236 matched (4 per case) on gender, age, and place of residence, and 118 unmatched (2 per case). The study focused primarily on the effect of background gamma radiation on the incidence of acute myeloid leukemia. A self-administered questionnaire was mailed to eligible participants and included questions on sources of radiation exposure, 10 different occupational exposures, medical care, and lifestyle exposures. The response rate for questionnaire completion was not specified. The method of assessing solvent and other chemical exposures from the "qualitative" information about solvent exposure provided on the questionnaires was also not clarified. Data were analyzed by logistic regression. The OR for 3 cases of acute myeloid leukemia following styrene exposure (vs. 1 referent) was 18.9 (95% CI = 1.9 to 357). [Note that it appears from the data presented that this OR is unadjusted for other potentially confounding variables.]

Guenel *et al.* (2002) conducted a nested case-control study among a population of French utility workers. Seventy-two cases of leukemia (ICD-9 204–208) among active workers below the age of 60 and 285 controls matched by birth year were identified for the study period of 1978 to 1989. Occupational exposures were assigned by company physicians, toxicologists, and epidemiologists, using a job-exposure matrix (JEM) based on job title, job tasks, and place of work. In addition, the cumulative duration (% of work time-years) but not intensity of exposure was estimated for a group of chemicals that included styrene. The OR (adjusted for benzene and several other chemical exposures) for potential exposure to styrene (estimated from a JEM) was 1.1 (95% CI = 0.2 to 5.9) based on 2 exposed cases and 9 exposed controls.

Seidler *et al*. (2007) conducted a population-based, case-control study of exposure to chlorinated and aromatic organic solvents and malignant lymphoma incidence among men and women between 18 and 80 years of age in six regions of Germany. Cases (710) were matched by age, region, and gender to equal numbers of population controls. Cumulative occupational exposures were evaluated by detailed personal work histories obtained by face-to-face questionnaire and assessment by an occupational physician; exposure was estimated by both duration (% of work time) and 3 levels of exposure. Data were analyzed by conditional logistic regression. In comparison with 542 cases with no estimated exposure to styrene, the ORs for malignant lymphoma associated with styrene exposure, after adjustment for smoking and alcohol consumption, were 0.7 (95% CI = 0.5 to 1.0, 70 cases) for > 1 to 1.5 ppm-years; 1.2 (95% CI = 0.8 to 1.7, 79 cases) for > 1.5 to 67.1 ppm-years; and 0.8 (95% CI = 0.3 to 1.4, 12 cases) for > 67.1 ppm-years. No significant trend with exposure was observed ($P = 0.43$). No elevated risks were observed when lymphoma subtypes were considered. No attempt was made in this study to adjust for potential confounding by multiple exposures, including other aromatic hydrocarbons and chlorinated hydrocarbons. [Note that chlorinated hydrocarbons, but not other aromatic hydrocarbons, were associated with an elevated risk of malignant lymphoma.]

3.5.2 Breast cancer

Cantor *et al.* (1995) conducted a population-based, case-control study of breast cancer mortality based on records gathered between 1984 and 1989 from 24 U.S. states. Cases were 33,509 women with breast cancer listed as the underlying cause of death on the death certificate, and 117,794 controls were randomly selected from non-cancer deaths matched for gender, age within 5 years, and race. Homemakers were excluded from the analysis. A JEM was used to classify cases and controls with respect to specific occupational exposures and the probability and level of exposure. The JEM was based on the usual occupation and industry listed on the death certificate, from which an industrial hygienist assigned a probability of exposure or a probable level of exposure for a total of 31 agents or groups of agents. Analyses were adjusted for age at death and, in some cases, socioeconomic status, and results were presented separately for black and white women. Among white women, in comparison with 27,610 cases with no estimated

styrene exposure, the ORs for breast cancer, adjusted for age and socioeconomic status, were 1.16 (95% CI = 1.1 to 1.3, 807 cases) for low exposure, 1.13 (95% CI = 1.0 to 1.3, 522 cases) for medium exposure and 1.19 (95% CI = 0.9 to 1.6, 70 cases) for the highest exposure category. Among black women, in comparison with 3,918 non-exposed cases, the adjusted ORs for low- and medium-exposure levels were 1.59 (95% CI = 1.2 to 2.1, 87 cases) and 1.41 (95% CI = 1.0 to 1.9, 63 cases), respectively. Thus, breast cancer showed a weak but statistically significant association with styrene exposure, with ORs generally about 1.2 for whites and 1.5 for blacks. No clear trend by exposure probability or exposure level was seen that was consistent across the two races. [Note that no other exposures investigated were significantly associated with breast cancer in this population, with the exception of a weak association with asbestos and non-ionizing radiofrequency and ionizing radiation.]

Coyle *et al.* (2005) conducted an ecological study to evaluate the relationship between invasive breast cancer incidence and releases of 12 selected environmental toxicants reported in the paper to be associated with breast cancer (the chemicals carbon tetrachloride, formaldehyde, methylene chloride, styrene, tetrachloroethylene, and trichloroethylene and the metals arsenic, cadmium, chromium, cobalt, copper, and nickel) that were reported to the Environmental Protection Agency as being released in one or more of 254 counties in Texas during 1988 to 2000. During the years 1995 through 2000, 54,487 cases of breast cancer (in both men and women) were identified from the Texas Cancer Registry. For each toxicant, the age-adjusted breast cancer rate for each of these counties was compared with the amount of toxicant released in that county, based on information obtained from the EPA Toxics Release Inventory (TRI) for 1988 to 2000. In a univariate analysis, the median age-adjusted annual breast cancer incidence was significantly higher in counties reporting releases of styrene (and several other compounds) than counties not reporting releases (66.2 cases in 61 counties vs. 59.8 cases in 193 counties, respectively, $P < 0.001$). A multivariate analysis model of breast cancer and exposure to the environmental toxicants (that were significantly associated with breast cancer in the univariate analyses) found significant positive associations between release of styrene and breast cancer in women and men ($P = 0.0004$), women ($P = 0.002$), and women aged 50 or older ($P = 0.002$). [No other data were presented, and it is not

clear why linear regression was used.] The model adjusted for age, ethnicity, race, and exposure to those environmental toxicants that were significantly associated with breast cancer in univariate analyses. [The criterion for exposure (one or more releases reported in the TRI in a given county) was unlikely to reflect individual styrene exposure. The ecological nature of the study did not allow for the evaluation of other factors (such as socioeconomic status) that may also differ between counties with high and low breast cancer rates but may correlate with exposure.]

3.5.3 Series of studies in a Canadian population

A series of population-based, case-control studies of occupational risk factors was conducted according to similar protocols and within the same population in Montreal, Canada (Dumas *et al.* 2000, Gérin *et al.* 1998, Parent *et al.* 2000). A total of 3,730 male cancer patients (with cancer of the esophagus, stomach, colon, rectum, pancreas, lung, prostate, bladder, and kidney, melanoma of the skin, NHL, and Hodgkin's disease) were evaluated from 1979 to 1986. As controls for each cancer site analyzed, Gérin *et al.* used a sample of 533 other cancer patients pooled with a sample of 533 male population controls; Dumas *et al.* used all other patients with cancer at other sites (excluding lung cancer and anatomically contiguous cancers); and Parent *et al.* used cancer patients (as in Dumas *et al.* 2000) pooled with the 533 population controls. Case and control subjects were interviewed about the characteristics of each job held, and chemists and hygienists translated each job case-by-case into potential exposure to styrene, styrene-butadiene rubber, and some 300 other substances. Data were analyzed by unconditional logistic regression with adjustment for age, smoking, and respondent status in all studies; body mass index (Dumas *et al*. 2000, Parent *et al*. 2000); family income and ethnic group (Gerin *et al*. 1998); and education and beer consumption (Dumas *et al.* 2000).

In analyses that focused on four different organic solvents, Gerin *et al.* (1998) found statistically significant increased ORs with respect to exposure to medium/high levels of styrene for rectal cancer (OR = 5.1, 95% CI = 1.4 to 19.4, 5 cases), and prostate cancer (OR = 5.5, 95% CI = 1.4 to 21.8, 7 cases), and statistically nonsignificant increased risks of NHL (OR = 2.0, 95% CI = 0.8 to 4.8, 8 cases), and Hodgkin's lymphoma (OR = 2.4, 95% CI = 0.5 to 11.6, 2 cases). No increases in the risk for cancer of the esophagus,

stomach, colon, pancreas, lung, bladder, or kidney or melanoma of the skin were observed. [Note that these ORs were adjusted for demographic and socioeconomic covariates, smoking, and respondent status but not for other exposures.] Only 2% of the population was classified as exposed to styrene and only 45% of those were considered to be "certainly" exposed. Dumas *et al.* (2000) focused on a broad spectrum of occupational factors and the risk of rectal cancer and found a statistically increased unadjusted OR for styrene exposure (for "substantial exposure," unadjusted OR = 3.9, 95% CI = 1.2 to 12.9, 5 cases; for "any" exposure, OR adjusted for demographic and lifestyle factors = 1.7, 95% CI = 0.7 to 4.5, 6 cases. Note that no adjustment for potential confounders was conducted for the "substantial" exposure group). Parent *et al.* (2000) examined the risk factors for renal-cell carcinoma and found that the OR for exposure to styrene-butadiene rubber was significantly increased for renal-cell carcinoma among the "any exposure" group (OR = 2.1, 95% CI = 1.1 to 4.2, 10 cases, adjusted for demographic and lifestyle variables, but the risk was somewhat attenuated when additionally adjusted for felt dust exposure (OR = 1.8, 95% CI = 0.9 to 3.7).

3.5.4 Lung cancer and styrene exposure

Scélo *et al.* (2004) conducted a clinic-based, case-control study of 2,861 patients (of a total of 3,403) diagnosed with lung cancer between 1998 and 2002 in Romania, Hungary, Poland, Russia, Slovakia, the Czech Republic, and the United Kingdom. There were 3,118 hospital or population controls. Cases and controls were interviewed about the characteristics of each job held, and chemists and hygienists translated each job case-by-case into potential exposure to styrene and 70 other agents. The OR for lung cancer among patients ever exposed to styrene (N = 51) was 0.7 (95% CI = 0.42 to 1.18; adjusted for center, gender, age, smoking, vinyl chloride, acrylonitrile, formaldehyde, and inorganic pigments). No trends with duration of exposure or cumulative exposure were observed.

Table 3-7. Case-control and ecological studies evaluating cancer risk and exposure to styrene

Study	Population and methods	Exposure	Effects	Comments
Flodin *et al.* 1986 Sweden	clinic-based case-control study *Cases:* 59 Swedish patients (aged 20–70) diagnosed with acute myeloid leukemia at medical clinics or departments in hospitals, 1977–82 *Controls:* 354 Swedish referents: 236 matched controls (4 per case) selected from general population register and matched for gender, age, and locality; 118 randomized controls (2/case) from the general population register of the hospital catchment area, aged 20–70 ORs calculated by logistic regression	Exposure to styrene and other agents (radiation, solvents), and information on smoking and other lifestyle exposures assessed by questionnaire	OR (95% CI), cases/controls (unadjusted) *Styrene exposure* acute myeloid leukemia 18.9 (1.9–357), 3/1	Self-reported exposure information obtained after diagnosis was made Only 3 styrene-exposed cases
Guenel *et al.* 2002 France	nested case-control study of active utility workers *Cases:* 72 cases of leukemia diagnosed 1978–89 (< 60 years old) *Controls:* 285 controls (4 per case) matched on birth year ORs calculated by conditional logistic regression	Exposure to styrene and other agents (electromagnetic fields, radiation, other solvents, asbestos, etc.) assessed by company by job title, type and place of work using a JEM	OR (95% CI), no. of cases/controls St*yrene exposure* Leukemia OR (adjusted for benzene + other chemicals) 1.1 (0.2–5.9), 2/9	
Cantor *et al.* 1995 U.S.	population-based, case-control study *Cases:* 33,509 women with breast cancer as underlying cause of death (1984–89) in a database of 24 states; homemakers excluded, leaving 29,397 white women and 4,112 black women *Controls:* 117,794 controls (4/case)	Occupation title and industry obtained from death certificates; JEM linked this information with occupational hygiene literature to estimate the probability (4 levels) and level (3 levels) of exposure to 31 specific occupational	adjusted OR (95% CI), no. of cases adjusted for age at death and socioeconomic status *Styrene exposure and breast cancer* *White women* probability 1 1.13 (1.0–1.2), 804 2 1.18 (1.1–1.3), 527	Styrene exposure based only on "usual occupation" on death certificate

Study	Population and methods	Exposure	Effects	Comments
	randomly selected from noncancer deaths; frequency matched for age (5-yr groups), gender, and race; homemakers excluded, leaving 102,955 white women and 14,839 black women ORs adjusted for age at death and socioeconomic status	agents, including styrene	3 1.38 (1.0–1.9), 64 4 NR level 1 1.16 (1.1–1.3), 807 2 1.13 (1.0–1.3), 522 3 1.19 (0.9–1.6), 70 *Black women* probability 1 1.49 (1.1–2.0), 80 2 1.52 (1.1–2.1), 61 3 1.32 (0.5–3.3), 7 4 NR level 1 1.59 (1.2–2.1), 87 2 1.41 (1.0–1.9), 63 3 NR	
Coyle *et al.* 2005 Texas, USA	ecological study 54,487 cases of invasive breast cancer in men and women reported to the Texas Cancer Registry, 1995–2000 Age-adjusted breast cancer rates for each of the 254 Texas counties compared with the amount of toxicant (for 12 toxicants) released in those counties *Univariate analysis:* Mann-Whitney U test used to compare median average annual age-adjusted breast cancer rates in counties reporting releases vs. those not reporting releases Stepwise multiple linear regression models included age, race, ethnicity, and toxicants	The amount of toxicant released in each county in 1988–2000 obtained from the EPA Toxics Release Inventory. Release information obtained for 12 chemicals or metals: carbon tetrachloride, formaldehyde, methylene chloride, styrene, tetrachloroethylene, trichloroethylene, arsenic, cadmium, chromium, cobalt, copper, and nickel. Toxicants were chosen based on (1) reporting of an association with breast cancer	*Univariate analysis* median average annual age-adjusted breast cancer rate in Texas counties *Reported release of styrene* yes 66.2 cases no 59.8 cases, $P < 0.001$ *Multiple linear regression: styrene and age-adjusted breast cancer rate* β, *P*, explained variance (%) men & women 0.219, 0.0004, 9 women 0.191, 0.002, 8 women ≥ 50 0.187, 0.002, 14 women < 50 NR, > 0.05	Criterion for exposure (≥ 1 releases reported in TRI in a given county) unlikely to reflect individual styrene exposure Ecological nature of the study did not allow for the evaluation of other factors that may also differ between counties with high and low breast cancer rates

Study	Population and methods	Exposure	Effects	Comments
	significantly associated with breast cancer	in the published literature, (2) designation by EPA as carcinogens or substances with estrogenic effects, and (3) consistent reporting of releases to TRI in 1988–2000		Unclear why linear regression analysis employed
Gérin *et al.* 1998[a] Dumas *et al.* 2000 Parent *et al.* 2000 Canada	population-based case-control study *Cases:* 3,730 men aged 35–70 with cancer identified 1979–86; cancers at 15 sites studied[a] *Cancer* — *no. of cases* renal-cell carcinoma — 142 rectal — 257 prostate — 449 NHL — 215 Hodgkin's disease — 54 *Controls:* *Cancer controls:* patients with cancer at different sites, excluding lung and anatomically contiguous sites *Population controls:* 533 men selected from electoral list, age distribution similar to cases Gérin *et al.* used the 533 population controls and a subset (533) of the cancer controls ORs computed by unconditional logistic regression analysis with adjustment for age, respondent status, cigarette smoking (all publications), plus (1) family income, ethnic group (Gérin *et al.*) (2) education, beer consumption, body mass index (Dumas *et al.*)	Case and control subjects interviewed about characteristics of each job; chemists and hygienists translated each job into potential exposure to styrene, styrene-butadiene rubber, and 300 other substances	*Styrene exposure and various cancers, using pooled (population & cancer) controls* (Gérin *et al.*) adjusted OR (95% CI), cases/controls *Cancers with increased ORs* *Medium or high exposure* rectum — 5.1 (1.4–19.4), 5/4 prostate — 5.5 (1.4–21.8), 7/3 esophagus — 1.4 (0.5–3.8), 5/40 *Ever exposed* NHL — 2.0 (0.8–4.8), 8/19 Hodgkin's disease — 2.4 (0.5–11.6), 2/19 *Styrene exposure and rectal cancer, using cancer controls* (Dumas *et al.*) adjusted OR (95% CI), no. of exposed cases any — 1.7 (0.7–4.5), 6 substantial — 3.9 (1.2–12.9), 5 *Styrene-butadiene rubber exposure and renal-cell cancer, using pooled controls* (Parent *et al.*) adjusted OR (95 % CI), no. of exposed cases Model 1 — 2.1 (1.1–4.2), 10 Model 2 — 1.8 (0.9–3.7), 10	Styrene-exposed workers dominated by firefighters (35%), mechanics and repairmen (20%), and painters (11%), which are not generally known as groups exposed to high levels of styrene Only 2% of the population had potential styrene exposure

Study	Population and methods	Exposure	Effects	Comments
	(3) body mass index (Parent *et al*.): Model 1 (4) body mass index and felt dust exposure (Parent *et al*.): Model 2			
Scélo *et al.* 2004 Romania, Hungary, Poland, Russia, Slovakia, Czech Republic, U.K.	clinic-based, case-control study of lung cancer *Cases:* 2,861 patients with newly diagnosed lung cancer that occurred 1998–2002; patients recruited from 15 hospital centers *Controls:* 3,118 controls; most (at 13 of the 15 centers) were hospital controls recruited from the same hospital or area as the cases and without tobacco-related diseases; 2 centers used population controls recruited from the population or general practitioners' registers. ORs calculated by unconditional logistic regression and adjusted for age, gender, center, tobacco consumption, and exposure to occupational agents	Case and control subjects interviewed about characteristics of each job; chemists and hygienists translated each job into potential exposure to styrene and 70 other agents 0.6% of controls had potential exposure to styrene based on jobs held	Adjusted OR (95% CI), cases/controls *Styrene exposure and lung cancer* Ever exposed 0.70 (0.42–1.18), 51/47 (adjusted for age, gender, center, smoking, vinyl chloride, acrylonitrile, formaldehyde, organic pigments) Risk of lung cancer did not increase with increasing duration of exposure, weighted duration of exposure, or cumulative exposure	80% statistical power to detect OR for ever exposure in the range of 1.5 to 1.6

Study	Population and methods	Exposure	Effects	Comments
Seidler *et al.* 2007 Germany	multi-center population-based case-control study of malignant lymphoma *Cases:* 710 newly diagnosed male and female patients 18–80 years old prospectively recruited from 6 regions. *Controls:* 710 controls recruited from population registers, matched on age, gender, region. ORs calculated by conditional and unconditional logistic regression and adjusted for smoking and alcohol consumption.	Cases and controls interviewed about detailed job histories and leisure activities; exposure assessments conducted by occupational physician blind to status of participants to organic solvents including styrene, toluene, xylene and benzene, and 4 chlorinated hydrocarbons. 161 cases had estimated exposure to styrene; 542 cases had no estimated styrene exposure.	Adjusted OR (95% CI) cases/controls *ppm yrs:* > 0 1.5 0.7 (0.5–1.0), 70/85 > 1.5–67.1 1.2 (0.8–1.7), 79/67 > 67.1 0.6 (0.3–1.4), 12/17 test for trend: $P = 0.43$	No adjustment made for potential confounding due to multiple exposures No association found between other subtypes of lymphoma and styrene Significant association found between chlorinated hydrocarbons and lymphoma, but not other aromatic hydrocarbons

CI = confidence interval, NHL = non-Hodgkin's lymphoma, NR = not reported, OR = odds ratio.
[a]Gerin *et al.* reported results for all cancer sites, Dumas *et al.* reported results on rectal cancer, and Parent *et al.* reported results for renal cell carcinoma.

3.6 [Strengths and limitations of the literature][3]

This section discusses the utility of the studies for assessing the possible carcinogenicity of styrene (3.6.1), limitations of studies due to potential misclassification (3.6.2), and other possible sources of bias or confounding (3.6.3).

3.6.1 Utility of the studies

With respect to cohort studies, the possible carcinogenicity of styrene has been assessed among more than 100,000 workers employed in styrene-related industries. Workers in the reinforced-plastics industry may have experienced styrene exposure levels that may be considerably higher than workers in the styrene-butadiene rubber and styrene monomer and polymer industries (Delzell *et al.* 2001, Jensen *et al.* 1990, Kogevinas *et al.* 1994b, Macaluso *et al.* 1996, Macaluso *et al.* 2004, Thiess and Friedheim 1978). Furthermore, the reinforced-plastics industry, unlike the two other industries, is characterized by exposure to few other suspected carcinogens (Jensen *et al.* 1990). Results for workers biomonitored for styrene are also informative for this industry, because their styrene exposure was well characterized and because most of the workers monitored were laminators in the reinforced-plastics industry (Anttila *et al.* 1998). However, this study did not examine cancer risk by duration or level of exposure.

On the other hand, studies of the reinforced-plastics industry included few long-term workers, with the exception of the large multi-country cohort of Kogevinas *et al.* (1994a, 1993). Of approximately 85,000 reinforced-plastics workers studied, the majority were employed for less than one year, and fewer than 7,500 were employed for more than 10 years. An estimated 40% of the latter workers were laminators (the workers with the highest styrene exposure level), so the database includes results for only 3,000 long-term workers exposed to styrene at high levels. Secondly, the average follow-up was less than 15 years for three of the most informative populations (Anttila *et al.* 1998, Kogevinas *et al.* 1994a, Kolstad *et al.* 1995). Thirdly, as in the case of the majority of other populations exposed to styrene, exposures have been considerably reduced over the past decades (for example, in the study by Anttila *et al.* (1998) exposures among laminators had been

[3] The title of this section is bracketed to indicate the presence of opinion throughout this section rather than bracketing specific statements.

reduced from approximately 200 ppm in the 1960s to less than 100 ppm by the 1970s). Thus, workers who started employment in earlier years are likely to have had higher exposures than those hired in recent years. Nevertheless few of the studies report any analyses by year of first hire. In addition, none of these cohort studies (except for Antilla *et al.* 1998) used quantitative measures of exposure.

With respect to studies of styrene-butadiene rubber workers, there are a large number of person-years of exposure among several large cohorts, which have been followed over several decades. In addition, most plants in the styrene-butadiene rubber industry were brought into operation at about the same time (the 1940s) and workers in them are likely to have experienced similar patterns of decline in exposure levels over time. As in the reinforced-plastics industry, exposure to styrene has decreased over the past several decades. However, none of the studies have fully examined the effect of year of hire on cancer rates, and analyses by cumulative exposure or duration of exposure do not reflect these changes in exposure over time. The other main limitation of these cohort studies, in addition to exposure misclassification, is co-exposure to butadiene, a known carcinogen; this is of particular concern when analyzing lymphohematopoietic cancers.

The cohort studies of styrene monomer and polymer workers are small and lack sufficient statistical power to detect moderate increases in risk. In addition, few cancer outcomes have been studied, and workers in this industry are exposed to multiple chemicals, several of which, e.g., benzene, are known or suspected carcinogens.

Overall, the statistical power of the total epidemiologic database of cohort studies is only sufficient to detect markedly increased risks. Negative findings, even among the highly exposed study populations, should therefore be interpreted with caution, because a relatively small number of workers may have experienced relevant cumulative styrene exposure and time since first exposure.

The clinic- and population-based, case-control studies provide only limited relevant evidence, in large part because of low statistical power to detect an effect, which in turn is due mainly to the fact that high-level styrene exposure is rare in the general population (probably below 0.1% according to Gérin *et al*. (1998); only 2% of the population in his

Montreal-based study were considered to be potentially exposed to any level of styrene). The one occupational case-control study, of utility workers by Guenel *et al.* (2002), was primarily focused on electromagnetic field exposure and yielded only 11 of 357 workers (3%) with potential styrene exposure. In addition, the general lack of precision with which exposures were assigned also reduced the power of these studies to detect an effect, as discussed below. However, in the case of the large case-control study of breast cancer (Cantor *et al.* 1995), this study may be of value in assessing the risk of this cancer among the women exposed to styrene, since there is generally insufficient power to detect breast cancer risk among women in cohort studies due to the small number of exposed women.

3.6.2 Misclassification of disease and exposure

Only three of the reviewed cohort studies used incidence data to classify health outcomes — one report on a U.K. cohort by Hodgson and Jones (1985), two reports on a Danish cohort by Kolstad *et al.* (1995, 1994), and a report on a Finnish cohort by Anttila *et al.* (1998). The other cohort studies were based on mortality data, which may provide less reliable information about diagnosis, and which may not include cases with other causes of death or cases resulting in death after the end of the follow-up period. Among the Danish reinforced-plastics workers, 74% of the male patients with a recorded diagnosis of lymphohematopoietic malignancy in the national cancer registry had this diagnosis recorded on the death certificate (Kogevinas *et al.* 1994b, Kolstad *et al.* 1994). In the cohort of styrene-butadiene rubber workers studied by Delzell and colleagures (Delzell *et al.* 2001), medical records were obtained for a subset (majority) of the workers who died of leukemia, NHL, multiple myeloma, and Hodgkin's lymphoma, and the majority of the diagnoses were confirmed. One of the main methodological challenges in the analysis by different types of lymphohematopoietic cancers, particularly when based only on death certificate data, is the possibility of misclassification of different subtypes of leukemia and between different types of lymphohematopoietic cancers, e.g., leukemias and lymphomas.

Only a few studies have assessed specific sub-diagnoses of leukemia (Delzell *et al.* 2006, Flodin *et al.* 1986, Graff *et al.* 2005, Kogevinas *et al.* 1994a, Kolstad *et al.* 1996,

Sathiakumar *et al.* 2005). (AML, CML, and adult ALL arise from the same pluripotential stem cell, based on observations of specific genetic re-arrangements in these 3 subtypes of leukemia, which comprise about 80% of adult leukemias. For example, the blast crisis of CML, 90% of which have the Philadelphia chromosome, cannot be distinguished from AML. An estimated 10% of adult ALL cases have the Philadelphia chromosome, which suggests a common stem-cell origin for these leukemias (Bloomfield *et al.* 1978, Jacobs 1989, Yunis 1983). There is considerable overlap between CLL and NHL; CLL and NHL (85%) are B-cell malignancies (Delzell *et al.* 2006) and CLL is the same disease as small lymphocytic lymphoma (Delzell *et al.* 2006, Harris *et al.* 2000). Delzell *et al.* 2006 grouped NHL+CLL in their data analyses.)

The major limitation of the studies reviewed is potential misclassification of styrene exposure and potential confounding by co-exposures. In particular, there are no ambient air monitoring data for earlier calendar years when exposure is known to have been considerably higher than in recent years. [Some of the analytical methods used in exposure analyses are old; see Section 2.3.] In the smaller cohort studies (Frentzel-Beyme *et al.* 1978, Hodgson and Jones 1985, Meinhardt *et al.* 1978, Nicholson *et al.* 1978), no attempts were made to differentiate workers according to styrene exposure, and an unknown proportion may actually have been unexposed. The same limitation also pertains to the Danish studies of the reinforced-plastics industry; however, misclassification may have been less, because most of the Danish workers were employed in small companies and thus virtually all employees would have exposure to styrene (Kolstad *et al.* 1995, Kolstad *et al.* 1994, Kolstad *et al.* 2005). The temporal variation in styrene exposure levels can be another source of misclassification over the study period (Kolstad *et al.* 2005, Macaluso *et al.* 2004). (See discussion of temporal and job/task variation in exposure reported by Macaluso *et al.* 2004 and Figuer 2-4 and 2-5 [Figures 1 and 2 from Kolstad *et al.* 2005] in Section 2.5.1.)

In several of the case-control studies, reliance is placed on self-administered or in-person questions to establish either jobs held or potential exposures among living respondents combined with assignation of exposure by a member of the research team (Dumas *et al.* 2000, Flodin *et al.* 1986, Gérin *et al.* 1998, Parent *et al.* 2000, Scélo *et al.* 2004, Seidler *et*

al. 2007), which raises questions about the accuracy of recall by respondents (as well as the possibility of misclassification bias in studies where disease status was known to researchers, as discussed below). In the breast cancer mortality case-control study by Cantor *et al*. (1995) potential exposure was assigned based only on usual occupation listed on the death certificate, and in the ecological study by Coyle *et al.* (2005) an indirect measure of exposure based on toxic releases in the county of residence was used; this method of estimating individual exposure is considerably less precise than the use of usual job titles in the case-control studies. In both studies, the likelihood of misclassification of cumulative styrene exposure is particularly high.

Classification of workers by individual job titles (McMichael *et al.* 1976a, Ruder *et al.* 2004) or job-exposure matrices (Bond *et al.* 1992, Delzell *et al.* 2001, Kogevinas *et al.* 1994a, Matanoski *et al.* 1997, Santos-Burgoa *et al.* 1992, Seidler *et al.* 2007, Wong *et al.* 1994) may, at least partly, have reduced misclassification of exposure. [Exposure classification by job-exposure matrices is preferable since workers may experience different exposures within given departments according to the particular job peformed and may move between one job and another within and across departments.] However, in a validation test within the styrene-butadiene rubber industry, styrene exposure ranks correlated poorly with styrene measurements (Matanoski *et al.* 1993), clearly illustrating that it may be difficult to obtain valid exposure estimates for styrene in this industry. If exposure ranks and actual measurements correlate poorly, this would tend to attenuate any apparent risk and bias the findings towards the null. Macaluso *et al.* (2004) generally found estimates of styrene exposure in the styrene-butadiene rubber industry to be lower than industrial hygiene measurements but did not conduct a thorough validation of their exposure estimates.

In the Danish studies of the reinforced-plastics industry, duration of employment was abstracted from national pension fund records. Based on a small validation study, the estimates of duration of employment from the national pension fund records did not correlate well with information obtained from a questionnaire from a sub-sample of 671 employees from 8 companies. It was determined that up to 40% of the workers classified as short-term workers by the national pension fund were classified as long-term workers

by the questionnaire, while the opposite misclassification occurred among 13% of the workers classified as long-term by the national pension fund (Kolstad *et al.* 1994).

The study by (Anttila *et al.* 1998) was the only one that relied on individual measurements of exposure; exposure status thus was well documented for these subjects. On the other hand, other studies have shown considerable intra-individual (Symanski *et al.* 2001) and intra-company (Kolstad *et al.* 2005) variability in styrene exposure in the reinforced-plastics industry; group-level exposure assessment (Delzell *et al.* 2001, Kogevinas *et al.* 1994a, Macaluso *et al.* 1996, Matanoski *et al.* 1997) may therefore be preferable (Armstrong 1998).

Such misclassification of styrene exposure was independent of health outcome and thus would be expected to be nondifferential and to bias any measures of association towards no effect. Exceptions were (1) the studies reported by Delzell and colleagues using the revised exposure assessment (Delzell *et al.* 2001, Graff *et al.* 2005, Delzell *et al.* 2006), because the investigators were aware of the employment histories of the workers who had died of leukemia when they revised their exposure estimates, and (2) the population or clinic-based, case-control studies, because they relied on patients' retrospective descriptions of exposures or working conditions (Dumas *et al.* 2000, Flodin *et al.* 1986, Gérin *et al.* 1998, Parent *et al.* 2000, Scélo *et al.* 2004, Seidler *et al.* 2007). Kolstad *et al.* (1994) compared exposure data obtained from employers in the reinforced-plastics industry with those obtained from dealers in raw materials and found indications that employers' reports were not independent of health outcome for some companies; the employers' reports therefore were omitted from the analyses.

3.6.3 Other possible biases and confounding

As noted above, the potential exists for coexposure to other chemicals in the various styrene-based industries. Apart from limitations in evaluating exposure to such chemicals addressed above, there are limitations in the ability of the statistical modeling methods used to adjust for such exposures to disentangle the effects of individual chemicals, particularly in cases where multiple co-exposures occur such as in the styrene monomer and polymer industry, where interaction effects might occur, and/or where a high degree

of correlation between exposures is observed, such as in the styrene-butadiene industry. Several studies adjusted for butadiene (Delzell *et al.* 2001, Delzell *et al.* 2006, Graff *et al.* 2005, Santos-Burgoa *et al.* 1992) and DMDTC (Delzell *et al.* 2001, Delzell *et al.* 2006, Graff *et al.* 2005). Graff *et al.* (2005) noted that styrene, butadiene, and DMDTC exposure are highly correlated, and it is difficult to separate the effects of one agent from the other two agents. Butadiene is classified as a known human carcinogen by IARC and the NTP, and is considered to be a risk factor for leukemia (IARC 1999, NTP 2004). DMDTC is less strongly correlated with styrene exposure than butadiene, 0.6 compared with 0.8 (Delzell *et al.* 2001). It is considered to be an immune system depressant (T-cell) (Delzell *et al.* 2006), but its carcinogenicity has not been evaluated outside the studies in the styrene-butadiene industry. Although there is potential exposure to benzene in the styrene-butadiene rubber industry, it was not considered to have an impact on leukemia based on studies by Macaluso *et al.* 1996 (Delzell *et al.* 2006).

In the styrene monomer and polymer industry, the confounding effects due to multiple co-exposures are difficult to distinguish from the potential effect of styrene (particularly as some co-exposures, such as benzene and ethylbenzene, are known or suspected carcinogens). In the case-control studies, there was generally little or no attempt to adjust ORs by other exposures or to take into account multiple comparisons due to the large number of potential exposures investigated.

Analyses in the studies reviewed generally were adjusted for age, sex, and, in some studies, calendar year. In addition, some studies included information about years since hiring (Delzell *et al.* 2001, Delzell *et al.* 2006, Graff *et al.* 2005) duration of employment (Matanoski *et al.* 1997, Wong *et al.* 1994), and race (Matanoski *et al.* 1997). Very few studies analyzed data by year of first hire, which may be important because there is evidence that overall exposures in the three industry sectors have been reduced over the past several decades. Estimates of cumulative exposure that are based on recent measurements or estimates of current exposure may not accurately reflect the higher exposures experienced by older workers. Wong *et al.* controlled for smoking in their nested case-control study of lung cancer in the reinforced-plastics industry, but this apparently did not affect the relative risk estimates related to styrene exposure (Wong

1990). Certain lifestyle factors and/or other occupational factors were included in analyses of the clinic- and population-based, case-control studies (Dumas *et al.* 2000, Flodin *et al.* 1986, Gérin *et al.* 1998, Parent *et al.* 2000, Scélo *et al.* 2004, Seidler *et al.* 2007), but not in the cohort-based studies. Internal analyses within the worker populations are expected to be less sensitive to confounding by lifestyle factors, because the populations are expected to be relatively homogenous with respect to socioeconomic factors (Delzell *et al.* 2001, Kogevinas *et al.* 1994a, Kolstad *et al.* 1995, Kolstad *et al.* 1994, Wong *et al.* 1994). However, confounding cannot be ruled out, because little is known about the causes of the majority of the malignant diseases studied.

Short-term workers in the reinforced-plastics industry showed generally higher cancer risk than long-term workers (Kogevinas *et al.* 1994a, 1995, Kolstad *et al.* 1994, Ruder *et al.* 2004, Wong *et al.* 1994). This might be because short-term workers are mainly assigned to jobs with high styrene exposure; however, no data are available to assess this hypothesis. The finding might also be explained by the healthy-worker effect — that is, a selection process by which workers who become unfit during employment tend to leave. However, the healthy-worker effect is generally less for malignant diseases than for chronic nonmalignant diseases (Arrighi and Hertz-Picciotto 1994). A third explanation might be confounding because of differences in other risk factors between short- and long-term workers. A separate study supported this hypothesis; it showed that Danish short-term reinforced-plastics workers had been hospitalized for lifestyle-related health conditions before employment in the industry more often than long-term workers (Kolstad and Olsen 1999). Thus, confounding by factors related to lifestyle is a likely explanation, at least to some extent, of the unexpected decline in risk with length of employment. One way of handling such confounding would be by comparison with a non-styrene–exposed group of short-term workers with expected comparable socioeconomic status and lifestyle factors. Such analysis of the Danish reinforced-plastics industry workers showed a statistically nonsignificant increased risk of leukemia (RR = 1.89, 95% CI = 0.78 to 4.59) (Kolstad *et al.* 1994).

3.7 Summary of previous evaluations (IARC and Cohen *et al.*)

As mentioned in the introduction, the 1979 and the 1994 IARC working groups characterized the evidence available to them at the time on carcinogenicity of styrene in humans as "inadequate" (IARC 1979, 1994a). The 2002 working group upgraded the human evidence to "limited" (IARC 2002).

In its 2002 evaluation of the human data, IARC considered case reports; cohort studies of workers in the reinforced-plastics, styrene-butadiene rubber, and the styrene monomer and polymer industries; nested case-control studies within the styrene-butadiene rubber industry; biomonitoring of workers for styrene exposure; environmental exposure of students to styrene; and clinic- and population-based, case-control studies of acute myeloblastic leukemia and 15 major cancer sites.

IARC (2002) regarded data from the reinforced-plastics industry as the most informative, because workers in that industry were exposed to the highest levels of styrene and had less potential for exposure to other substances within the occupational setting than the other cohorts studied. The IARC evaluation emphasized a small, nonsignificantly increased incidence of leukemia among Danish reinforced-plastics workers and a statistically significant excess among those workers with the earliest first years of employment, the highest styrene exposure levels, or latency of at least 10 years. However, among all workers exposed for 1 year or more, the incidence of leukemia was not increased. In a European multinational cohort of reinforced-plastics workers (that partially overlapped with the Danish study), mortality from lymphatic and hematopoietic neoplasms was not increased, based on comparison with national reference rates. However, in an internal analysis using the unexposed workers as the comparison group, mortality was increased in exposed workers after 20 years since the first exposure to styrene and also increased with increasing intensity of exposure, but not with increasing cumulative exposure to styrene. A large U.S. mortality study of reinforced-plastics workers found no overall excess of lymphohematopoietic malignancies. IARC stated that problems with mortality ascertainment in the European study and underestimation of duration of exposure in the Danish study might have influenced the findings.

Studies of the styrene monomer and polymer industry showed weak association between styrene exposure and lymphohematopoietic cancers, and studies of the styrene-butadiene rubber industry showed increasing mortality from leukemia with increasing cumulative exposure to styrene. IARC considered these findings difficult to interpret because of potentially confounding coexposures; in the styrene-butadiene rubber industry, styrene exposure was highly correlated with butadiene exposure. IARC mentioned increased risks of rectal, pancreatic, and nervous system cancers in some studies, but considered those findings of limited importance.

Cohen *et al.* (2002) reviewed epidemiologic studies relevant to the carcinogenicity of styrene. The authors concluded that the balance of epidemiologic evidence did not suggest a hazard of cancer in humans from exposure to styrene. The authors emphasized that there were no consistent patterns of increased risks for the various lymphatic and hematopoietic cancers (NHL, Hodgkin's disease, multiple myeloma, and leukemia) across studies of the reinforced-plastics industry, which they considered the most informative because subjects had high styrene exposure levels and few other potentially confounding occupational exposures. They stressed the absence of exposure-response patterns for these cancers. The only study identified as showing a statistically significant increased risk of lung cancer was that of Wong *et al.* (1994), and that risk was confined to short-term workers, indicating confounding related to socioeconomic status. Cohen *et al.* also stressed the finding of no increased risk of lung cancer in the European study conducted by Kogevinas *et al.* (1994a). As general problems of the studies reviewed, the authors emphasized nondifferential misclassification of exposure with respect to disease outcome and imprecise diagnoses in studies relying on death certificates. Cohen *et al.* mentioned that some other cancers (of the esophagus, pancreas, urinary tract, and genital organs) showed increased risks in some studies, but they did not consider them related to styrene exposure, because the increases were small and statistically nonsignificant, and they did not concentrate in groups with high exposure.

3.8 Summary of the findings for selected cancer sites

The results for 12 separate study populations are presented in Table 3-8 for major cancer sites. This tabulation did not include the study by Coggon *et al.* (1987) because this

population was included in the Kogevinas *et al.* (1994a) study of the European reinforced-plastics industry. It includes the Kolstad studies (one report on lymphohematopoietic cancers from 1994 and one report on solid cancers from 1995), but it should be emphasized that 15,867 of the 36,610 Danish workers were also included in the Kogevinas *et al.* study (1994a). The findings by Ruder *et al.* (2004) are also included, but not those of Okun *et al.* (1985), similarly, those of Wong *et al.* (1994) but not Wong *et al.* (1990) are included, and Bond *et al.* (1992) but not Ott *et al.* (1980) are described because the reports included were based on the last and longest follow-up of the same cohorts.

For the styrene-butadiene rubber industry, the results for the cohort described in Sathiakumar *et al.* 2005 and Delzell *et al.* 2006 are included, but results from other studies of this industry were omitted due to major overlap of study populations (Matanoski *et al.* 1997, Matanoski *et al.* 1993, Matanoski *et al.* 1990, Matanoski and Schwartz 1987, Meinhardt *et al.* 1982, Santos-Burgoa *et al.* 1992), or because of shorter follow-up (Sathiakumar *et al.* 1998), or because they did not tabulate results for the major cancer sites but focused on exposure response for leukemia and other lymphohematopoietic cancers (Delzell *et al.* 2001, Delzell *et al.* 1996, Graff *et al.* 2005, Macaluso *et al.* 1996).

[This tabulation suggests that the strongest indications of consistently increased risks across the individual studies were for cancer of esophagus, pancreas, larynx, lung, and lymphohematopoietic tissues (NHL, Hodgkin's disease, multiple myeloma, and leukemia)]. Pooled results for these selected cancers obtained from studies of workers in the reinforced-plastics industry are presented in Table 3-9. For each study, results for the well-defined worker category with the highest styrene exposure (defined by title or task) are presented when possible. Table 3-10 presents these results for workers in the reinforced-plastics industry.

Among all 85,000 workers included in studies of the reinforced-plastics industry, 2,238 cases of cancer were identified from death certificates and cancer incidence registrations, which is close to the expected number (2,210.5). In this tabulation (Tables 3-9 and 3-10)

the male Danish workers (results were not presented for female workers [(Kolstad *et al.* 1995, Kolstad *et al.* 1994)] were omitted from the dataset of the European reinforced-plastics industry (Kogevinas *et al.* 1994a). This was done based on country-specific findings published in an IARC report describing details of the European study (Kogevinas *et al.* 1994b). [This was done to eliminate overlap between the Danish and the European datasets and thus erroneously pooled estimates. Another option would be to exclude the total Danish dataset (as Cohen *et al.* 2002 did in their review), but this would mean that the results based on that part of the Danish population not included in the European data set would be left out. Furthermore, the Danish study reported incidence data while the European study only reported mortality data, and incidence data is regarded the most relevant for several of the cancers studied with relatively low mortality. Therefore, inclusion of the Danish population and exclusion of the male Danish workers from the European study when pooling observed and expected number of cases, is expected to have improved the validity of the overall evaluation.]

[The data in Tables 3-8 to 3-10 permit a general comparison of multiple studies and the identification of trends in the data (e.g., several studies reporting nonsignificant increases in a specific site). However, there are several limitations inherent in pooling data across different studies. Study populations may differ with respect to, for example, the size and type of population studied, their racial or age composition, inclusion and exclusion criteria (such as minimum duration of employment or exposure time), latency periods, duration of follow-up, the nature and intensity of exposure, and the type of cases reported (incidence vs. mortality). In addition, studies clearly vary in quality, e.g., with respect to the power of the study, (especially for specific cancer sites) and which, if any, potentially confounding variables (e.g., potential exposure to other carcinogens) are adjusted for. This methodology also does not incorporate information on subgroup analyses (such as exposure-response relations) that may be important in evaluating causality. However, as mentioned previously, one of the major limitations of the body of literature is the small numbers of highly exposed workers, which limits the ability to detect an effect, especially for uncommon tumors. This approach (summing observed and expected cases of highly exposed workers from all studies) facilitates the evaluation of the relationship between styrene exposure and cancer risk of these tumors.]

Table 3-8. Relative occurrence of cancer in 12 cohort studies of populations exposed to styrene (total study populations)

Cancer site	Reinforced-plastics industry				Styrene monitored workers	SBR industry		Styrene monomer & polymer industry					Environ. exp.	
											Hodgson and Jones 1985		Loughlin *et al. 1999*	
	Ruder *et al. 2004*	Wong *et al. 1994*	Kolstad *et al.* 1994, 1995	Koge-vinas *et al.* 1994[a]	Anttila *et al.* 1998	McMi-chael *et al.* 1976	Sathia-kumar *et al.* 2005[c] Delzell *et al.* 2006	Frentzel-Beyme *et al. 1978*	Bond *et al.* 1992	Nicholson *et al. 1978*	SMR	SIR	M	F
all cancer	+	+		–	(–)		–		–	(–)	(–)	(–)	(+)	–
buccal cavity & pharynx	(–)	(–)		–			–		–					
lip			(+)											
tongue			(–)											
salivary gland			(+)											
mouth			(+)											
pharynx			(–)											
digestive sys.	(+)								(–)				(+)	(–)
esophagus[d]	+	+	(–)	(–)			(–)		(–)		(+)			
stomach	(+)	(–)	(–)	(–)	(+)	+	(–)	(–)	(+)					
small intestine				(+)										
large intestine		(+)	(–)	(–)	(–)		(-)	(–)	(–)					

Cancer site	Reinforced-plastics industry				Styrene monitored workers	SBR industry		Styrene monomer & polymer industry					Environ. exp.	
											Hodgson and Jones 1985		Loughlin *et al. 1999*	
	Ruder *et al. 2004*	Wong *et al. 1994*	Kolstad *et al.* 1994, 1995	Koge-vinas *et al.* 1994[a]	Anttila *et al.* 1998	McMi-chael *et al.* 1976	Sathia-kumar *et al.* 2005[c] Delzell *et al.* 2006	Frentzel-Beyme *et al. 1978*	Bond *et al.* 1992	Nicholson *et al. 1978*	SMR	SIR	M	F
intestine except rectum	(–)													
rectum		(+)	(–)	–	+			(–)	(–)					
large intestine & rectum						(–)	(+)							
liver & gallbladder	(–)	(+)		(–)					(–)					
liver			(–)		(+)		(–)							
gallbladder			(–)											
pancreas[d]	(+)	(+)	(+)	(+/–)	(+)		(–)	(+)	(–)					
peritoneum			(+)											
respiratory sys.	(+)	+				(–)			(–)					
nose & nasal cavities			(+)	(–)										
larynx[d]		(+)	(+)	(+)			(–)		(–)			+		
lung[d]	(+)	+	(+)	(–)	(–)		–	(–)	(–)	(–)	(+)		(+)	(–)

Cancer site	Reinforced-plastics industry				Styrene monitored workers	SBR industry		Styrene monomer & polymer industry					Environ. exp.	
											Hodgson and Jones 1985		Loughlin *et al. 1999*	
	Ruder *et al. 2004*	Wong *et al. 1994*	Kolstad *et al.* 1994, 1995	Koge-vinas *et al.* 1994[a]	Anttila *et al.* 1998	McMi-chael *et al.* 1976	Sathia-kumar *et al.* 2005[c] Delzell *et al.* 2006	Frentzel-Beyme *et al. 1978*	Bond *et al.* 1992	Nicholson *et al. 1978*	SMR	SIR	M	F
pleura			(+)											
mediastinum			(+)											
breast	(–)	(–)		–	(–)									(–)
female genital organs	(+)	+[e]			(+)									
uterus		(+)[e]												
cervix		+		(–)										
ovary	(+)			(+)										
male genital organs														
prostate	+	(+)	(–)	(+)		(–)	(+)		(–)					
testis		(–)	(+)	(–)					(–)					
external male genital organs			(+)											
urinary organs	(+)							(+)						
kidney	(+)	(+)	(–)	(–)	(–)		(–)		(–)					

Cancer site	Reinforced-plastics industry				Styrene monitored workers	SBR industry		Styrene monomer & polymer industry					Environ. exp.	
											Hodgson and Jones 1985		Loughlin *et al. 1999*	
	Ruder *et al. 2004*	Wong *et al. 1994*	Kolstad *et al.* 1994, 1995	Koge-vinas *et al.* 1994[a]	Anttila *et al.* 1998	McMi-chael *et al.* 1976	Sathia-kumar *et al.* 2005[c] Delzell *et al.* 2006	Frentzel-Beyme *et al. 1978*	Bond *et al.* 1992	Nicholson *et al. 1978*	SMR	SIR	M	F
bladder	(+)	(–)	(+)	(–)		(–)	(–)		(–)					
skin				(–)					(–)					
melanoma			(–)											
other skin			(–)											
eye			–						(–)					
brain & nervous sys.	(+)	(–)	(–)	–	(+)		(–)		(–)					
thyroid			(+)	(–)					(–)					
other endocrine glands			(+)											
bone			(–)						(+)					
connective tissue			(–)	(–)										
all LH[d]	(–)	(–)	(+)	(–)	(–)	+	(+)		(+)			(+)	(+)[f]	(–)
all lymphoma[d]										(–)	+	+		
NHL[d]	(–)	(–)	(+)	(–)			(+/–)		(+)	(+)		(+)		
Hodgkin's disease[d]	(–)	(–)	(+)	(–)	(+)		(+)		(+)			(–)	(+)	(–)

Cancer site	Reinforced-plastics industry				Styrene monitored workers	SBR industry		Styrene monomer & polymer industry					Environ. exp.	
											Hodgson and Jones 1985		Loughlin *et al. 1999*	
	Ruder *et al. 2004*	Wong *et al. 1994*	Kolstad *et al.* 1994, 1995	Koge-vinas *et al.* 1994[a]	Anttila *et al.* 1998	McMi-chael *et al.* 1976	Sathia-kumar *et al.* 2005[c] Delzell *et al.* 2006	Frentzel-Beyme *et al. 1978*	Bond *et al.* 1992	Nicholson *et al. 1978*	SMR	SIR	M	F
multiple myeloma[d]			(–)	(–)			(–)		(+)			(–)		
leukemia[d]	(–)	(–)	(+)	(+)		+[c]	(+)		(+)	(–)	(–)	(+)	(+)	(–)

+ = Statistically significant excess of cancer; (+) = statistically nonsignificant excess of cancer; – = statistically significant deficit of cancer; (–) = statistically nonsignificant deficit of cancer; (+/–) = no excess/deficit of cancer, i.e., SMR = 1.0.

[a] Male Danish workers were excluded from the European data set in this calculation to eliminate overlap between the Danish and the European datasets.

[b]Results based on exposure ratios (colorectal, prostate, bladder, respiratory cancers) or risk ratios (stomach, all lymphohematopoietic, leukemia).

[c]Data are from the follow-up reported by Sathiakumar *et al.* 2005 of 17,924 workers from 1944 to 1998 except for esophagus, which was not reported in the 2005 analysis, so is from the earlier follow-up of the smaller cohort (N = 15,649) followed up to 1991 and reported in Sathiakumar *et al.* 1998.

[d]Cancer site selected for more thorough evaluation, see Tables 3-8 and 3-9.

[e]The paper reported statistically significant increases for cervix (SMR = 2.835, 1.359–5.213, $P < 0.01$) and other female genital organs (SMR = 2.016, 1.074–3.448, $P < 0.05$), while uterus (SMR = 1.973, 0.985–3.531) approached statistical significance.

[f]Incident cases overlap with deaths; 2 cases of lymphohematopoietic cancer (one lymphoma and one leukemia) were not recorded as the underlying cause of death.

3.8.1 Esophageal cancer

Reinforced plastic workers: Statistically significantly increased risks were observed in the two U.S. studies (Ruder *et al.* 2004 and Wong *et al*. 1994), and a nonsignificantly increased risk was observed for laminators, but not for workers with unspecified tasks or other exposed jobs, in the European cohort (Kogevinas *et al*. 1994a). The SIR in the Danish study was close to unity (Kolstad *et al.* 1995). Among the worker categories with the highest potential styrene exposure — laminators (Kogevinas *et al.* 1994a, Ruder *et al.* 2004), open-process mold workers (Wong *et al.* 1994), and workers at companies employing 50% to 100% laminators (Kolstad *et al.* 1995) – a total of 14 cases of esophageal cancer were observed (≥ 7.2 expected[4]; see Table 3-10). A statistically nonsignificant trend toward increased esophageal cancer mortality with increasing cumulative styrene exposure was seen among European reinforced-plastics workers (Kogevinas *et al*. 1994a), and mortality was highest at ≥ 20 years since first exposure. Mortality was increased (SMR = 2.74, 95% CI = 0.004 to 22.3, 1 observed death) among workers from Washington state with high exposure for more than 1 year (Ruder *et al.* 2004).

Other industries: Among workers in the styrene-butadiene rubber industry, the SMR for esophageal cancer was close to unity (SMR = 0.94, 95% CI = 0.68 to 1.26, 44 observed deaths) (Sathiakumar *et al.* 1998). Among styrene monomer production workers, 4 cases were identified, compared with 5.1 expected (Bond *et al.* 1992, Hodgson and Jones 1985). [Evaluation of site-specific cancer risks in this industry is limited by the small numbers of subjects; three of the four studies had low numbers (fewer than 20) of expected and observed cases of all malignant tumors.] Risk estimates for esophageal cancer were not reported for the biomonitoring study (Anttila *et al.* 1998) or the study of environmental exposure to styrene-butadiene (Loughlin *et al.* 1999).

With respect to the case-control studies, only the study of Gerin *et al.* (1998) investigated esophageal cancer, in association with potential exposure to four solvents (benzene, toluene, xylene, and styrene). No statistically significant association between esophageal

[4] "≥" is used when at least one study reported the number of observed cases but not expected cases.

cancer and styrene exposure was observed (OR adjusted for demographic, socioeconomic and lifestyle factors = 1.4 [95% CI = 0.5 to 3.8, based on 5 cases] for "medium/high exposure").

3.8.2 Pancreatic cancer

Reinforced-plastics workers: Increased risks (1 significant and 2-nonsignificant) of pancreatic cancer were observed among the high-exposure groups in three of the four reinforced-plastics worker populations (Kogevinas *et al.* 1994a, Kolstad *et al.* 1995, Ruder *et al.* 2004), but not in the fourth (Wong *et al.* 1994) (see Table 3-8. A total of 34 cases were registered across all four populations (high-styrene–exposure groups) compared with 19.2 expected (Table 3-10 [corresponding to an SMR value of 1.77 (95% CI = 1.23 to 2.47)]. In internal analyses, Kolstad *et al.* (1995) reported significant risks of pancreatic cancer among individuals with probable high styrene exposure (workers from plants employing 50% to 100% laminators), and among individuals exposed to styrene for greater than one year. The risk of pancreatic cancer increased with increasing cumulative styrene exposure ($P = 0.068$) (Kogevinas *et al.* 1994a), and a slightly higher risk was seen among long-term than among short-term workers and earlier start dates (Kolstad *et al.* 1995), but not in all studies (Ruder *et al.* 2004).

Other industries: In styrene-butadiene rubber industry workers, the SMR was 0.87 (95% CI = 0.68 to 1.08; 76 observed deaths) (Sathiakumar *et al.* 1998). The findings from the styrene monomer and polymer industry were divergent; decreased mortality (non-significant) was reported by Bond *et al.* (1992) and an increased mortality (non-significant) was reported by Frentzel-Beyme *et al*. (1978) and the pooled number of cases was less than expected (7 observed vs. 11 expected). The biomonitored workers (Anttila *et al.* 1998) showed a 3-fold increased risk of pancreatic cancer (SIR = 3.64; 95% CI = 0.75 to 10.6, 3 cases) 10 years or more after the first measurement. No risk estimate was reported in the environmental exposure study.

No increased risk of pancreatic cancer (based on 1 exposed case and 22 exposed controls) was reported in the population-based, case-control study reported by Gérin *et al.* (1998).

3.8.3 Laryngeal cancer

Reinforced-plastics workers: Among all reinforced-plastics workers, 36 cases of laryngeal cancer were observed (vs. 32.7 expected) (Table 3-9), yet only 3 cases were identified among the workers classified with the highest styrene exposure (vs. ≥ 1.9 expected) (Table 3-10).

Other industries: The SMR value was non-significantly decreased (SMR = 0.71, 95% CI = 0.41 to 1.13, 17 observed deaths) in the styrene-butadiene rubber industry (Sathiakumar *et al.* 2005). In the styrene monomer and polymer industry, only 1 case (death) was reported (vs. 2.9 expected) (Bond *et al.* 1992). Hodgson and Jones (1985) reported an excess of incidence cases (3 observed vs. 0.5 expected, $P = 0.041$); however, no mortality was reported. The authors stated that laryngeal cancer is often amenable to treatment. Risk estimates were not calculated in the biomonitoring or environmental studies, and there were no case-control studies evaluating laryngeal cancer.

3.8.4 Lung cancer

Reinforced-plastics workers: Lung cancer risk was significantly increased among U.S. workers (Wong *et al.* 1994), and increased but not statistically significant among workers from Denmark (Kolstad *et al.* 1995) and Washington state (Ruder *et al.* 2004). Among the highest-styrene–exposure group in the reinforced-plastics industry, 158 cases of lung cancer were observed, compared with 151.5 expected (Table 3-10). Lung cancer risk was lower among styrene-exposed workers in a nested case-control study that controlled for smoking, in long-term workers, and among workers with higher cumulative styrene exposure (Kogevinas *et al.* 1994a, Kolstad *et al.* 1995, Ruder *et al.* 2004, Wong *et al.* 1994).

Other industries: In the styrene-butadiene rubber and the styrene monomer and polymer industries, fewer cases were observed than expected. No increased risk of lung cancer was seen in workers biomonitored for styrene exposure (Anttila *et al.* 1998), in the styrene-butadiene rubber industry (McMichael *et al.* 1976a, Sathiakumar *et al.* 1998), or in the styrene polymer manufacturing industry (Frentzel-Beyme *et al.* 1978) (see Table 3-8). No significant association with lung cancer was observed among potentially styrene-

exposed cases in a population-based, case-control study by Scelo *et al.* (2004) or the population-based study of Gerin *et al.* (1998), although the power to detect an effect in the latter study is low.

3.8.5 Lymphohematopoietic cancers

Not all studies reporting on lymphohematopoietic cancers analyzed by type. In addition, the power to detect increased risks for subtypes of lymphohematopoietic cancers is limited by the small number of total lymphohematopoietic cancers observed in some cohorts.

3.8.5.1 All lymphohematopoietic cancers combined

Reinforced-plastics workers: Kolstad *et al.* 1994 reported a non-significantly increased incidence for all lymphohematopoietic malignancies (SIR = 1.20; 95% CI = 0.98 to 1.44, 112 observed cases) among Danish workers (which overlaps with the international study reported by Kogevinas *et al.* (1994a, 1993). No increase in lymphohematopoietic cancer mortality was observed for the two U.S. studies (Rude*r et al*. 2004, and Wong *et al*. 1994). Among all workers in the reinforced plastic industry, 196 cases were observed compared with 199.2 expected (Table 3-9). Observed among the high-styrene–exposure groups in the reinforced-plastics industry were 52 cases of any lymphohematopoietic malignancy (53 expected) (see Table 3-10). In the largest study (the multi-country) the risk of all lymphohematopoietic malignancies increased with average exposure (P = 0.019) and time since first exposure (P = 0.012), but did not increase with increasing cumulative styrene exposure (Kogevinas *et al.* 1994a). No increased risk was observed with duration of employment in the other studies (Kolstad *et al.* 1994, Ruder *et al.* 2004, Wong *et al.* 1994).

Styrene-butadiene rubber workers: The principal methodological challenge in these studies lies in teasing out possible independent or synergistic effects of butadiene, which is highly correlated with styrene exposure in this industry. 1,3-Butadiene is listed as *known to be a human carcinogen* in the 11th Report on Carcinogens (NTP 2004). In the synthetic rubber industry, McMichael *et al.* (1976a) reported a significant increase in the age-standardized relative risk of all lymphohematopoietic cancers (RR = 6.2, 99% CI =

4.1 to 12.5) among workers engaged in synthetic rubber tire manufacture (primarily styrene-butadiene), [but no adjustment for other exposures was attempted].

In the cohort of styrene-butadiene rubber workers established by Delzell and colleagues, a slightly increased mortality from all lymphohematopoietic malignancies (SMR = 1.06, 95% CI = 0.90 to 1.23, 162 observed deaths) was observed in the 1998 follow-up by (Sathiakumar *et al.* 2005). This cohort included styrene-butadiene rubber workers from a smaller cohort reported by Meinhardt *et al.* and most of the workers from a larger cohort studied by Matanoski and coworkers. There were numerous publications on both cohorts or subpopulations of the cohorts (Matanoski and Delzell), and interpretation of the studies are complicated by overlapping populations, different exposure assessments and different types of analyses.

Risk estimates for quantitative exposure to styrene and the risk of lymphohematopoietic cancers (combined) was not reported in the most recent updates of the most comprehensive cohort (e.g., Delzell *et al.* 2006); however, it was studied in two nested case-control studies from the Matanoski cohort, which reported findings for workers employed from 1943 to 1976 and followed until 1982. The nested case-control study (59 cases and matched controls) reported by Santos-Burgoa *et al.* (1992) found non-significant increases for cumulative exposure to styrene (greater than average exposure) and lymphohematopoietic mortality using matched and unmatched analyses; however, the magnitude of the OR was decreased in matched models that controlled for butadiene exposure. The second case-control study (58 cases and 1,242 controls) found a two-fold significantly increased risk for lymphohematopoietic cancers (combined) and time-weighted average (working lifetime) exposure to 1-ppm styrene after taking into account butadiene exposure and other variables in a step-down logistical regression analysis (Matanoski *et al.* 1997). This analysis used an exposure assessment based on measurements of styrene air levels (taken in 1978 to 1983) and used controls sampled without individual matching by plant and other variables, whereas exposure was assessed by job-exposure matrix in the study by Santos-Burgoa e*t al.*

Other industries: In the largest study of the styrene monomer and polymer industry, the risk of all lymphohematopoietic malignancies increased with increasing duration of exposure (if workers with < 1 year are compared with those with > 1 year of exposure) but not with increasing styrene exposure level (Bond *et al.* 1992). Among all workers at the four styrene monomer and polymer plants studied, there were 34 deaths due to lymphohematopoietic malignancies, compared with 23.1 expected (Bond *et al.* 1992, Frentzel-Beyme *et al.* 1978, Hodgson and Jones 1985, Nicholson *et al.* 1978). Among workers biomonitored for styrene exposure, the incidence of all lymphohematopoietic malignancies was not increased (SIR = 0.39, 95% CI = 0.05 to 1.40, 2 cases) (Anttila *et al.* 1998). No cases of lymphohematopoietic cancer occurred 10 years or more after the first measurement, but the study included only 2 cases. In the study of environmental exposure to styrene (Loughlin *et al.* 1999), a non-significantly increased risk of all lymphohematopoietic malignancies was reported among men.

3.8.5.2 Leukemias

Reinforced plastic workers: Among the reinforced-plastics workers, Kolstad *et al.* 1994 reported a non-significant increased incidence in leukemia (SIR = 1.22, 95% CI = 0.88 to 1.65, 42 observed cases) among Danish workers (which overlaps with the international study reported by Kogevinas *et al.* (1994a, 1993). Kogevinas *et al.* reported non-significant increases for myeloid leukemia mortality, but no increase with increasing average or cumulative exposure was observed; a non-significant trend was observed with time since first exposure ($P = 0.094$). In the Danish study, significantly increased mortality from leukemia was observed among workers with more than 10 years after first styrene exposure and for workers with earlier years of first employment (Kolstad *et al.* 1994). No relationship between cumulative exposure or duration was observed among the U.S. workers reported by Wong *et al.* (1994). Among the high-styrene–exposure groups in the reinforced-plastics industry, a total of 19 cases of leukemia was observed (19.6 expected) (see Table 3-10). In analyses of subtypes of leukemia, the risk of myelogenous leukemia (chronic and acute) was slightly higher than for all leukemia (Kogevinas *et al.* 1994a), and increased risk was also seen for myeloid leukemia with chromosomal aberrations in a nested case-control study of the Danish workers (Kolstad *et al.* 1996).

Styrene-butadiene rubber workers: McMichael *et al.* (1976a) reported a statistically significant increase in the age-standardized risk for lymphatic leukemia (RR = 3.9, 99% CI = 2.6 to 8.0) among rubber tire workers engaged in synthetic rubber manufacture (primarily styrene-butadiene) [but no adjustment for other exposures was attempted].

In the latest follow-up (to 1998) of the most comprehensive cohort of styrene-butadiene workers, the SMR for leukemia among all workers was 1.16 (95% CI = 0.91 to 1.47, 71 deaths) (Sathiakumar *et al.* 2005). Compared with workers with other combinations of duration of employment and time since hire, the highest risk of leukemia in this cohort was observed among workers with > 10 years of employment and 20 to 29 years since hire (SMR = 2.58, 95% CI = 1.56 to 4.03, 19 deaths). Among this subgroup, those who had been hired between 1950 and 1959 had the highest risk of leukemia (SMR = 3.92, 95% CI = 1.96 to 7.03, 11 deaths (Delzell *et al*. 2006). Statistically significant increased risks of leukemia (SMR ranging from 2.58 to 4.31) were observed among workers involved in production (polymerization and coagulation) job groups) and labor (maintenance and laboratories job groups) (Sathiakumar *et al.* 2005). (Note that production and maintenance workers had high exposure to both styrene and butadiene, and coagulation workers had low to moderate exposure to styrene, but only background exposure to butadiene.) Significant SMRs (approximately 2-fold increased) were also reported among the two highest categories of cumulative levels of styrene exposure.

Exposure to styrene and leukemia risk were evaluated in the two nested case-control studies from the Matanoski cohort and in several reports from the Delzell cohort [note that these cohorts overlap]. Santos-Burgoa *et al.* (1992) reported a significantly increased risk of leukemia for cumulative exposure greater than average exposure in both matched and unmatched analysis; however, the risk was no longer significant after controlling for butadiene exposure. In the nested case-control study from the Matanoski cohort, no significant risks were found for leukemia and 1-ppm time-weighted average exposure to styrene; however, a significant association between leukemia and cumulative exposure was found in a final model that included styrene, butatdiene exposure, and duration of employment (Matanoski *et al.* 1997).

Graff *et al.* (2005) and Delzell *et al.* (2006) conducted a series of internal analyses in which exposure to butadiene and DMDTC were adjusted for either in models of cumulative exposure or in analyses of cross-classified categories of styrene and butadiene exposure, for the 1998 update of the cohort established by Delzell. Statistically nonsignificant increases in the relative risk of leukemia for categories of styrene and butadiene exposure or quartiles of cumulative exposure to styrene in the single-, and two-chemical models; however, the RRs were below one in the three-chemical model. [There was a trend towards higher risk with increasing exposure to styrene alone and when adjusted for butadiene and styrene; however, tests for trend, were not reported by the authors.] In a similar analysis using cumulative exposure due to styrene total peaks > 50 ppm and butadiene total peaks > 100 ppm, increasing risks of leukemia with increasing levels of exposure were observed in single-, two- and three-chemical models. In external analysis of cumulative exposure to styrene in this cohort, significantly increased SMRs were observed for the two highest categories of styrene exposure; [however, there was no adjustment for exposure to butadiene or DMDTC.]

Statistically nonsignificant increases in relative risk were observed in internal analyses by subtypes of leukemia (CLL, AML, and other) for terciles of styrene exposure; no increasing risks with exposure were observed for CML (Graff *et al.* 2005, Delzell *et al.* 2006). (These analyses were restricted to workers over 40 years of age and, in some cases, to > 20 years since hire). In external analyses of CLL, AML, and CML, non-significant increases in CML and CLL were observed, and CLL was significantly increased at cumulative styrene exposures exceeding 61.1 ppm-years (SMR = 3.10, 95% CI = 1.01 to 7.24, 5 deaths).

Other studies: Among all workers at the four styrene monomer and polymer plants studied, slightly increased mortality was seen for leukemia (10 observed deaths vs. 8.7 expected (Bond *et al.* 1992, Frentzel-Beyme *et al.* 1978, Hodgson and Jones 1985, Nicholson *et al.* 1978). A statistically nonsignificant increase in leukemia was observed in the U.S. cohort of styrene monomer and polymer workers (SMR = 1.18, 95% CI = 0.54 to 2.24, 9 deaths) (Bond *et al.* 1992). In the study of environmental exposure to

styrene (Loughlin *et al.* 1999), non-significant increases in leukemia were reported among men.

The 2 case-control studies that addressed leukemia (Flodin *et al.* 1986, Guenel *et al.* 2002) had small numbers of exposed subjects (Guenel – 2 cases of leukemia and 9 controls, and Flodin – 3 cases of myeloid leukemia and 1 control), [precluding any firm conclusions (despite the high OR found in the study of Flodin *et al*.)].

3.8.5.3 Other lymphohematopoetic cancers

Reinforced plastic workers: Among reinforced-plastics workers, statistically nonsignificant elevations in lymphomas were observed by Kolstad *et al.* (1994) (SIR = 1.33, 95% CI = 0.96 to 1.80, 42 cases) and Kogevinas *et al.* (1994a) (among laminators) (SMR = 1.40, 95% CI = 0.56 to 2.88, 7 cases), but no increased risks were observed in the smaller cohorts. Among the high-styrene–exposed groups in the entire industry, a total of 14 cases of NHL (vs. 15.1 or more expected) and 11 cases of Hodgkin's disease (vs. 7.9 or more expected) were observed (Table 3-10). Kogevinas *et al.* reported that the risk of malignant lymphoma increased with averge exposure ($P = 0.052$) and with time since first exposure ($P = 0.072$), but not cumulative exposure.

Styrene-butadiene rubber workers: In the styrene-butadiene industry, several subtypes of lymphohematopoietic cancers were investigated in the overlapping cohorts established by Matanoski and Delzell *et al.* The nested case-control study from the Matanoski cohort of 58 lymphohematopoietic cases and 1,242 controls found two- to three-fold increased risks for lymphoma, lymphosarcoma, and myeloma and styrene exposure (increase of 1 ppm in TWA) (Matanoski *et al.* 1997), and the risk of myeloma increased with increasing cumulative exposure to styrene using the step-down regression analysis and taking into account butadiene exposure and other variables. Styrene exposure was not associated with Hodgkin's disease. However, no associations between other types of lymphohematopoietic cancers (lymphosarcoma, Hodgkin's disease, and other lymphatic cancers) were observed in the nested case-control study reported by Santos-Burgoa *et al.* (1992).

In the 1998 follow-up of the Delzell *et al.* cohort, no significant increases in risks were observed for Hodgkin's disease, multiple myeloma, or NHL (Delzell *et al.* 2006). No significant increases in the risk of NHL, Hodgkin's lymphoma, or multiple myeloma were observed in subgroup analyses of years since hire and latency or by work group/job area. Borderline significanty increased risks of NHL+CLL were observed among all ever-hourly workers, and significantly increased risks were observed among these workers with greater than 10 years of employment and 20 to 29 years or 30+ years since hire; significantly increased risks were also observed for styrene-butadiene rubber workers in polymerization and finishing workshops. Statistically significant SMRs for NHL or NHL+CLL were also observed for the highest cumulative levels of styrene, but no association was found between multiple myeloma and exposure to styrene. In internal analyses, increasing risks of NHL and of NHL+CLL with increasing quartiles of styrene exposure were observed before and after controlling for butadiene and/or DMDTC exposure (although the trends were attenuated in models with DMDTC), but no one quartile was significant (Graff *et al.* 2005, Delzell *et al.* 2006). In single-chemical models, the risk for NHL and NHL+CLL also were increased at the two highest levels of butadiene exposure; however, no increased risk was observed after controlling for styrene, [suggesting that butadiene was not a risk factor for these cancers; however, butadiene is a risk factor for leukemia. Tests for trend were not performed.] No such trend was seen for multiple myeloma. Similar results were seen in SMR analyses. When all lymphoid and all myeloid cancers were considered in two separate groups, no significant increases in relative risks with increasing styrene exposure were observed in single- or multiple-chemical models, with the exception of myeloid cancers at styrene levels of 1.8 to < 61.1 ppm-years (RR = 2.6, 95% CI = 1.2 to 5.5, 13 deaths) (Delzell *et al.* 2006).

Other studies: Among all workers at the four styrene monomer and polymer plants studied, slightly increased mortality was seen for lymphoma (11 observed deaths vs. 7.9 expected) and leukemia (10 observed deaths vs. 8.7 expected (Bond *et al.* 1992, Frentzel-Beyme *et al.* 1978, Hodgson and Jones 1985, Nicholson *et al.* 1978). Among workers biomonitored for styrene exposure, the incidence of Hodgkin's disease was slightly, but

nonsignificantly increased (SIR = 1.89, 95% CI = 0.23 to 6.84, 2 cases) (Anttila *et al.* 1998).

The population and clinic-based, case-control studies evaluated different types of lymphohematopoietic cancer and, as discussed above, were limited principally by potential misclassification of exposure. The population-based, case-control study by Seidler *et al.* (2007) included a sufficient number of cases and controls, but no significant increase in malignant lymphoma was found and no trend with increasing exposure detected. Non-significant increases in non-Hodgkin's lymphoma (8 cases and 19 controls) and in Hodgkin's lymphoma (2 cases and 19 controls) were observed in the Canadian population-based, case-control study, but the number of exposed subjects was too small to draw firm conclusions(Gérin *et al.* 1998).

3.8.6 Other sites

Findings are less consistent across cohort or case-control studies for other sites. Significantly increased risks for cancer of the stomach (McMichael *et al.* 1976a), benign neoplasms (which were brain tumors) (Loughlin *et al*. 1999), cervix and other female genital organs (Wong *et al.* 1994) have been reported in a single study; however, other studies reported either nonsignificantly increased or decreased risks. For other sites (prostate, rectum, and urinary system), significant increases were reported in at least 2 studies or there was supporting exposure-response data.

Prostate: Ruder *et al.* (2004) reported a significant increase in prostate cancer mortality among reinforced plastic workers; SMRs were elevated in both the high- and low-exposure groups although the SMR was slightly higher in the high-exposure cohort. Mixed results (nonsignificant increases or decreases) were observed in the other cohort studies; however, Gerin *et al*. reported a significant risk (OR = 5.5, 95% CI = 1.4 to 21.8, 7 exposed cases and 3 controls) for medium to high styrene exposure in the Canadian case-control study. In the most recent update of the Delzell *et al.* cohort, a slight but non-significant increase in the SMR for prostate cancer was observed (SMR = 1.04, 95% CI = 0.88 to 1.21, 154 deaths) (Sathiakumar *et al.* 2005), but no increase in the relative risk of prostate cancer was observed by increasing levels of cumulative styrene exposure in

either single- or multiple-agent models (analysis restricted to workers 50+ years of age) (Delzell *et al.* 2006).

Rectal: A significantly increased incidence of rectal cancer was observed among the biomonitored workers; the incidence was higher among individuals with > 10-year follow-up, but did not increase with increasing lifetime urinary metabolite levels (Anttila *et al.* 1998). A significant risk (OR = 3.9, 95% CI = 1.2 to 12.9, 5 exposed cases) was also observed in the Canadian case-control study for substantial exposure to styrene (Dumas *et al.* 2000). However, non-significant decreases or null results were reported in most of the other studies. A slight but non-significant increase in the SMR for colorectal cancer was observed by Sathiakumar *et al.* (2005) (SMR = 1.09, 95% CI = 0.94 to 1.25, 193 deaths). In an internal analysis of increasing exposure to styrene, the relative risk exceeded 1.0 in 3 of 4 quartiles (1.2, 1.2, 0.6 and 1.5, respectively) but none of the estimates were significant (Delzell *et al.* 2006).

Urinary: Ruder *et al.* reported an increase in urinary cancer mortality among the high-exposure group of reinforced plastic workers from Washington state (SMR = 3.44, 95% CI = 1.26 to 7.50, 6 observed deaths), and there was a trend towards increasing SMRs with increasing duration of exposure in this group. In the multi-country European cohort (Kogevinas *et al.* 1994a), the relative risk for kidney cancer increased with increased cumulative exposure (although the test for trend was not significant), but decreased with time since first exposure. The SMR was not elevated among the low-exposure group. An increased risk of renal-cell cancer was also associated with exposure to styrene-butadiene rubber in the population case-control study from Canada (Parent *et al.* 2000). Results from other studies were not consistent, with some studies reporting nonsignificant increases and others nonsignificant decreases.

Increased risk of breast cancer was suggested in an ecological study (Coyle *et al.* 2005), which assessed styrene exposure by toxic release inventory data; [however, this study was limited by the ecological design and poor characterization of styrene exposure, which was based only on residence in counties with varying environmental toxic releases]. A population-based, case-control study (Cantor *et al.* 1995) from the United

States also reported a statistically significant increased risk for breast cancer; [however, exposure was assigned based only on occupation listed on the death certificate]. No increased risk of breast cancer was shown in the industry-based cohort studies. [The body of literature from the occupational cohort studies has limited power to detect an effect. The studies from the styrene-butadiene rubber and the styrene monomer and polymer industries have been of men (except for Frentzel-Beyme *et al.* 1978, which did not state the sex of the population, did not report a risk estimate for breast cancer, and was limited by small numbers of expected (18.5) and observed cases (12) of malignant tumors). Studies by Ruder *et al*. (2004), Wong *et al.* (1994), Kogevinas *et al.* (1994a), and Antilla *et al.* (1998) included women; however, they were limited by small numbers of expected and observed cases of breast cancer mortality (Ruder *et al.*, Kogevinas *et al*. or low levels of styrene exposure (Wong *et al.*). It seems reasonable that women are more likely to have low-exposure jobs, and Kolstad *et al.* (1993, 1994) omitted females from subsequent studies because the majority were not involved in the production of reinforced plastics.

[The cohort studies have not attempted to control for confounding factors that affect breast cancer risk (such as body mass index, family history of breast cancer, alcohol use, menopausal status, parity, hormone use, and age at first birth). In addition, cohort studies often do not have sufficient follow-up time to detect effects on incidence or mortality because of the long latency (sometimes in excess of 40 years) from the initiation to detection of breast cancer. A decreased risk of breast cancer was found among women in the cohort study of environmental exposure to styrene, but this study was limited by small numbers of expected (7) and observed (4) deaths from breast cancer, and questionable completeness of follow-up and identification of death certificates (Loughlin *et al*. 1999). Note that a marginally significant increase in the incident risk ratio for breast cancer was observed among a cohort of women army personnel in occupations with medium to high potential exposure to volatile organic compounds (VOC), including potential styrene exposure, (IRR = 1.48, 95% CI = 1.01 to 2.07 [95% CI = 1.03 to 2.12 also reported in a table]) compared with women with no or low VOC exposure (Rennix *et al.* 2005), but no specific inferences for styrene can be drawn from this study.]

Table 3-9. Mortality or incidence of selected cancers among all workers in the reinforced-plastics industry

	Washington state (Ruder *et al.*)				United States (Wong *et al. 1994*)				Denmark (Kolstad *et al. 1994, 1995*)				Europe[a] (Kogevinas *et al. 1994a*)				Total Obs/Exp
	SMR	95% CI	Obs	Exp	SMR	95% CI	Obs	Exp	SIR	95% CI	Obs	Exp	SMR	95% CI	Obs	Exp	
esophagus	2.30	1.19–4.02	12	5.2	1.92	1.05–3.22	14	7.3	0.92	0.50–1.57	13	14.2	0.82	0.47–1.31	17/12	20.9/16.0	51/42.7
pancreas	1.43	0.78–2.41	14	9.8	1.13	0.68–1.77	19	16.8	1.20	0.86–1.63	41	34.2	1.00	0.71–1.38	37/21	36.9/26.5	95/87.3
larynx	NR	NR	NR	NR	1.02	0.28–2.61	4	3.9	1.10	0.71–1.63	25	22.6	1.11	0.53–2.05	10/7	9.0/6.2	36/32.7
lung	1.14	0.90–1.43	76	66.7	1.41	1.20–1.64	162	115.2	1.12	0.98–1.26	248	222.4	0.99	0.87–1.13	235/168	237.3/175.0	654/579.3
all LH	0.74	0.42–1.20	16	21.6	0.82	0.56–1.17	31	37.7	1.20	0.98–1.44	112	93.7	0.93	0.71–1.20	60/37	64.4/46.2	196/199.2
lymphoma	0.39	0.01–2.19	1	2.6	0.72	0.20–1.85	4	5.5	1.33	0.96–1.80	42	31.5	0.77	0.43–1.28	15/11	19.4/14.2	58/53.8
Hodgkin's disease	0.61	0.02–3.40	1	1.6	0.90	0.25–2.30	4	4.5	1.08	0.62–1.76	16	14.8	0.90	0.36–1.84	7/6	7.8/5.9	27/26.8
multiple myeloma	NR	NR	NR	NR	NR	NR	NR	NR	0.99	0.51–1.73	12	12.1	0.99	0.48–1.83	10/5	10.1/7.5	17/19.6
leukemia	0.60	0.19–1.40	5	8.3	0.74	0.37–1.33	11	14.8	1.22	0.88–1.65	42	34.4	1.04	0.69–1.50	28/15	27.0/18.6	73/76.1

Note that caveats regarding the pooling of data across cohort studies are discussed in Section 3.8, above.
[a]The number of expected and observed cases after the Danish male workers were excluded in the European study is presented after the slash (/). These numbers were used to pool the total number of observed and expected cases across the four studies to prevent any overlap between the Danish population and the European population.
"≥" is used because for some cancer sites, the pooled number of expected cases was a slight underestimate because the expected number of cases was not given for all studies reporting observed number of cases.

Table 3-10. Mortality or incidence of selected cancers among workers in high-styrene–exposure groups (laminators and others)* in the reinforced-plastics industry

	Washington State[a] (Okun *et al.*, Ruder *et al.*)				United States[b] (Wong, Wong *et al.*)				Denmark[c] (Kolstad *et al.*)				Europe[d] (Kogevinas *et al.*)				Total Obs/Exp
	SMR	95% CI	Obs	Exp	SMR	95% CI	Obs	Exp	SIR	95% CI	Obs	Exp	SMR	95% CI	Obs	Exp	
esophagus	1.85	0.22–6.67	2	1.1	3.57	NR	2	0.6	NR	NR	NR	NR	1.81	0.87–3.34	10	5.5	14/≥ 7.2
pancreas	1.88	0.51–4.81	4	2.1	0.80	NR	1	1.3	2.20	1.1–4.5	17	7.7	1.48	0.76–2.58	12	8.1	34/19.2
larynx	NR	NR	NR	NR	0.0	NR	0	NR	NR	NR	NR	NR	1.55	0.32–4.52	3	1.9	3/≥ 1.9
lung	1.29	0.76–2.04	18	14.0	0.90	NR	8	8.9	1.00	0.7–1.3	72	72.0	1.06	0.81–1.36	60	56.6	158/151.5
all LH	0.72	0.20–1.84	4	5.6	1.41	NR	4	2.8	1.09	0.74–1.55	31	28.4	0.81	0.43–1.39	13	16.0	52/52.8
lymphoma	0.0	NR	0	NR	2.55	NR	1	0.4	0.62	0.23–1.35	6	9.7	1.40	0.56–2.88	7	5.0	14/≥ 15.1
Hodgkin's disease	1.78	0.05–9.89	1	0.6	0.00	NR	0	NR	1.41	0.57–2.91	7	5.0	1.33	0.27–3.88	3	2.3	11/≥ 7.9
multiple myeloma	NR	NR	NR	NR	NR	NR	NR	NR	1.18	0.32–3.02	4	3.4	0.00	0.0–1.55	0	NR	4/≥ 3.4
leukemia	0.47	0.01–2.63	1	2.1	0.90	NR	1	1.1	1.38	0.75–2.32	14	10.1	0.48	0.10–1.39	3	6.3	19/19.6

* Note that in the Kolstad *et al.* studies, high-styrene exposure groups were defined as those who worked in plants where 50% to 100% of the workers were laminators. Note also that caveats regarding the pooling of data across cohort studies are discussed in Section 3.8, above.
LH = lymphohematopoietic.
[a]Workers employed in fibrous glass or lamination departments.
[b]Open-mold process workers for more than two years.
[c]All workers employed in companies with 50% to 100% laminators.
[d]Laminators, excluding the Danish workers included by Kolstad *et al*.

3.9 Summary

Numerous epidemiological studies have evaluated the relationship between styrene and cancer in humans. Most of the studies are cohort studies of workers in three major industries: (1) the reinforced-plastics industry, (2) the styrene-butadiene rubber industry, and (3) the styrene monomer and polymer industry. Two additional cohort studies (one on biomonitored workers, and the second on environmental exposure to styrene-butadiene), several case-control studies, and an ecological study have also been published.

The limitations of these studies include potential misclassification of styrene exposure and disease, small numbers of long-term workers, inadequate follow-up, and the potential for co-exposure to other chemicals. Thus, although more than a hundred thousand workers have been studied to assess a possible carcinogenic effect of styrene exposure, only a small fraction of well-characterized, high-level, and long-term styrene-exposed workers have been followed for a sufficiently long time. In addition, most of the available studies of occupational cohorts have focused only on male workers (who constitute the majority of exposed workers) or have not performed gender-specific risk analyses. [Thus, comparatively few data are available on cancer incidence or mortality among exposed female workers, limiting the ability to evaluate breast cancer or cancers at tissue sites specific for females.]

Workers in the reinforced-plastics industry have the highest levels of exposure and few other potentially carcinogenic exposures, but many of the workers in this industry have short-term exposure, often of less than a year. Cancer mortality or incidence was studied in the following four populations of reinforced-plastics workers: (1) in Washington state in the United States (Ruder *et al.* 2004), (2) in 30 manufacturing plants in unspecified U.S. locations (Wong *et al*. 1994), (3) in Denmark (Kolstad *et al.* 1994), and (4) in Europe (Denmark, Finland, Italy, Norway, United Kingdom, and Sweden) (Kogevinas *et al.* 1994a). (The Danish and the European populations were partly overlapping, as 13,682 Danish male workers were included among the 36,610 male workers making up the European cohort.)

In the styrene-butadiene industry, the cohort studies are among the largest, with the longest follow-up times. The principal methodological challenge is to separate the potentially independent or synergistic effects of butadiene, a known human carcinogen, which is highly correlated with styrene in this industry. Two independent (non-overlapping populations) are available, a small cohort of 6,678 male workers at a rubber tire manufacturing plant (a subset of the workers were engaged in the production of styrene-butadiene and other rubbers) (McMichael *et al.* 1976a) and a larger cohort established by Delzell and colleagues (Delzell *et al.* 1996, 2006) of 13,130 to 16,610 styrene-butadiene rubber industry workers from multiple plants in the United States and Canada. The cohort established by Delzell includes most (but not all) of the workers from two cohorts — a 2-plant cohort (Texas) (Meinhardt *et al.* 1982) and an 8-plant cohort originally established by Matanoski and colleagues (United States and Canada) and reported in a series of previous publications (7 of the 8 plants were included in the Delzell cohort). Thus, there is considerable overlap between these populations. Two nested case-control studies (Matanoski *et al.*1997, Santos-Burgoa *et al.* 1992) of a single group of cases with lymphohematopoietic cancers were available from the Matanoski cohort. The Delzell cohort expanded the previous cohorts to include workers employed from 1943 to January 1, 1991 and followed to 1998, whereas the earlier cohort included workers employed until 1976 and followed until 1982. In addition, the individual study populations were established by different procedures and exclusion criteria (which may partly explain the lack of complete consistency in the number of study subjects across the published studies) and often used different exposure assessments, selection of study subjects, and types of analysis. Two types of analyses were conducted on the Delzell cohort: external analyses reporting on standardized mortality ratios (SMRs) for the total cohort or subsets of the cohorts for multiple cancers sites (Sathiakumar *et al.* 1998, 2005), and, secondly, internal analyses of relative risk (RR) estimates for quantitative exposure to styrene and lymphohematopoietic cancers (Delzell *et al.* 2001, 2006, Macaluso *et al.* 2006, Graff *et al.* 2005). (Dimethyldithiocarbamate [DMDTC] was also included as a potential confounder in some analyses of lymphohematopoietic cancer in the Delzell cohort, according to the authors, because of its potential immunosuppressant activity in CD4+ lymphocytes, although its carcinogenicity has not been evaluated

outside of this series of studies). Workers in the styrene monomer and polymer industry may be exposed to a variety of chemicals, including benzene, toluene, ethylbenzene, and various solvents, and the cohorts are smaller, with many short-term workers, and few cancer outcomes.

The potential effect of styrene on lymphohematopoietic cancers has been studied most extensively. Findings for lymphohematopoietic cancer and other tumor sites of interest are discussed below.

Lymphohematopoietic cancers

Statistically significant increases were observed for all lymphohematopoietic cancers combined and leukemia among rubber-tire manufacturing workers (McMichael *et al.* 1976) and statistically nonsignificant increases were observed for combined lymphohematopoietic cancers and some specific lymphohematopoietic cancers in the Meinhardt and Matanoski cohorts, but the potentially confounding effects of butadiene and other exposures were not analyzed. Two nested case-control studies (using different types of analyses and exposure assessments and the same group of cases) from the Matanoski cohort attempted to evaluate the relative contribution of styrene and butadiene to lymphohematopoietic cancer mortality. Santos-Burgoa *et al.* (1992) found no significant excess risks for combined and specific lymphohematopoietic cancers and mean exposure after controlling for butadiene exposure. Matanoski *et al.* (1997) calculated risks for both average and cumulative exposure to styrene. Taking into account butadiene exposure, and demographic and employment variables in step-down regression analyses, these models found, for an average exposure of 1 ppm vs. no exposure, significant associations for all lymphohematopoietic cancers combined, lymphomas, and myeloma, but not leukemia. For cumulative exposure, significant positive associations between styrene exposure and combined lymphohematopoietic cancers, leukemia, and myeloma were found, with butadiene exposure dropping out of each of the final models except for leukemia.

Specific lymphohematopoietic cancers have been studied more extensively in the Delzell cohort. With respect to leukemia, statistically significant increases have been reported

among subgroups of workers with longer durations of employment and longer latency, with the highest cumulative exposure, and in certain specific job groups (Sathiakumar *et al.* 2005, Delzell *et al.* 2006). Internal analyses by Delzell *et al.* involving single-chemical (styrene only), 2-chemical (styrene and butadiene), and 3-chemical (styrene, butadiene, and DMDTC) models of cumulative exposure have shown increased relative risks of leukemia with increasing cumulative styrene exposure. However, the response was attenuated when controlling for exposure to butadiene and was no longer apparent (RRs were less than or equal to one) after additionally controlling for DMDTC. Elevated risks for leukemia were also observed with increasing exposure to styrene peaks in single-chemical, 2-chemical and 3-chemical models (although it was attenuated somewhat in the 2- and 3-chemical models) (Graff *et al.* 2005, Delzell *et al.* 2006).

No statistically significant increased risks were found for other lymphohematopoietic cancers in all employees of the Delzell cohort, but statistically significant risks of NHL and CLL combined were found among workers with higher exposure in an external (SMR) analysis, and in internal analyses among ever-hourly workers, ever-hourly workers with 10+ years of employment and 20 to 29 years or 30 years since first hire, and among specific job groups. Risks of NHL or NHL and CLL combined appeared to increase with increasing cumulative styrene exposure; the risks increased when butadiene was added to the model, and were somewhat attenuated in models that included DMDTC. Exposure to butadiene did not appear to be related to NHL and CLL combined or NHL risk. [However, it should be noted that no trend analyses were performed on these data.] (Graff *et al.* 2005, Delzell *et al.* 2006). No associations were found for other types of lymphohematopoietic cancers and styrene exposure in the Delzell cohort.

In the reinforced-plastics industry, among the highest-exposure groups, the total number of observed versus expected deaths or cases across the four cohorts were comparable for all lymphohematopoietic (52 observed vs. 52.8 expected), lymphomas (14 vs. 15.1), or leukemia (19 vs. 19.8), and were slightly higher than expected for Hodgkin's disease (11 observed vs. 7.9 expected) and multiple myeloma (4 vs. 3.4). Significantly increased risks for leukemia incidence were reported in the Danish study among workers with earlier first date of exposure, and who had worked at least 10 years since first

employment, but not for workers employed for 1 year or more (Kolstad *et al.* 1994). In the European multi-country cohort (which overlaps with the Danish study), no excess of leukemia mortality was found, and no exposure-response relationships with cumulative or average exposure were observed, although a non-significant trend was observed with time since first exposure (Kogevinas *et al.* 1994a). With respect to other lymphohematopoietic cancers, non-significantly increased risks for non-Hodgkin's lymphoma were found in the Danish and European multi-country cohorts. Positive exposure-response relationships with average styrene exposure and time since first exposure was observed for lymphohematopoietic cancers ($P = 0.019$ and 0.012, respectively) and for malignant lymphoma ($P = 0.052$ and 0. 072, respectively) in the European multi-country cohort, but no relationship with cumulative exposure was observed (Kogevinas *et al.* 1994a). No excesses in mortality from any lymphohematopoietic cancers were observed in the two smaller cohort studies (Ruder *et al.* 2004 and Wong *et al*. 1994). In the styrene monomer and polymer industries, the risk of lymphohematopoietic malignancies was also increased in most of the studies (as well as the total number of observed cases across studies), but these workers might also have been exposed to benzene.

Pancreatic cancer

Among the highest styrene-exposed group in the reinforced-plastics industry, there was an excess in the total number of observed cases of pancreatic cancer across the four cohort studies compared with the total number of expected cases [corresponding to an SMR of 1.77 (95 % CI = 1.23 to 247)]. Increases in pancreatic cancer risk were observed in three of the four reinforced-plastics industry cohorts (one of which was statistically significant [Kolstad *et al.* 1995], and the other two of which were nonsignificant [Kogevinas *et al.* 1994a, Ruder *et al.* 2004]). The risk of pancreatic cancer was slightly higher among the Danish workers with longer term employment and earlier start date, and increased with cumulative exposure in the multi-plant cohort. No indications of exposure-response relationships were found in the smaller U.S. cohorts. Statistically nonsignificant increased risks were also observed in one study in the styrene monomer and polymer industry (Frentzel-Beyme *et al.* 1978), and among biomonitored workers

(10 years after the first measurement) (Anttila *et al.* 1998). However, no increased risk of pancreatic cancer was reported among styrene-butadiene workers (Sathiakumar *et al.* 2005).

Esophageal cancer

Among workers with high potential exposure to styrene, increases in esophageal cancer risk were reported in three of the four cohorts (statistically significant increases in mortality were observed among all exposed workers in the two U.S. studies of reinforced-plastics workers [Ruder *et al.* 2004, and Wong *et al.* 1994] and a statistically nonsignificant increase among a subset of laminators in the European cohort [Kogevinas *et al.* 1994a]). Risks were not elevated among the Danish reinforced-plastics workers (Kolstad *et al.* 1994). Across the industry, an approximately 2-fold excess of esophageal cancer was observed among high-exposed groups (laminators and others). A nonsignificant trend with cumulative exposure was reported in the European multi-country study. No increases in risk were reported among styrene-butadiene rubber workers or among styrene monomer and polymer workers.

Other sites

Findings were less consistent for cancer at other sites. Significantly increased risks were observed for cancers of the lung, larynx, stomach, benign neoplasms, cervix and other female tumors, prostate, rectum, and urinary system in either a single study or two studies. There were some supporting exposure-response data for cancers of the urinary system and rectum. A significant increase in breast cancer mortality was observed in a case-control study of occupational exposures among adult females (Cantor *et al.* 1995), although there was no evidence of increased risk between low- and high-exposure categories. An ecological study reported a significant increase in the risk of invasive breast cancer in the general population, but exposure estimates were based on environmental releases of styrene, which are the least precise measures of exposure.

This Page Intentionally Left Blank

4 Studies of Cancer in Experimental Animals

The carcinogenicity of styrene has been investigated in experimental animals (primarily mice and rats) by several routes of administration, and IARC (1994a, 2002) has evaluated the carcinogenicity of styrene. The 1994 IARC review included four studies in mice (three gavage and one intraperitoneal [i.p.] injection study) and seven studies in rats (three gavage, one drinking water, one inhalation, one i.p., and one subcutaneous (s.c.) injection study) and concluded that there was limited evidence in experimental animals for the carcinogenicity of styrene. IARC (2002) also concluded that there was limited evidence in experimental animals for the carcinogenicity of styrene. The latter review included two inhalation studies (one in mice and one in rats) that were not available for the previous review, and the IARC working group considered the earlier gavage studies in mice as inadequate.

The data and findings from the publicly available, peer-reviewed carcinogenicity studies of styrene in experimental animals are summarized in this section. This includes the studies reviewed by WHO (1983), Huff (1984), Bond (1989), McConnell and Swenberg (1993, 1994), and IARC (1994a, 2002). In addition, information from one unpublished study (Jersey *et al*. (1978), a two-year inhalation study in rats conducted by Dow Chemical), is included based on reviews by WHO (1983), Huff (1984), McConnell and Swenberg (1993, 1994), and Cohen *et al.* (2002)[5].

Section 4.1 presents carcinogenicity data for mice, and Section 4.2 presents data for rats. These sections are organized by route of administration. Section 4.3 includes data from one carcinogenicity study with a mixture containing styrene and β-nitrostyrene, and Section 4.4 briefly reviews carcinogenicity data for styrene-7,8-oxide, the primary metabolite of styrene. All the data are summarized in Section 4.5.

4.1 Mice

Two oral studies (NCI 1979a, Ponomarkov and Tomatis 1978), one inhalation study (Cruzan *et al.* 2001), and one i.p. study (Brunnemann *et al.* 1992) are reviewed below.

[5] The expert panel evaluation conducted by the Harvard Center for Risk Analysis and funded by the Styrene Information and Research Center (SIRC).

The Ponomarkov and Tomatis (1978) study included two strains of mice and included both pre- and postnatal exposure.

4.1.1 Oral

Styrene [one impurity (0.3%) was reported in 1 of the 6 batches purchased for the bioassay, but purity was not specified for the remaining 5 batches] was administered by gavage in corn oil to groups of 50 male and 50 female B6C3F$_1$ mice for 78 weeks (NCI 1979a). The mice were approximately 6 weeks old at the beginning of the study. Test groups received styrene at 150 or 300 mg/kg b.w., 5 days per week, while control groups of 20 male and 20 female mice were exposed to corn oil alone. Mice were held for an additional 13 weeks after the last treatment. There was a slight dose-related body weight depression in female mice. Survival in male mice was 78% (high dose), 92% (low dose), and 100% (controls); survival in female mice was 76% (high dose), 80% (low dose), and 90% (controls). The Tarone test for dose-related mortality was significant in male mice ($P = 0.003$). Therefore, animals that did not survive at least 52 weeks or died before the first appearance of the tumor(s) of interest were not included in the analysis. The Cochran-Armitage exact trend analysis also indicated a significant dose-response relationship for combined alveolar/bronchiolar neoplasms in male mice. This was supported by an increased incidence of alveolar/bronchiolar neoplasms (adenoma and carcinoma combined) in male mice in the high-dose group compared with controls (Table 4-1). Because the incidence of lung tumors in the male vehicle-treated controls (0%) in this study was unusually low compared with historical untreated controls (32 of 271, 12%), there was some uncertainty regarding the significance of the lung tumors. NCI (1979a) reported that the historical incidence of these tumors in vehicle control male mice was 0 of 40 (2 studies from Litton Bionetics, including the styrene study); however, this was considered by NCI to be too small a number of animals for meaningful use as historical controls. [The NTP reviewed (for the purpose of this document) lung tumor incidences in historical vehicle controls from NCI studies conducted at other laboratories. However, although the studies were performed at different laboratories, the historical vehicle control animals were from the same source and same study protocol, and the tests were performed in the same chronological window. The selection criteria included data for corn oil vehicle controls for gavage studies in male B6C3F$_1$ mice conducted prior to

1979 with a similar duration (total of 91 weeks) and from the same source as the styrene study. In addition to the two studies from Litton Bionetics (NTP 1979a, 1979b), there were 12 applicable studies conducted by Hazleton Laboratories (NTP 1976a, 1976b, 1977, 1978a, 1978b, 1978c, 1978d, 1978e, 1978f, 1978g, 1978h, 1978i). The incidence of combined lung tumors in historical vehicle controls from these 14 studies was 11 of 273 (4%). Therefore, the incidence of lung tumors in control male mice in the NCI (1979a) study was not unusually low and support the finding that lung tumors as a result of styrene exposure are statistically significant.] In addition to the lung tumors, hepatocellular carcinomas were reported in male mice but occurred at a higher incidence in controls (20%) than in the treatment groups (6% to 14%). Thus, there were no significant hepatocellular tumor findings in male mice. No other tumors were considered as dose-related. Although there was a significant trend for hepatocellular adenoma (P = 0.03) in female mice, there were no hepatocellular carcinomas in any female mice, and the NCI did not consider this marginal adenoma effect to be related to styrene. NCI (1979a) concluded that there was suggestive evidence for the carcinogenicity of styrene in male $B6C3F_1$ mice, but no convincing evidence was obtained for either sex.

Groups of pregnant O20 (a strain susceptible to lung tumors) and C57Bl mice were administered a single dose of styrene (O20 mice, 1,350 mg/kg; C57Bl mice, 300 mg/kg) [the rationale for choosing these doses was not discussed], dissolved in olive oil, or a single dose of olive oil (vehicle control) by gavage on gestation day 17 (Ponomarkov and Tomatis 1978). The purity of styrene used in this study was > 99%. After weaning, their progeny were administered the same dose of styrene or olive oil once per week. Separate groups received no treatment and served as untreated controls. Styrene treatment of O20 mice was suspended after 16 weeks because of toxicity, while C57Bl mice received weekly treatments until their deaths or 120 weeks. Litter sizes were similar in all groups except in the C57Bl vehicle control group, which had less than one-half the number of animals in the other study groups. Preweaning mortality was higher in the styrene-treated group of O20 mice (43%) compared with the vehicle control group (22%). Mortality remained high in O20 mice after styrene treatment was suspended at 16 weeks; however, body weights were similar in all groups. Mortality was not increased in C57Bl mice treated with styrene.

Table 4-1. Tumor incidences in B6C3F$_1$ mice exposed to styrene by gavage for 78 weeks and surviving for at least 52 weeks

Sex	Dose (mg/kg)	Initial no. mice	Tumor incidence [%]			
			Hepatocellular adenoma	Alveolar/bronchiolar		
				Adenoma	Carcinoma	Combined
Male	0	20	1/20 [5]	0/20 [0]	0/20 [0]	0/20 [0][b]
	150	50	5/47 [10.6]	3/44 [6.8]	3/44 [6.8]	6/44 [13.6]
	300	50	7/43 [16.3]	4/43 [9.3]	5/43 [11.6]	9/43 [20.9]*
	[Trend[a]]		[NS]	[NS]	[P = 0.08]	[P = 0.02]
Female	0	20	0/20 [0]	0/20 [0]	0/20 [0]	0/20 [0]
	150	50	1/44 [2.3]	1/43 [2.3]	0/43 [0]	1/43 [2.3]
	300	50	5/43 [11.6[	3/43 [7.0]	0/43 [0]	3/43 [7.0]
	[Trend[a]]		[P = 0.03]	[NS]	[NS]	[NS]

Source: NCI 1979a.
NS = not significant.
* $P < 0.05$ (compared with concurrent controls, one-tailed Fisher's exact test).
[a][Cochran-Armitage exact test for positive dose-response trend performed by NTP].
[b] Incidences in untreated historical controls were 32/271 or 12% [reported by NCI 1979a] and in vehicle controls were 11/273 or 4% [calculated by NTP for the present report].

Tumor incidences are shown in Tables 4-2a for O20 mice and 4-2b for C57Bl mice. There was a statistically significant ($P < 0.01$) increased incidence of total lung tumors in both male and female O20 mice treated with styrene compared with the vehicle control groups. [When compared with the untreated groups, the difference was statistically significant ($P < 0.001$) only for the females.] Lung tumors were reported to occur at an earlier age in the styrene-treated progeny than in control progeny, [but this may be the result of higher mortality in the styrene-treated mice rather than an effect of styrene. Information necessary to interpret the significance of this observation (whether the lung tumors were incidental or fatal) was not reported.] The authors noted that this study had severe limitations because of the severe toxicity and early mortality in O20 mice but concluded that there was weak evidence for the carcinogenicity of styrene in O20 mice when administered at a high dose level.

The predominant tumors occurring in C57Bl mice included lymphoma and lung or liver tumors (Table 4-2b). The incidences of these tumors in styrene-treated mice (dams or progeny) were not significantly higher than controls. While the authors reported that the higher incidence of liver tumors in styrene-treated male mice (3 carcinomas; 12.5%) was

cause for some concern, one adenoma was also observed in a vehicle control male (8.3%) and one adenoma was observed in an untreated control male (2.1%).

Table 4-2a. Lung tumor incidences in O20 mice exposed to styrene *in utero* and weekly by gavage for 16 weeks after weaning

Treatment	Group	Initial no. mice	Lung tumor incidence (%)[a]		
			Adenoma	Carcinoma	Total
Untreated	Male	54	22/53 (41.5)	12/53 (22.6)	34/53 (64.2)
	Female	47	11/47 (23.4)	14/47 (29.8)	25/47 (53.2)
Vehicle control	Dams[b]	9	1/8 (12.5)	4/8 (50)	5/8 (62.5)
	Progeny				
	Male	20	4/19 (21.1)	4/19 (21.1)	8/19 (42.1)
	Female	22	10/21 (47.6)	4/21 (19.0)	14/21 (66.7)
Styrene (1,350 mg/kg)	Dams[b]	29	4/20 (20)	7/20 (35)	11/20 (55)
	Progeny				
	Male	45	12/23 (52.2)	8/23 (34.8)	20/23 (87)**
	Female	39	14/32 (43.8)	18/32 (56.3)	32/32 (100)**[c]

Source: Ponomarkov and Tomatis 1978.
** $P < 0.01$ compared with vehicle control-treated group significance levels reported only for total tumors; statistical test not reported].
[a] Based on the number of animals surviving until the time the first tumor was observed.
[b] On gestational day 17, dams received a single dose by gavage of 1,350 mg/kg; after weaning, progeny received weekly doses of 1,350 mg/kg.
[c] $P < 0.001$ when female progeny of styrene-treated dams compared with untreated females, and the authors reported a non-significant difference for males [statistical method not reported]; [NTP calculated $P = 0.037$ by Fisher's exact test for male progeny of styrene-treated dams compared with untreated males.]

Table 4-2b. Tumor incidences in C57Bl mice exposed to styrene *in utero* and weekly by gavage for 120 weeks after weaning

Treatment	Group	Initial no. mice	Tumor incidence (%)[a]			
			Lymphoma	Lung	Liver[b]	Other
Untreated	Male	51	13/47 (27.7)	5/47 (10.6)	1/47 (2.1)	3/47 (6.4)[c]
	Female	49	20/47 (42.5)	1/47 (2.1)	0/47 (0)	4/47 (8.5)[d]
Vehicle control	Dams[e]	5	3/5 (60.0)	0/5 (0)	0/5 (0)	2/5 (40.0)[f]
	Progeny					
	Male	12	3/12 (25.0)	3/12 (25.0)	1/12 (8.3)	2/12 (16.7)[g]
	Female	13	5/13 (38.4)	1/13 (7.7)	0/13 (0)	1/13 (7.7)[h]
Styrene (300 mg/kg)	Dams[e]	15	10/12 (83.3)	1/12 (8.3)	0/12 (0)	3/12 (25.0)[i]
	Progeny					
	Male	27	9/24 (37.5)	1/24 (4.2)	3/24 (12.5)	1/24 (4.2)[j]
	Female	27	13/24 (54.2)	1/24 (4.2)	0/24 (0)	4/24 (16.7)[k]

Source: Ponomarkov and Tomatis 1978.
[a] Based on the number of animals surviving until the time the first tumor was observed.
[b] Liver tumors in males in the 2 control groups were adenomas; and in the styrene-treated group were carcinomas.
[c] Forestomach papilloma, duodenum polyp, kidney adenocarcinoma.
[d] Uterine sarcoma (2), lacrimal gland adenoma, ovary theca-cell tumor.

[e] On gestational day 17, dams received a single dose by gavage of 300 mg/kg; after weaning, progeny received weekly doses of 300 mg/kg.
[f] Pituitary adenoma.
[g] Hemangioendothelioma of the leg, hemangioma (s.c.).
[h] Hemangioma (s.c.).
[i] Jaw osteosarcoma, ovary granulosa-cell tumor, pituitary adenoma.
[j] Urinary bladder papilloma.
[k] Uterine sarcoma (2), adenoma of the lacrimal gland, ovary theca-cell tumor.

4.1.2 Inhalation

Cruzan *et al.* (2001) exposed groups of 70 male and 70 female CD-1 mice to styrene vapor (whole-body exposure) at concentrations of 0, 20, 40, 80, or 160 ppm, 6 hours/day, 5 days/week for 98 (females) or 104 weeks (males). The purity of styrene used in this study was 99.5% to 99.8%. The mice were 4 weeks old when received. Interim sacrifices of 10 animals per sex per group were conducted after 52 and 78 weeks. Styrene exposure did not affect survival in male mice, and apart from two deaths in the 160-ppm group during the first 2 weeks, survival was slightly increased in styrene-exposed female mice. Body weight gains were significantly less in the high-dose groups of both sexes during the first 13 weeks of the study. At the end of the study, males (80 and 160 ppm) and females (160 ppm) gained significantly less weight than controls.

There was an increased incidence of benign lung tumors (alveolar/bronchiolar adenomas) at several exposure levels in both sexes and malignant lung tumors (alveolar/bronchiolar carcinomas) in high-exposure female mice at the end of the study (Table 4-3). Incidences of adenomas and carcinomas combined were not evaluated by the study authors, but Cohen *et al.* (2002) reported that the combined tumor incidences were significantly higher than controls at exposures of 40, 80, and 160 ppm (male mice) and 20, 40, and 160 ppm (female mice). [These results for combined tumor incidences were confirmed by NTP by Fisher's exact test for pairwise comparisons and Cochran-Armitage exact trend test (see Table 4-3).] Cruzan *et al.* (2001) also reported that there was a significant positive trend for benign lung tumors in both sexes and for benign plus malignant tumors in female mice. The incidence of lung tumors was not increased in styrene-exposed mice sacrificed at 52 or 78 weeks.

Table 4-3. Lung tumor incidence in CD-1 mice exposed to styrene by inhalation for 98 or 104 weeks[b]

Sex	Exposure conc (ppm)	Alveolar/bronchiolar tumor incidence[a] [%]		
		Adenoma	Carcinoma	Combined
Male	0	15/50 [30]	4/50 [8]	17/50 [34]
	20	21/50 [42]	5/50 [10]	24/50 [48]
	40	35/50 [70]**** [c]	3/50 [6]	36/50 [72]***
	80	30/50 [60]** [c]	6/50 [12]	30/50 [60]**
	160	33/50 [66]*** [c]	7/50 [14]	36/50 [72]***
	[Trend]	[$P < 0.001$]	[NS]	[$P < 0.001$]
Female	0	6/50 [12]	0/50 [0]	6/50 [12]
	20	16/50 [32]* [c]	0/50 [0]	16/50 [32]*
	40	16/50 [32]* [c]	2/50 [4]	17/50 [34]**
	80	11/50 [22]	0/50 [0]	11/50 [22]
	160	24/50 [48]**** [c]	7/50 [14]** [c]	27/50 [54]****
	[Trend]	[$P < 0.001$]	[$P < 0.001$]	[$P < 0.0001$]

Source: Cruzan *et al.* 2001; Cohen *et al.* 2002.
NS = not significant.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, [Fisher's exact test for pairwise comparisons and the Cochran-Armitage exact trend test conducted by NTP.]
[a](Number of mice with tumor) / (number of animals examined for each tissue type).
[b]Due to high mortality, females in this study were terminated early at 98 weeks; males were exposed until planned study termination at 104 weeks.
[c] Reported by Cruzan *et al.* 2001 as $P < 0.05$.
.

4.1.3 Intraperitoneal injection

In a screening study, Brunnemann *et al.* (1992) exposed 25 female A/J mice (a strain susceptible to lung tumors) to a total dose of 200 μmol [~100 mg/kg b.w.] styrene (> 99% purity) given by i.p. injection three times per week for 20 doses. This study also included a positive control group exposed to 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK). The mice were 6 to 8 weeks old at the beginning of the study. Test animals were held for 20 weeks after the last injection and examined for lung tumors. Three mice exposed to styrene developed lung adenoma compared with one in the control group. The difference was not significant, and the authors concluded that styrene was not tumorigenic under the conditions of this bioassay. [The short duration of the study, single sex, and small group size limit this study as a test for carcinogenic activity.]

4.2 Rats

The carcinogenicity of styrene in rats has been investigated following oral administration (Beliles *et al.* 1985, Conti *et al.* 1988, Maltoni *et al.* 1982, NCI 1979a, Ponomarkov and Tomatis 1978), inhalation (Conti *et al.* 1988, Cruzan *et al.* 1998, Jersey *et al.* 1978), and i.p. and s.c. injection (Conti *et al.* 1988). These studies are reviewed in the following sections.

4.2.1 Oral

Styrene was administered by gavage in three studies and in the drinking water in one study. These studies are reviewed briefly below and the results are summarized in Table 4-5.

Ponomarkov and Tomatis (1978) investigated the carcinogenic effects of prenatal and postnatal exposure to styrene (purity 99%). On the 17th day of gestation, 21 pregnant BD IV rats received a single oral dose of 1,350 mg/kg styrene dissolved in olive oil. After weaning, the offspring (73 males and 71 females) were given weekly doses of 500 mg/kg by stomach tube throughout their lifespan (all survivors were killed at 120 weeks) [Only one treatment group was used and dosing was only once per week.] The control groups were similarly treated with olive oil. Litter sizes were not affected by styrene treatment, and no differences in survival or body weights were noted. The incidences of tumors in the styrene-treated rats were not significantly higher than those of controls. The authors

reported that stomach tumors observed in one styrene-treated dam and two styrene-treated female progeny were of "some concern" because they were "rarely seen in controls." While the histologic types of these stomach tumors were not specified in the table, they were described in the text as an adenoma, a fibrosarcoma, and a carcinosarcoma without specific attribution to a particular dose group. A stomach fibrosarcoma was observed in one of the vehicle control female progeny. [The low incidence of stomach tumors and inadequate reporting of tumor types limits concluding that these tumors are associated with treatment.]

NCI (1979a) treated groups of 50 male and female F344 rats with 500, 1,000, or 2,000 mg/kg styrene (purity described in Section 4.1.1) in corn oil by gavage 5 days per week for 78 weeks (mid- and high-dose groups). The rats were 6 weeks old at the beginning of the study. Surviving animals were sacrificed after 104 or 105 weeks. Only 6 of 50 male rats in the high-dose group survived past week 53, and only 7 of 50 female rats survived past week 70. Because of poor survival, the high-dose groups were not included in the statistical analysis of tumors. Due to excessive mortality in the high- and medium-dose groups, additional groups of male and female rats were placed on test in week 23. These dosed rats were intubated with styrene at a level of 500 mg/kg for 103 weeks, followed by a 1-week observation period, when no test chemicals were used. Separate vehicle controls were also started for this group. Survival of low- (44/50) and medium-dose rats (47/50) at week 90 was considered adequate by the study authors. No increased tumor incidences were observed in any of the treatment groups.

Conti *et al*. (1988) investigated the long-term carcinogenicity of styrene (purity 99.8%) in Sprague-Dawley rats. A previous publication from this study focused only on brain tumors (Maltoni *et al.* 1982), while the complete results were reported by Conti *et al.* Groups of 40 male and female rats (13 weeks old at the start of the experiment) were exposed by stomach tube to 50 or 250 mg/kg styrene, 4 or 5 days per week for 52 weeks and held until death [less than lifetime exposure duration, low doses]. The control group received olive oil. Females in the high-dose group had a higher mortality rate compared with controls. Body weight was not affected by styrene treatment; [however, there was limited reporting of results]. No increased tumor incidences were reported.

Sprague-Dawley rats (7 weeks old at the start of the study) were exposed to styrene (purity ≥ 98.9%) in their drinking water for two years (Beliles *et al.* 1985). [This study was identified as the Chemical Manufacturers Association study by Huff (1984) before it was published.] Nominal concentrations were 125 or 250 ppm. The authors noted that the calculated daily doses in this study (7.7 to 14 mg/kg in males and 12 to 20.5 mg/kg in females) were at least an order of magnitude lower than doses used in other chronic oral toxicity studies with styrene, such as the NCI study above (500, 1,000, or 2,000 mg/kg) [solubility of styrene in water limited the dosage]. Chronic toxicity and reproductive performance were evaluated. The test groups included 50 male and 70 female rats, while the control group consisted of 76 males and 106 females. This study also evaluated the effects of styrene on reproductive function through three generations (see Section 5.4.2 for reproductive toxicity). The only treatment-related effect identified was a decrease in water consumption. There was no effect on mortality. The authors reported that all tumors observed were either common, spontaneously occurring tumors of Sprague-Dawley rats or were uncommon tumors that affected only individual rats in the treatment groups and concluded that styrene administered in drinking water did not produce deleterious dose-related effects in rats. Tumors were identified only by tissue (number of tissues examined and total number of tumors) [actual tumor rates not reported]. However, Huff *et al.* (1984) reexamined these data and reported specific mammary tumor incidences for fibroadenoma, adenoma, adenocarcinoma, and combined mammary tumors (Table 4-4). The authors reported marginal increase in combined mammary gland tumors (fibroadenoma, adenoma, and adenocarcinoma) in female rats. Incidences were 49 of 96 (51%) in controls, 18 of 30 (60%) in the low-dose group and 40 of 60 (66.7%) in the high-dose group. Huff reported that there was a significant dose-related trend (P = 0.032), and the incidence in the high-dose group was significantly higher than the control group (P = 0.039, Fisher's exact test).

Table 4-4. Mammary gland tumor incidence in Sprague-Dawley rats exposed to styrene in drinking water for 104 weeks

Exposure ppm (mg/kg)	Mammary Gland Tumor Incidence (%)			
	Fibroadenoma	Adenoma	Adenocarcinoma	Combined
0	45/96 (49)	1/96 (1)	8/96 (8)	49/96 (51)
125 (12)	15/30 (50)	0/30 (0)	5/30 (17)	18/30 (60)
250 (21)	37/60 (62)*	0/60 (0)	8/60 (13)	40/60 (67)
[Trend]	[*P* = 0.046]	[NS]	[NS]	[–[a]]

Source: Huff *et al.* 1984.
NS = not significant.
**P* = 0.05 [Fisher's exact test for pairwise comparison and Cochran-Armitage exact trend test *P* values calculated by NTP.]
[a] [Statistics not reported by NTP for benign and malignant tumors combined because of lack of information on the histogenesis of the tumors.]

Table 4-5. Summary of carcinogenicity studies in rats exposed to styrene by oral administration

Reference	Strain	Dose (mg/kg)[a]	No. rats[b]	Duration (wk)		Results/comments
				Exposure	Study	
Ponomarkov and Tomatis 1978	BD IV	0 500[c]	36–39 71–73	120	120	No treatment-related increases of any type of tumor [*Limitations*: Only one treatment group, dosing schedule only once per week]
NCI 1979a	F344	0 500 1000 2000	40 50 50 50	104–105 103 78 78	104–105 104 105 105	No treatment-related increases of any type of tumor [*Limitations:* High mortality in high-dose group (both sexes) resulted in an inadequate number of animals for statistical analyses]
Maltoni *et al.* 1982, Conti *et al.* 1988	Sprague-Dawley	0 50 250	40 40 40	52	Held until death	No increase in tumors [*Limitations:* High mortality rate in high-dose females, limited reporting, less than lifetime exposure duration]
Beliles *et al.* 1985	Sprague-Dawley	<u>Males</u> 0 7.7[d] 14[d] <u>Females</u> 0 12[d] 20.5[d]	 76 50 50 106 70 70	 104 104	 104 104	No treatment-related increases of any type of tumor reported by study authors. [*Limitations*: Low doses]

[a] Administered by gavage 4 or 5 days per week unless otherwise noted.
[b] Includes numbers for each sex unless otherwise noted.
[c] Dams received a single dose of 1,350 mg/kg on gestation day 17. After weaning, progeny received weekly doses of 500 mg/kg for life or until study termination.
[d] Administered in drinking water (125 or 250 ppm); conversion to mg/kg as reported by Beliles *et al.* (1985).

4.2.2 Inhalation

Conti *et al.* (1988) exposed groups of 30 male and 30 female Sprague-Dawley rats to styrene vapors (purity 99.8%) in stainless steel inhalation chambers at concentrations of 25, 50, 100, 200, or 300 ppm for 4 hours/day, 5 days/week for 52 weeks and held the animals until death. The animals were 13 weeks old at the beginning of the study. The control groups included 60 rats of each sex. There were no significant differences in body weight or mortality between exposed and control groups. A higher incidence, but not statistically significant, of total malignant tumors that was not due to an increase in any specific tumor was observed in male (8 of 30, 26.7%) and female (13 of 30, 43.3%) rats exposed to 100 ppm compared with controls (10 of 60, 16.7% in males and 16 of 60, 26.7% in females). Total malignant tumors were not increased at the two highest dose levels. The incidence of malignant mammary tumors was higher in female rats (all exposed groups) compared with controls (Table 4-6). [Therefore, it appears that the reported incidence of malignant mammary tumors was too high, or the incidence of total malignant tumors was too low.] The authors concluded that the increased incidence of malignant mammary tumors in female rats was “treatment-related and statistically significant” and that this study demonstrated a weak carcinogenic effect for styrene. IARC (1994a) considered this study to be inconclusive because of incomplete reporting and the high incidence of spontaneous mammary tumors.

Table 4-6. Incidence of mammary tumors in Sprague-Dawley rats exposed to styrene by inhalation for 52 weeks

Sex	Exposure conc. (ppm)	Initial no. rats	Mammary tumor incidence[a] (%)	
			Benign + malignant[b]	Malignant[c]
Male	0	60	8/60 (13.3)	1/60 (1.7)
	25	30	6/30 (20.0)	1/30 (3.3)
	50	30	3/30 (10.0)	1/30 (3.3)[d]
	100	30	6/30 (20.0)	0/30 (0)
	200	30	4/30 (13.3)	1/30 (3.3)
	300	30	5/30 (16.7)	0/30 (0)
	[Trend]		[NR[b]]	[NS]
Female	0	60	34/60 (56.7)	6/60 (10.0)
	25	30	24/30 (80.0)	6/30 (20.0)
	50	30	21/30 (70.0)	4/30 (13.3)
	100	30	23/30 (76.7)	9/30 (30.0)*
	200	30	24/30 (80.0)	12/30 (40.0)[e]***
	300	30	25/30 (83.3)	9/30 (30.0)*
	[Trend]		[NR[b]]	[P = 0.002]

Source: Conti *et al.* 1988.
* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. [Table provides significance values calculated by NTP: Fisher's exact test for pairwise comparison and Cochran-Armitage exact test for trend.]
conc. = concentration, NR = not reported, NS = not significant.
[a] (number mice with tumor) / (number of animals examined for each tissue type)
[b] Authors noted higher incidence in all exposed groups of females compared with controls, but increases were not reported to be statistically significant and specific tumor types were not reported. [Statistics not reported for benign plus malignant tumors because of lack of information on the histogenesis of the tumors.]
[c] Authors reported to be treatment-related and statistically significant for females; however, no specific dosed group(s) was identified.
[d] Reported as 3% by Conti *et al.*
[e] [Reported incidence may be in error because it exceeds the incidence reported for total malignant tumors of 10 of 30 (33.3%). When the Cochran-Armitage exact test was recalculated with 10/30 as the tumor incidence for the 200-ppm group of females, the P value was 0.004. Trend tests were performed by NTP.]

An unpublished study (Jersey *et al.* 1978) was reviewed by WHO (1983), Huff (1984) [note that Huff referenced the paper as Dow 1978], ATSDR (1992), McConnell and Swenberg (1993, 1994), and Cohen *et al.* (2002) and is included here based on information from these secondary sources. [However, without the original data provided in the unpublished laboratory report data essential to the interpretation of this study are missing.] Groups of 96 or 97 male and 96 female Sprague-Dawley rats (7 to 8 weeks of age) were exposed to 600- or 1,200-ppm styrene (purity 99.5%) for 6 hours/day, 5 days/week. After 2 months, the concentration for the high-dose group was reduced to 1,000 ppm because of excessive toxicity. Interim sacrifices of 5 or 6 animals of each sex were conducted after 6 and 12 months. Styrene exposure was stopped after 18.3 months in males and 20.7 months in females because mortality had reached 50%. Animals were

observed until their deaths, or 24 months. Survival was lower in males than in females (attributed to a high incidence of chronic murine pneumonia in males). At 24 months, the number of surviving animals was as follows: control group (5 males and 30 females), 600-ppm group (18 males and 30 females), 1,000-ppm group (6 males and 22 females) (Cohen *et al.* 2002). Although the incidence of mammary adenocarcinoma in females in the 600-ppm group was 8.2% and was significantly higher than in controls, the authors concluded that there was no causal association with styrene exposure because mammary adenocarcinoma did not occur in the high-dose group, the incidence of mammary adenocarcinoma in the control group (1.2%) was low compared with historical controls (mean of 5.8%), and the range among historical controls (0% to 9%) contained the rate observed in the treatment group. Incidences of mammary fibroadenoma showed no evidence of a styrene effect (WHO 1983). Combined incidences of lymphosarcoma and leukemia in female rats were 1.2% in controls and 7.1% in both exposed groups; incidences in males were 1.2% in controls, 5.8% in the 600-ppm group, and 1.2% in the 1,000-ppm group (Table 4-7). Incidences of lymphosarcoma and leukemia were not statistically significant compared with the concurrent controls but were significant when compared with historical controls. [The combined incidence of leukemia and lymphosarcoma in historical controls was not provided; however, these tumors are not typically combined in carcinogenicity studies.] Huff (1984) mentioned that the authors concluded that the data were "suggestive of an association between the exposure of these female rats to styrene vapor and an increased incidence of tumors of the leukemia-lymphosarcoma type. In males, the results are even more inconclusive but tend to support the suggestive association found in the females." McConnell and Swenberg (1994) noted that "this study was seriously flawed by the presence of chronic murine pneumonia, which caused a high rate of mortality in both control and exposed male rats; it was less a factor in females."

Table 4-7. Mammary tumors and leukemia or lymphosarcoma in Sprague-Dawley rats exposed to styrene by inhalation for 18 to ~21 months

Sex	Exposure conc (ppm)	Initial no. rats[a]	Treatment duration (mo)	Tumor incidence (%)	
				Mammary adenocarcinoma	Leukemia or lymphosarcoma
Male	0	96	24	0/85 (0)	1/85 (1.2)
	600	97	18.3	0/86 (0)	5/86 (5.8)
	1,000	96	18.3	0/84 (0)	1/84 (1.2)
	[Trend] [e]			[NS]	[NS]
Female	0	96	24	1/85 (1.2)[c]	1/85 (1.2)
	600	96	20.7	7/85 (8.2)*	6/85 (7.1) [d]
	1,000[b]	96	20.7	0/85 (0)	6/85 (7.1) [d]
	[Trend] [e]			[*P* = 0.002]	[*P* = 0.035]

Source: unpublished study by Jersey *et al.* 1978, cited in Huff 1984; WHO 1983, McConnell and Swenberg 1993, 1994, Cohen *et al.* 2002.
* Significantly different from the control, *P* value and statistical method not reported. [*P* = 0.032 by Fisher's exact test calculated by NTP.]
conc. = concentration, NS = not significant.
[a] Interim sacrifice of 5 animals of each sex at 6 months and 6 animals of each sex at 12 months.
[b] Initial concentration was 1,200 ppm for the first 2 months then decreased to 1,000 ppm because of toxicity.
[c] Incidence in historical controls was 5.8%.
[d] Reported to be significant when compared with historical controls (1.36% [11/808]; range = 0%–2.64%), but historical control data were not provided in a published report.
[e][Cochran-Armitage exact trend test conducted by NTP.]

Cruzan *et al*. (1998) exposed groups of 70 male and 70 female Sprague-Dawley rats to styrene vapor (purity 99.5% to 99.7%) at 0, 50, 200, 500, or 1,000 ppm 6 hours/day, 5 days/week for 104 weeks in inhalation chambers. Surviving animals were killed during weeks 105 to 107. Styrene exposure did not affect survival in males, but survival in females in the 500- and 1,000-ppm groups was higher than in the control group. Eight males in the 1,000-ppm group and 6 males in the 500-ppm group were not included in the mortality or tumor analysis because they died or were taken off study after an accidental massive dermal exposure to styrene during week 61. Body-weight gain was lower in males in the 500- and 1,000-ppm groups and in females in the 200-, 500-, and 1,000-ppm groups. A complete histological examination was conducted for the control and high-exposure groups. The histologic examination for the lower-exposure groups was limited to the target organs (nasal passages, lungs, liver, kidney, testes, and epididymides), gross lesions, and all masses. Styrene exposure did not affect hematology, clinical chemistry, urinalysis, or organ weights. No treatment-related effects were reported in animals necropsied at week 52. The authors reported that there was no evidence that styrene exposure caused significant increases of any tumor type in males or females. Treated

female rats had decreases in pituitary adenomas and mammary adenocarcinomas compared with controls (Table 4-8). Incidences of mammary tumors were based on the total population rather than the number examined because these tumors are rarely found microscopically when not seen macroscopically (Cruzan *et al.* 1998). There was a positive trend for testicular tumors, but none of the pairwise comparisons was significant, and tumor incidences were within the historical control range (0% to 13.5%). Therefore, the differences were judged to be incidental and not treatment related.

Table 4-8. Tumor incidences in Sprague-Dawley rats exposed to styrene by inhalation for 104 weeks

Sex	Exposure conc. (ppm)	Tumor incidence (%)		
		Testes (interstitial cell)	Pituitary gland (adenoma)	Mammary gland (adenocarcinoma)
Male	0	2/60 (3.3)[a]	31/60 (51.7)	0/60 (0)
	50	2/60 (3.3)	17/60 (28.3)	0/60 (0)
	200	2/60 (3.3)	28/60 (46.7)	0/60 (0)
	500	4/54 (7.4)	24/54 (44.4)	1/54 (1.9)
	1,000	6/52 (11.5)	20/52 (38.5)	0/52 (0)
	[Trend][b]	[*P* = 0.015]	[NS]	[NS]
Female	0	–	45/60 (75)	20/60 (33.3)
	50	–	42/60 (70)	13/60 (21.7)
	200	–	35/60 (58.3)	9/60 (15)
	500	–	29/60 (48.3)	5/60 (8.3)
	1,000	–	31/60 (51.7)	2/59 (3.4
	[Trend]		[*P* = 0.002N]	[*P* = < 0.0001N]

Source: Cruzan *et al.* 1998.
conc. = concentration.
[a]Historical control range = 0 to 13.5%
[b][Cochran-Armitage exact trend test conducted by NTP. NS = not significant. A negative trend in an exposure group is indicated by N.]

4.2.3 Parenteral administration

Conti *et al.* (1988) exposed groups of Sprague-Dawley rats to styrene by either i.p. or s.c. injection. Groups of 40 male and 40 female rats were given four i.p. injections containing 50 mg styrene in olive oil at 2-month intervals. Controls were given i.p. injections of olive oil. Other groups of 40 male and 40 female rats received a single s.c. injection of 50 mg of styrene in olive oil. Animals were 13 weeks old at the beginning of the study and were held until death. No treatment-related neoplasms were reported. [The studies were markedly limited by the low and infrequent doses, short duration of styrene exposure, and incomplete reporting.]

4.3 Mixtures containing styrene

NCI (1979b) also conducted carcinogenicity studies of a mixture containing 30% β-nitrostyrene and 70% styrene in B6C3F$_1$ mice and F344 rats. Exposed groups included 50 animals of each sex, while control groups included 20 animals of each sex. Mice were administered solutions in corn oil containing 87.5 or 175 mg/kg β-nitrostyrene [204 or 408 mg/kg styrene] by gavage 3 days/week for 78 weeks followed by a 14-week observation period. Male rats were administered 150 or 300 mg/kg β-nitrostyrene [350 or 700 mg/kg styrene], and female rats were administered 75 or 150 mg/kg β-nitrostyrene [175 or 350 mg/kg styrene] 3 days/week for 79 weeks followed by a 29-week observation period. Control groups were gavaged with corn oil on the same schedule as the treatment groups. All animals were approximately 6 weeks old at the beginning of the study. The authors concluded that a sufficient number of animals survived to the end of the study in all groups. Survival was not significantly affected by exposure in rats (both sexes) or female mice. The probability of survival was dose-related in male mice (90% in controls, 86% in the low-dose group, and 66% in the high-dose group). Body weights were depressed in high-dose male rats and female mice. Male mice in the low-dose group had a significantly ($P = 0.016$) increased incidence (11 of 49; 22.4%) of alveolar/bronchiolar adenoma or carcinoma compared with controls (0 of 20) (Table 4-9). The incidence in the high-dose group was 2 of 36 (5.5%) and was not significant by pairwise comparison. No other neoplasms in mice or rats were associated with exposure to the styrene mixture. [However, because of poor survival of the high-dose male mice there were substantially fewer animals at risk for late-occurring lung tumors.] The NCI concluded that "under the conditions of this bioassay, there was no convincing evidence that a mixture of β-nitrostyrene and styrene was carcinogenic in B6C3F$_1$ mice or F344 rats."

Table 4-9. Tumor incidences in B6C3F$_1$ mice exposed to a mixture of β-nitrostyrene and styrene for 79 weeks

Sex	Styrene dose (mg/kg)	Initial no. mice	Alveolar/bronchiolar tumor incidence (%)		
			Adenoma	Carcinoma	Combined
Male	0	20	0/20 [a] (0)	0/20 (0)	0/20 (0)
	175	50	8/49 (16.3)	3/49 (6.1)	11/49 (22.4)*
	350	50	1/36 (2.8)	1/36 (2.8)	2/36 (5.5)
	[Trend][b]		[NS]	[NS]	[NS]
Female	0	20	0/19 (0)	0/19 (0)	0/19 (0)
	175	50	1/49 (2.0)	1/49 (2.0)	2/49 (4.1)
	350	50	0/46 (0)	0/46 (0)	0/46 (0)
	[Trend]		[NS]	[NS]	[NS]

Source: NCI 1979b.
NS = not significant.
[a] (Number mice with tumor) / (number of animals examined for each tissue type).
[b] Cochran-Armitage exact trend test conducted by NTP. *NS*, non-significant.
* $P < 0.05$ (compared with controls, Fisher's exact test).

4.4 Styrene metabolites

Styrene-7,8-oxide is a primary metabolite of styrene (see Section 1.3) and is listed in the Report on Carcinogens as *reasonably anticipated to be a human carcinogen* based on sufficient evidence in experimental animals (see NTP (2004) for detailed information on the carcinogenicity of styrene oxide). IARC (1994b) also reviewed this compound and concluded that there was "*sufficient evidence* in experimental animals for the carcinogenicity of styrene-7,8-oxide." Styrene oxide induced high incidences of both benign and malignant tumors of the forestomach in both sexes of rats (three strains tested) and in one strain of mice (IARC 1994b) (see Table 4-10). In addition, Lijinsky (1986) reported liver tumors in male mice.

Table 4-10. Summary of neoplastic lesions in mice and rats exposed to styrene-7,8-oxide by gavage

Studies	Design: dose, duration and initial group size	Comments on study	Results- male	Results- female
B6C3F1 mice Lijinsky 1986, as reviewed in IARC 1994b	0, 375, or 750 mg/kg (in corn oil) 3 days/wk, 104 wk, (97% purity) 52/sex/group	Study termination 3–4 wk after last dose	Significant increase in hepatocellular neoplasms at low dose; significant increase in forestomach tumors at both doses	Significant increase in forestomach tumors at both doses
F344 rats Lijinsky 1986, as reviewed in IARC 1994b	0, 275 or 550 mg/kg (in corn oil) 3 days/wk, 104 wk, (97% purity) 52/sex/group	Study termination 3–4 wk after last dose	Significant increase in forestomach tumors at both doses	Significant increase in forestomach tumors at both doses
Sprague-Dawley rats Conti *et al.* 1988, Maltoni *et al.* 1979, as reviewed in IARC 1994b	0, 50, 250 mg/kg (in olive oil) 4–5 d/wk, 52 wks (purity not specified) 40/sex/group	Rats held after dosing until death	Significant dose-dependent increase in forestomach tumors	Significant dose-dependent increase in forestomach tumors
BD IV inbred rats Ponomarkov *et al.* 1984, as reviewed in IARC 1994b	200 mg/kg (in olive oil) 14 dams dosed on prenatal day 17; progeny dosed once per wk at 100–150 mg/kg, 96 wk starting at 4 wk of age (99% purity) 62 females and 43 males	Dams of control progeny were not dosed; control progeny of 55 females and 49 males dosed with vehicle; study terminated at 120 wk	Significant increase in forestomach tumors	Significant increase in forestomach tumors

4.5 Summary

The carcinogenicity of styrene has been investigated in rats and mice by several routes of exposure and the results are summarized in Tables 4-11 and 4-12. [Many of the studies were severely limited in their ability to detect carcinogenic effects because of inadequate study design (low doses, short treatment or short study duration, small group size) or intercurrent disease and high mortality (e.g., pneumonia), or the studies were inconclusive because of limited reporting (tumor diagnosis, statistical methodology).]

In mice, gavage studies in both sexes for three strains, an inhalation study in both sexes of one strain, and one i.p. study in females were found in the literature and reviewed. [The oral gavage studies in B6C3F$_1$ mice (NCI 1979a) and the inhalation studies in CD-1 mice (Cruzan *et al.* 2001) were the most informative of the carcinogenicity studies.]

Male B6C3F$_1$ mice had a statistically significant dose-response trend for alveolar/bronchiolar adenoma and carcinoma (combined) that was supported by a significantly increased incidence of these lung tumors in the high-dose group. The authors questioned the significance of these lung tumors because the incidence in the control group was unusually low compared with historical untreated controls. However, the concurrent vehicle controls were within the range of historical vehicle controls from the same source, same study protocol, and same chronological window. Further, the tumor incidence in the high-dose group was significantly increased compared with these historical vehicle controls. A dose-related trend in female B6C3F$_1$ mice was also observed for hepatocellular adenoma. Significantly increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) were also observed in male and female CD-1 mice exposed to styrene by inhalation. In each sex, three treatment groups (males, 40, 80, 160 ppm; females, 20, 40, 160 ppm) showed increases in these tumors. The high-dose (160-ppm) female mice had an increased incidence of alveolar/bronchiolar carcinoma. A significant trend in hepatocellular adenoma in female mice was also reported, but the pairwise comparison between treated and control animals was not significant.

In a short-term oral gavage study in O20 mice, a strain with a high spontaneous incidence of lung tumors, significantly higher incidences of lung tumors (adenoma and carcinoma combined) were observed in both males and females compared with vehicle controls.

In rats, gavage studies in three strains, and three inhalation studies in one strain, and a drinking-water study in one strain were reviewed. The oral gavage studies in F344 rats (NCI 1979a) and the inhalation studies in Sprague-Dawley rats (Cruzan *et al.* 1998) were the most informative of the carcinogenicity studies. Neither study showed an increase in tumor incidences in styrene-treated rats, although Sprague-Dawley rats exhibited a dose-

related reduction in pituitary and mammary gland tumors. A significant trend in interstitial testicular tumors was found in rats, but the pairwise comparison between treated and control animals was not significant. In the inhalation study reported by Conti *et al.* (1988), there was a dose-related increase in the incidences of malignant mammary gland tumors; treatment-related and statistically significant incidences of these tumors were seen in the top three dose groups. The drinking-water study in Sprague-Dawley rats did not report any dose-related carcinogenic effects; however, statistical reanalyses of study data indicated a marginal increase in mammary fibroadenoma in high-dose female rats and a significant dose-related trend. For the unpublished inhalation study by Jersey *et al.* (1978), a statistically significant increase in mammary adenocarcinoma in the low-dose, but not high-dose group was reported in several reviews of this study. [There was an inconsistent association of mammary-gland tumors and styrene treatment across these studies]. Elevated leukemia/lymphosarcoma were observed in both treatment-related groups of female Sprague-Dawley rats in one inhalation study (Jersey *et al.* 1978). [However the study was limited by lack of information on whether the leukemia was lymphocytic in nature.]

No increase in alveolar/bronchiolar tumor incidence was observed in female rats exposed to a mixture of 70% styrene and 30% β-nitrostyrene. An increase in lung tumors (low-dose group only) was observed in male mice exposed to this styrene/β-nitrostyrene mixture. [Substantial mortality in the high-dose group could have precluded the observation of late-occurring tumors, such as the lung, in many animals.]

Uncertain findings include hepatocellular adenomas in female mice (NCI 1979a) and interstitial testicular tumors in rats (Cruzan *et al.* 1998), both of which were statistically significant by trend but not by pairwise comparison between treated and control animals.

Table 4-11. Summary of studies in mice

Studies	Design: dose, duration and initial group size	Limitations of study	Results	
			Males	Females
Oral				
B6C3F$_1$ mice NCI 1979a	150 or 300 mg/kg (in corn oil by gavage), 5 days/wk by gavage, 78 wk Controls – 20/sex Treated – 50/dose level/sex	Limited control group size	Significant increase and dose-related trend in lung adenoma and carcinoma combined	Dose-related increase in hepatocellular adenoma
O20 mice Ponomarkov and Tomatis 1978	1,350 mg/kg (in olive oil by gavage) once on prenatal day 17 & weekly postweaning for 16 wk. Controls – 20 males, 22 females Dosed – 45 males, 39 females	High mortality in treated animals; only one treatment group; short dosing duration; small control groups	Significant increase in lung tumors	Significant increase in lung tumors
C57Bl mice Ponomarkov and Tomatis 1978	300 mg/kg (in olive oil by gavage) once prenatal day 17 and weekly postweaning until death. Controls – 12 males, 13 females Dosed – 27 males, 27 females	Only one treatment group; low dose; limited reporting; small group size, particularly controls	No significant increase in tumors	No significant increases in tumors
Inhalation				
CD-1 mice Cruzan *et al.* 2001	20, 40, 80 or 160 ppm, 6 h/d, 5 d/wk, 98–104 wk 50 animals/group/sex	No major limitations	Significant increase and dose-related trend in lung adenoma and combined adenoma and carcinoma	Significant increase and dose-related trend in lung adenoma, carcinoma, and combined adenoma and carcinoma
Intraperitoneal				
Female A/J mice Brunnemann *et al.* 1992	Total 100 mg/kg in divided doses, 3/wk, held for 20 wk after last injection 25 animals/group	Only one treatment group, limited reporting, small group size	NA	No significant increase in lung tumors

NA = not applicable.

Table 4-12. Summary of studies in rats[a]

Studies	Design: dose, duration and initial group size	Limitations of study	Results male	Results female
Oral				
F344 rats NCI 1979a	500, 1000, or 2000 mg/kg (in corn oil, by gavage), 5 d/wk, 78 wk (mid- & high dose) or 103 wk (low dose) Controls – 20/sex Treated – 50/ dose group/sex	Poor survival of high dose groups, small control group	No significant increase in tumors	No significant increase in tumors
BD IV rat Ponomarkov and Tomatis 1978	1,350 mg/kg (in olive oil by gavage) once prenatal day 17 & 500 mg/kg weekly postweaning for lifespan Controls – 39 females/36 males Dosed – 71 females/73 males	Limited dosage regimen, once/wk dosing, limited reporting	No significant increase in tumors	No significant increase in tumors
Sprague-Dawley rats Conti *et al.* 1988	50 or 250 mg/kg, 4–5 d/wk (in olive oil by gavage) for 52 wk and held until death 40/dose group/sex	Mortality in high-dose females, short treatment duration, low doses, limited reporting	No significant increase in tumors	No significant increase in tumors
Sprague-Dawley rats Beliles *et al.* 1985	125 or 250 ppm in drinking water (7.7–14 mg/kg/d in males (50/group) and 12–20.5 mg/kg/d in females (70/group) for 104 wk; controls 104 females and 76 males	Low doses, limited reporting	No significant increase in tumors	Small increase in mammary fibroadenoma
Inhalation				
Sprague-Dawley rats Conti *et al*. 1988	25, 50, 100, 200 or 300 ppm (99.8% purity), 4 h/d, 5 d/wk for 52 wk and held until death Controls – 60/sex Treated – 30/sex/dosegroup	Limited dosing regimen, limited reporting	No significant increase in tumors	malignant mammary tumors increased in all groups, with significant trend
Sprague-Dawley rats	600 or 1,200/1,000 ppm, 6 h/d, 5 d/wk, for 18.3 mo (males) or 20.7	Original report and data not available in	No significant increase in tumors	Small increase in leukemia/lympho-sarcoma, with a

Studies	Design: dose, duration and initial group size	Limitations of study	Results male	Results female
Jersey *et al.* 1978, as cited cited in Huff 1984, WHO 1983, McConnell and Swenberg 1993, 1994, Cohen *et al.* 2002.	mo (females) 96/sex/treatment group	published literature; limited reporting in reviews, high incidence of pneumonia		significant trend Increase in malignant mammary tumors in low-dose group
Sprague-Dawley rats Cruzan *et al.* 1998	0, 50, 200, 500 or 1000 ppm, 6 h/d, 5 d/wk for 104 wk 70/sex/group	No major limitations	Positive dose-related trend in benign interstitial testicular tumors (incidence within historical control range 0-13.5%)	Pituitary and malignant mammary tumors decreased in all dose groups (negative trend)

[a]The parenteral administration (i.p. or s.c.) study by Conti is not presented here because of the limitations of low and infrequent doses, short duration of exposure, and incomplete reporting.

This Page Intentionally Left Blank

5 Other Relevant Data

This section discusses relevant mechanistic data and other information needed to understand the toxicity and potential carcinogenicity of styrene. It includes information for styrene on absorption, distribution, metabolism, and excretion (Section 5.1), toxicity (Section 5.2), interspecies differences in metabolism, toxicity, and toxicokinetics (Section 5.3), genetic and related effects (Section 5.4), and mechanistic studies and considerations (Section 5.5). A summary is provided in Section 5.6.

5.1 Absorption, distribution, metabolism, and excretion

This section describes absorption (5.1.1), distribution (5.1.2), metabolism (5.1.3), and excretion (5.1.4) of styrene in humans and experimental animals.

5.1.1 Absorption

Studies in humans and experimental animals show that styrene is absorbed following inhalation, ingestion, or skin contact. Human data are presented in Section 5.1.1.1 and experimental animal data are presented in Section 5.1.1.2.

5.1.1.1 Humans

Styrene may be absorbed following inhalation, ingestion, or skin contact; however, the predominant route in occupational settings is inhalation (ATSDR 1992, IARC 1994a, 2002). Food is also an important source of exposure for the general population (see Section 2.3.4 and 2.4). In humans, approximately 60% to 70% of inhaled styrene is absorbed. No data were identified regarding oral absorption of styrene in humans, but several studies were available that evaluated dermal absorption. Dutkiewicz and Tyras (1968) reported that the rates of absorption of liquid styrene through the skin of the hand and forearm of a man were 9 to 15 mg/cm^2/h. When applied as an aqueous solution at concentrations of 66.5 to 269 mg/L, the rates of absorption were 0.040 to 0.18 mg/cm^2/h. Dermal absorption of residual styrene monomer from polystyrene-containing personal care products was demonstrated using *in vitro* diffusion-cell techniques (Kraeling and Bronaugh 2005). When ^{14}C-styrene (4.1 μg/cm^2) was applied to human skin as an oil-in-water emulsion that simulated cosmetic products, only 1.3% of the applied styrene was absorbed (1.2% absorbed into the receptor fluid and 0.1% remaining in the skin after 24

hours). Although absorption was low, it was rapid with peak absorption occurring at about 6 hours. The total recovery of styrene in this study was only 1.5%. The low recovery was attributed to volatilization of styrene from the emulsion. The dermal absorption rate of styrene in human volunteers who dipped one hand into liquid styrene for 10 to 30 minutes was low (about 1 $\mu g/cm^2/min$) (Berode *et al.* 1985). In another study, dermal absorption was determined by measuring styrene and styrene metabolites in blood, exhaled air, and urine in 10 volunteers who were exposed to styrene vapors (with respiratory protection) at 600 ppm for 3.5 hours (Riihimäki and Pfäffli 1978). Dermal absorption of styrene vapors was estimated to be about 0.1% to 2% of the estimated exposure from inhalation. In a similar experiment, Wieczorek (1985) measured styrene metabolites in the urine of four volunteers exposed to styrene vapor at 1,300 to 3,200 mg/m^3 [300 to 740 ppm] for 2 hours and estimated that dermal absorption was about 5% of the amount absorbed via the respiratory tract. Limasset *et al.* (1999) compared urinary excretion of styrene metabolites in four groups of workers in the fiberglass-reinforced polyester (reinforced plastics, see Section 2.5.1) industry. The groups performed the same task at the same time and place but wore different types of protective equipment (total body protection, skin protection only, respiratory protection only, or no protection). There was no significant difference in urinary excretion of styrene metabolites in the group with total protection compared with the group using respiratory protection only. The authors concluded that percutaneous absorption of styrene vapor did not make an important contribution to the body burden of styrene-exposed workers. However, Luderer *et al.* (2005) estimated that in situations of prolonged and repeated contact with liquid styrene, dermal uptake could be equivalent to inhalation exposure at the lower range of occupational styrene concentrations (1 to 2 ppm).

5.1.1.2 Experimental animals

Styrene was absorbed in laboratory rodents exposed to styrene vapors or by oral administration, intraperitoneal injection, and skin application (ATSDR 1992, IARC 1994a, 2002). Inhalation studies in rats at concentrations ranging from 50 to 2,000 ppm for 5 hours or 80 to 1,200 ppm for 6 hours indicated rapid uptake with styrene concentrations in blood reaching maximal values at the end of the exposure period. In one study, a 15-fold increase in exposure concentration (80 to 1,200 ppm for 6 hours)

resulted in a 63-fold increase in blood levels and indicated saturation of styrene metabolism at high concentrations. Morris (2000) examined the uptake of styrene in surgically isolated upper respiratory tracts of Sprague-Dawley rats and CD-1 mice. The average uptake efficiency in rats ranged from 24% with exposure to styrene at 5 ppm to about 9% or 10% with exposure at 100 or 200 ppm. The steady-state uptake decreased with increasing concentration. In mice exposed to the same range of styrene concentrations, the average uptake efficiency ranged from 42% (5 ppm) to 10% (200 ppm); however, uptake efficiency did not maintain a steady state, but declined steadily during exposure. Pretreatment with the cytochrome P450 (CYP450) inhibitor metyrapone significantly reduced uptake efficiency in both rats and mice and abolished the concentration dependence. The loss of concentration dependence and the observation that metyrapone pretreatment also caused uptake efficiency to achieve steady state in mice led the author to conclude that both the concentration dependence and the non-steady–state behavior in mice likely were due to styrene metabolism.

In a study of dermal absorption of styrene vapor in male F344 rats, the maximum blood concentration of about 10 μg/mL was achieved after 4 hours of exposure to 3,000 ppm (McDougal *et al.* 1990). The skin permeability constant was 1.75 cm/h. When exposure was by both inhalation and skin absorption, styrene uptake via skin exposure was estimated to be 9.4% of the total absorbed. In another study of dermal absorption in F344 rats, the peak blood concentration was 5.3 μg/mL when 2 mL of neat [undiluted] styrene was administered in a sealed dermal cell; absorption was less when the styrene was diluted with water (Morgan *et al.* 1991). Sandell *et al.* (1978) exposed adult male Wistar rats to cutaneous doses (0.5 or 3.0 g/kg) of styrene daily for 7 consecutive days. They reported that rat skin was easily penetrated by styrene as evidenced by changes in detoxifying enzyme activity in the liver but not in the lung.

5.1.2 Distribution

This section discusses distribution of styrene and its metabolites in humans (5.1.2.1) and rodents (5.1.2.2). Absorbed styrene is widely distributed from the blood to other body tissues (ATSDR 1992).

5.1.2.1 Humans

The observation that partition coefficients for styrene between body tissues and air are high (4,100 for fat, 84 to 154 for other organs, and 59 for blood) led to the suggestion that styrene would accumulate in subcutaneous fat (IARC 1994a, 2002). However, in a study of styrene-exposed workers, urinary excretion of mandelic acid and phenylglyoxylic acid did not increase during a work week, leading the authors to conclude that styrene did not accumulate (Pekari *et al.* 1993). IARC (2002) noted that pharmacokinetic analysis of the disposition of styrene does not indicate that styrene accumulates in subcutaneous fat. Ramsey *et al.* (1980) exposed four healthy volunteers to 80-ppm styrene for 6 hours and concluded that styrene would not accumulate in the human body. The estimated half-life of styrene in subcutaneous fat in humans is between 2 and 5 days (ATSDR 1992, IARC 1994a).

5.1.2.2 Experimental animals

In a study of tissue distribution of styrene and its metabolites in mice exposed to styrene via i.p. injection, the highest concentrations of unchanged styrene were detected in fat, pancreas, liver, and brain. However, polar metabolites were detected in the liver, kidneys, lungs, and plasma only (Löf *et al.* 1983). In rats orally exposed to styrene, Plotnick and Weigel (1979) found the highest concentrations of styrene in the kidney, liver, and pancreas.

In one study reviewed by IARC (1994a), the concentration of styrene in the blood of male Wistar rats exposed to styrene vapor at 50 to 2,000 ppm, or injected with styrene intravenously (i.v.) at doses of 1.3 to 9.4 mg/kg b.w., indicated saturation of metabolic elimination at higher concentrations. The apparent volume of distribution, however, was not dependent on exposure level and was approximately 10 times the blood volume of the animals, indicating that styrene distributed extensively to the tissues. The concentration of styrene in perirenal fat was about 10 times the concentration seen in any other organ. The reported biological half-life of styrene in rats [strain not specified] was 6.3 hours, and the half-lives in blood, liver, kidney, spleen, muscle, and brain were between 2.0 hours and 2.4 hours.

Boogaard *et al.* (2000a) exposed rats and mice to [ring-U-^{14}C]styrene by nose-only inhalation and performed quantitative whole-body autoradiography on sections taken from one rat and two mice. Radioactivity was detectable in over 40 different tissues, but its concentration in most tissues was lower than in blood. Tissues where the concentration was higher than in blood included the liver and kidney cortex in both rats and mice, with higher levels in mice for both tissues. Radioactivity levels were clearly higher in the lungs than in the blood of mice, but were higher in the blood than in the lungs of rats. The radioactivity in the lungs of mice was located in discrete regions that the authors equated with the bronchi. In both species, the nasal mucosa contained higher levels of radioactivity than the blood (> 3 times as much in rats and 2 to 13 times as much in mice), with most of it residing in the olfactory mucosa rather than the respiratory mucosa. The authors also noted that their measurements of radioactivity in fat indicated that styrene was stored in fat during exposure, but was released rapidly from fat after the exposure period ended. The high levels of radioactivity in the kidney also were transient and were most likely related to clearance of radiolabeled styrene through the kidney.

5.1.3 Metabolism

This section describes the metabolic pathways for styrene in humans (5.1.3.1) and experimental animals (5.1.3.2), differences in styrene metabolism among tissue and cell types (5.1.3.3), metabolic enzyme activity in human lung in general (5.1.3.4), the roles of specific metabolic enzymes in biotransformation of styrene (5.1.3.5), and detoxification of styrene metabolites (5.1.3.6).

5.1.3.1 Humans

The primary and secondary metabolic pathways for styrene in humans are shown in Figure 5-1. The available data indicate that styrene metabolism becomes saturated at air concentrations greater than 200 ppm in humans, rats, and mice (ATSDR 1992).

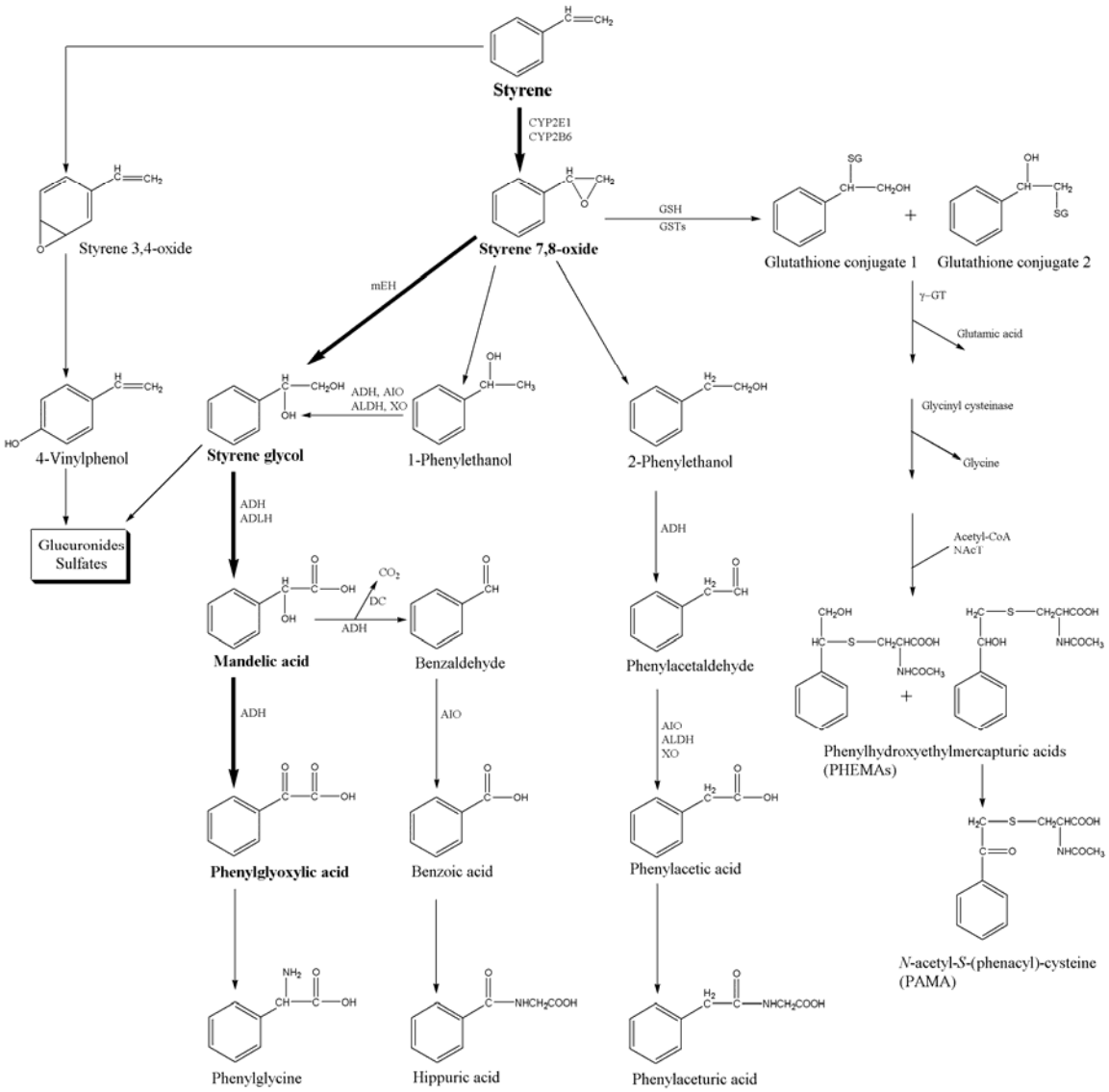


Figure 5-1. Styrene metabolism in humans

Source: Manini *et al.* 2002b.

Bold arrows show the main pathway. PHEMAs are four diastereoisomers: (*R,R*)- and (*S,R*)-*N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)-L-cysteine and (*R,R*)- and (*S,R*)-*N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)-L-cysteine.

Abbreviations: CYP2E1 and CYP2B6 = cytochrome P450 monooxygenase, mEH = microsomal epoxide hydrolase, ADH = alcohol dehydrogenase, AlO = aldehyde oxidase, ALDH = aldehyde dehydrogenase, XO = xanthine oxidase, DC = decarboxylase, GSH = glutathione, GSTs = glutathione S-transferases, γ-GT = gammaglutamyl transpeptidase, NAcT = N-acetyltransferase.

The main route of styrene metabolism in humans produces the terminal metabolites mandelic and phenylglyoxylic acids by way of the intermediate styrene-7,8-oxide, which is subsequently hydrolyzed to styrene glycol (phenylethylene glycol) (IARC 1994a, Sumner and Fennell 1994). Styrene-7,8-oxide contains a chiral carbon and can exist as

either the *R*- or *S*-enantiomer. More than 90% of the styrene retained in humans is initially activated to genotoxic styrene-7,8-oxide, with subsequent conversion to detoxification products. Carlson *et al.* (2000) detected the metabolism of styrene to styrene-7,8-oxide in 6 of 6 human liver microsomal preparations and 1 of 6 lung microsomal preparations collected from 12 individuals during surgical procedures or at autopsy. Liver microsomes showed a much higher metabolic activity than lung microsomes.

Korn *et al.* (1994) reported a linear correlation between the concentrations of styrene-7,8-oxide in the blood of workers and the concentration of styrene in air. The steady-state level of styrene-7,8-oxide was about 1 μg/L at styrene concentrations of 20 ppm. Johanson *et al.* (2000) exposed 4 healthy male volunteers to 50-ppm styrene for 2 hours during light physical activity. Maximum concentrations of styrene-7,8-oxide in blood ranged from 2.5 to 12.2 nM and were observed in the first samples collected shortly after exposure had stopped. No styrene-7,8-oxide was detected in blood samples collected 23.5 hours after exposure. Minor styrene metabolites identified in humans include mercapturic acid derivatives of styrene-7,8-oxide (Maestri *et al.* 1997) (which arise from glutathione conjugation of styrene-7,8-oxide), 4-vinylphenol (4-hydroxystyrene) (Pfäffli *et al.* 1981), 1-phenylethanol (Korn *et al.* 1985), 2-phenylethanol (Korn *et al.* 1985), and glucuronic acid and sulfur conjugates of hydroxylated styrene metabolites (Manini *et al.* 2002b). Formation of 4-vinylphenol indirectly indicates intermediate formation of the 3,4-arene oxide; formation of 2-vinylphenol (not shown in Figure 5-1), indirectly indicates intermediate formation of styrene-2,3-oxide. Urinary 4-vinylphenol sulfates and glucuronates have been identified in volunteers and occupationally exposed workers, and this metabolic pathway was shown to account for approximately 0.5% to 1% of the total excretion of styrene metabolites (Manini *et al.* 2003).

5.1.3.2 Experimental animals

Metabolism of styrene in various animal species has been reviewed by IARC (1994a, 2002) and by Sumner and Fennel (1994). As in humans, the first step in metabolism is usually the epoxidation of styrene to styrene-7,8-oxide in a NADPH-dependent reaction catalyzed by CYP enzymes (Figure 5-1). Styrene-7,8-oxide is further metabolized to

styrene glycol by epoxide hydrolase or conjugated with glutathione to form mercapturic acid metabolites. 1-Phenylethanol and 2-phenylethanol also have been identified as urinary metabolites in rats. The liver has the highest activity for formation of styrene-7,8-oxide and its subsequent conversion to styrene glycol. These metabolic steps also occur in lung and kidney, but not in heart, spleen, or brain. This preferential metabolism of styrene in the liver was found consistently in all species examined (male and female Sprague-Dawley rats, CD-1 mice, New Zealand rabbits, and Dunkin-Hartley guinea-pigs).

A second metabolic pathway results in formation of 4-vinylphenol, which is produced in very small amounts in rats (0.1% of styrene dose) (Bakke and Scheline 1970) and humans (Manini *et al.* 2003); this pathway is postulated to involve styrene-3,4-oxide as an intermediate (Pantarotto *et al.* 1978). No 4-vinylphenol was detected in *in vitro* experiments with mouse and rat lung and liver microsomal preparations incubated with styrene, but the authors suggested that rapid metabolism of 4-vinylphenol might explain their failure to detect the metabolite (Carlson *et al.* 2001). When the metabolism of 4-vinylphenol was tested in the same system, the metabolic rate was 3 times as high in mouse liver microsomes as in rat liver microsomes and 8 times as high in mouse lung microsomes as in rat lung microsomes. Boogaard *et al.* (2000a) reported that the percentage of $^{14}CO_2$ derived from ring-labeled styrene was 3 to 4 times as high in mice as in rats and suggested that this might indicate formation of reactive ring-opened metabolites in mouse lung, which would likely involve ring oxidation, as postulated for the formation of 4-vinylphenol. Differences in styrene metabolites formed by ring oxidation have been proposed as a possible explanation for the interspecies differences in susceptibility to lung tumors in experimental animals (see Sections 5.1.3.5, 5.2.2.2, and 5.5.3).

5.1.3.3 Tissue type, lung cell types, and metabolism

Green *et al.* (2001b) examined metabolism of styrene to styrene-7,8-oxide and detoxification of styrene-7,8-oxide *in vitro* by nasal epithelium of mice, rats, and humans. The rates of styrene metabolism to styrene-7,8-oxide were higher in rat and mouse nasal tissues, both olfactory and respiratory, than in liver. No metabolism of styrene to styrene-

7,8-oxide was detected in 9 samples of human nasal epithelium (8 S9 fractions and 1 microsomal fraction) at a limit of detection of 0.04 nmol/min per mg of protein.

A significant proportion of the oxidative metabolizing capacity of the rodent lung occurs in Clara cells in mice and type II cells in rats (Pinkerton *et al.* 1997). Clara cells are the target cells for styrene-induced pneumotoxicity (Cruzan *et al.* 1997). Hynes *et al.* (1999) investigated the roles of Clara cells and type II cells in styrene metabolism in rats and mice. Enriched Clara-cell and type II alveolar-cell fractions were obtained from lungs of male CD-1 mice and male Sprague-Dawley rats. The mouse and rat cell preparations metabolized styrene to *R*- and *S*-styrene-7,8-oxides; however, the *R/S* ratio was higher in mice than in rats. The metabolizing activity of mouse Clara cells was several-fold higher than that of rat Clara cells (Table 5-1). Metabolism was higher in fractions enriched for Clara cells compared with fractions enriched for type II cells. When the activities for the two fractions were solved as simultaneous equations (considering the percentage enrichment of each fraction), practically all the metabolizing activity was attributed to Clara cells.

Table 5-1. Production of *R*- and *S*-enantiomers of styrene-7,8-oxide by cell preparations enriched in either Clara cells or type II cells from rat and mouse lungs[a]

% Clara cells	% Type II cells	Production (pmol/10^6 cells per min)[b]		*R/S* ratio
		R-enantiomer	*S*-enantiomer	
Male CD-1 mouse (4 experiments)				
18.3 ± 3.5	33.5 ± 4.9	19.4 ± 4.1	6.9 ± 2.2	3.62 ± 1.09
55.8 ±8.0	6.5 ± 2.5	83.3 ± 27.7	23.0 ± 8.2	3.98 ± 0.75
Male Sprague-Dawley rat (3 experiments)				
12.8 ± 3.2	42.3 ± 4.1	3.7 ± 1.1	8.0 ± 2.6	0.47 ± 0.01
37.3 ± 9.0	4.0 ± 1.0	11.2 ± 3.6	11.0 ± 3.2	1.02 ± 0.09

Source: Hynes *et al.* 1999.
[a]All values are mean ± SE.
[b]Calculated on the basis of total number of nucleated cells.

Boogaard *et al.* (2000b) compared DNA adduct formation in rat and mouse liver and lung, and in fractions enriched in type II and Clara cells isolated from rat and mouse lung (see Section 5.4.3.1). DNA adduct profiles in liver and lung tissue were similar, but the adduct levels were significantly lower in lung. However, DNA adduct profiles in mice

and rats showed both quantitative and qualitative differences. These differences suggest that different reactive metabolites are formed in rats and mice. Clara cells are the predominant cell type in mouse lung, while type II cells predominate in rat lung.

Human lung also contains Clara cells (primarily in the bronchiolar epithelium) but the morphology is different from that seen in rodents. Pinkerton *et al.* (1997) reported that the most striking difference was the low proportion of agranular endoplasmic reticulum in human nonciliated bronchiolar epithelial cells (3.1% of the cellular components) compared with 55% in the mouse and 66% in the rat. Human terminal airways do not have significant numbers of Clara cells; however, the contribution of Clara cells to the proliferation compartment of normal human tracheobronchial epithelium is substantial, demonstrating a role of the Clara cell in the maintenance of the normal epithelium of the distal conducting airways in humans. This concept was demonstrated in the study by Boers *et al.* (1999). These authors evaluated the number of Clara cells from normal tissue taken from seven lungs obtained by autopsy. The number of Clara cells in the terminal and respiratory bronchioles were 11 ± 3% and 22 ± 5%, respectively. The proximal airway epithelium (bronchi and bronchioles) was virtually devoid of Clara cells. The overall proliferation compartment of the conducting airway epithelium was 0.83 ± 0.47%; the contribution of Clara cells was 9%. In the terminal bronchioles 15% of proliferating airway epithelial cells were Clara cells, and in the respiratory bronchioles this percentage increased to 44%.

5.1.3.4 Metabolic enzyme activity in human lung

The ability of lung cells to metabolize styrene to potentially tumorigenic molecules could be an important mechanistic factor in explaining the differences in the formation of lung tumors in experimental animals, particularly the development of lung tumors in mice but not in rats exposed to styrene. To understand the relevance of these findings to humans, it is important to examine the potential for human lung cells to metabolize styrene to the molecules identified as potential tumorigenic intermediates in animal studies, and that metabolism will depend on the expression cytochrome P450 isozymes. This section discusses that expression.

Human lung has been reported to contain either mRNA or protein for the following P450 isozymes: CYP1A1, CYP1A2, CYP2A6, CYP2A13, CYP1B1, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2F1, CYP2J2, CYP2S1, CYP3A4, CYP3A5, CYP4B1, CYP5A1, CYP7B1, CYP8A1, CYP27, and CYP51 (Ding and Kaminsky 2003, Hukkanen *et al.* 2002, Karlgren *et al.* 2005, Nishimura *et al.* 2003, Pelkonen and Raunio 1997, Seliskar and Rozman 2007, Somers *et al.* 2007, Zhang *et al.* 2006). Although levels of most P450 enzymes are reported to be lower in lung compared with liver (Somers *et al.* 2007), CYP2A13, CYP2F1, CYP2S1, CYP3A5, and CYP4B1 are preferentially expressed in the lung (Ding and Kaminsky 2003, Thum *et al.* 2006).

Xenobiotic metabolism in human lung occurs primarily in bronchial epithelial cells, Clara cells, type II pneumocytes, and alveolar macrophages; while in rodents and rabbits, metabolism is highest in Clara cells and type II pneumocytes. The CYP2F1 isoform of cytochrome P450 is the human homolog of the Cyp2f2 isoform expressed in mouse lung (see below) and the CYP2F4 isoform expressed in rat lung (Baldwin *et al.* 2005). The presence of a cDNA for CYP2F1 in a human lung library was first reported by Nhamburo *et al.* (1990). The mRNA for CYP2F1 has been shown by RT-PCR amplification to be present in human lung tissue and broncho-alveolar macrophages (Raunio *et al.* 1999) and in human bronchial biopsy and trachea and lung tissue (Bieche *et al.* 2007, Thum *et al.* 2006).

Sheets *et al.* (2004) reported that A549 human alveolar epithelial type II (adenocarcinoma) lung cells were capable of metabolizing benzene, and the activity decreased significantly (51%; $P < 0.05$) in the presence of 5-phenyl-1-pentyne (5P1P), a P450 inhibitor. 5-Phenyl-1-pentyne is an effective inactivator of CYP2E1 as well as CYP2F2 and CYP2F1 (Roberts *et al.* 1998, Simmonds *et al.* 2004). The authors concluded that CYP2F1 was important in benzene metabolism in this human lung cell line. BEAS-2B cells overexpressing CYP2F1 also were reported to have a significant ($P < 0.05$) increase in cytotoxicity resulting from bioactivation of 3-methylindole to 3-methyleneindolenine (Nichols *et al.* 2003).

5.1.3.5 Metabolic enzyme studies

Most styrene metabolism is mediated enzymatically, but nonenzymatic epoxidation of styrene has been demonstrated in human erythrocytes *in vitro* (Tursi *et al.* 1983). These experiments showed a linear relationship between styrene oxidation and the molar fraction of oxyhemoglobin, indicating that oxyhemoglobin rather than free oxygen radicals are involved in the reaction. The enzymatic metabolism of styrene, and the contributions of various cytochromes P450 in animal tissues have been studied through the use of chemical inhibitors and antibodies to specific cytochromes (IARC 2002), and *Cyp2E1* knockout mice (Carlson 2003, 2004a). These studies show that there are tissue differences in the enzymes responsible for styrene oxidation. A large number of human liver and lung CYP isoenzymes are capable of oxidizing styrene at the 7,8-position, but the most important appear to be CYP1A2, CYP2B6, CYP2E1, CYP2F1, CYP2C8, and CYP3A4. The enzymes involved in the formation and detoxification of styrene metabolites in humans and experimental animals are discussed in this section.

The biotransformation of styrene may be affected by inducers or inhibitors of xenobiotic metabolism. Metabolism of styrene *in vivo* in female Wistar rats and *in vitro* in liver microsome preparations from male Wistar rats was increased by administration of sodium pentobarbital (IARC 1994a). Co-exposure to acetone increased urinary styrene metabolites in male Han/Wistar rats, and i.p. injection of toluene suppressed styrene metabolism in Wistar rats. In a perfused rat liver system, co-administration of ethanol decreased the uptake and metabolism of styrene.

Nakajima *et al*. (1994a) compared the rate of formation of styrene glycol from styrene in human, rat, and mouse liver microsomes, and in human lung microsomes. At low styrene concentrations (0.085 mM), the rate was highest in mouse liver microsomes (2.43 ± 0.29 nmol/(mg of protein·min)) and lowest in humans (0.73 ± 0.45 nmol/(mg of protein·min)); however, at a higher styrene concentrations (1.85 mM), the rate was highest in the rat (4.21 ± 0.72 nmol/(mg of protein·min)) but remained lowest in humans (1.91 ± 0.84 nmol/(mg of protein·min)). The rate of styrene glycol formation in human lung was less than 1% of that in human liver. The specific P450 forms responsible for the metabolic activity were determined by analyzing cDNA-expressed individual P450 forms produced

in cultured hepatoma G2 cells by recombinant vaccinia viruses. Of the 12 human P450 forms studied, 9 were able to catalyze styrene oxidation. All the P450 forms studied, except CYP2F1 and CYP4B1, are expressed in the liver. CYP2B6 (119.6 nmol/(dish·2h)) and CYP2E1 (63.4 nmol/(dish·2h)) were the most active forms in human liver microsomes, while CYP2F1 (103.9 nmol/(dish·2h)) was most active in human lung microsomes. CYP1A2 (63.2 nmol/(dish·2h)) and CYP2C8 (47.6 nmol/(dish·2h)) showed intermediate activity. Less active forms included CYP3A3, CYP3A4, CYP3A5, and CYP4B1 (~12 to 24 nmol/(dish·2h)), while little detectable activity was reported for CYP2A6, CYP2C9, and CYP2D6. Mouse Cyp1a2 (85.0 nmol/(dish·2h)) was more active than mouse Cyp1a2 (17.2 nmol/(dish·2h)). Mouse Cyp1a2 and human CYP1A2 are orthologous counterparts, but the human form had about 3.5-fold greater activity than the mouse form. Rat CYP2B1 was the most active P450 investigated (198.8 nmol/(dish·2h)) and had more than twice the catalytic activity of rat CYP2B2 even though they have similar amino acid sequence homology.

Fukami *et al.* (2008) reported that CYP2A13, a human cytochrome P450 expressed predominantly in the respiratory tract, had the highest catalytic activity for the formation of styrene-7,8-oxide from styrene when compared with CYP2A6 and CYP2E1. These enzymes have overlapping substrate selectivity. The *CL*int values calculated from the initial slope of velocity plotted against the substrate concentration values were 46.8 for 2A13, 17.2 for 2A6, and 18.5 for 2E1.

The effects of CYP-specific inhibitors on formation of *R*- and *S*-enantiomers of styrene-7,8-oxide were investigated by Hynes *et al.* (1999) through the use of enriched Clara cell and type II alveolar cell fractions from lungs of male CD-1 mice and male Sprague-Dawley rats. Cyp2e1 and Cyp2f2 were found to be the most important isoforms. Hynes *et al.* reported that the mouse Clara cell preparation metabolized styrene to both enantiomers. CYP-specific inhibitors (diethyldithiocarbamate for Cyp2e1, 5-phenyl-1-pentyne for Cyp2f2, α-naphthoflavone for Cyp1a, and α-methylbenzylaminobenzotriazole for Cyp2b) were used to identify the cytochromes responsible. The Cyp1a inhibitor did not inhibit styrene-7,8-oxide formation, and the Cyp2b inhibitor had only a minor effect. 5-Phenyl-1-pentyne inhibited formation of both

the *R*- and *S*-enantiomers by approximately 34%, similar to the effect of SKF-525A, a nonspecific CYP inhibitor. In a previous study, Carlson (1997b) reported that diethyldithiocarbamate inhibited styrene metabolism in lung microsomal preparations by more than 50%. In contrast, Hynes *et al.* did not find a significant effect of this Cyp2e1 inhibitor on styrene metabolism in the Clara cell preparation and concluded from the results of both microsomal and isolated cell studies that Cyp2e1 and Cyp2f2 were the primary cytochromes responsible for styrene metabolism in the lung.

In another study, styrene metabolism in rat liver microsomes was decreased by antibodies to CYP2C11/6, CYP2B1/2, CYP1A1/2, and CYP2E1, with the strongest effect for anti-CYP2C11/6 (Nakajima *et al.* 1994b). CYP2C11/6, CYP2B1/2, and CYP1A1/2 were more important at high substrate concentrations, while CYP2E1 contributed more at low substrate concentrations. Metabolism by lung microsomes was inhibited only by anti-CYP2B1/2.

Kim *et al.* (1997) used antibodies against specific human CYP isoenzymes and compared rates of styrene glycol formation by microsomes isolated from human livers. They identified CYP2E1 and CYP2C8 as the most important metabolic enzymes at a low styrene concentration (0.085 mM) and CYP2B6 and CYP2C8 as most prominent at a high styrene concentration (1.8 mM). CYP2E1 was the primary enzyme in styrene metabolism, based on inhibition of metabolism in human liver microsome preparations by the CYP2E1 inhibitor 4-methylpyrazole (Wenker *et al.* 2001b). These authors also demonstrated that the maximum velocity (V_{max1}) and Michaelis-Menten (K_{m1}) enzyme kinetics constants varied 6- to 8-fold among 20 human microsomal liver samples; however, no relationship was found between the interindividual variations in enzyme kinetics and CYP2E1 polymorphisms.

Assessments of metabolic capacity and interindividual variation in styrene toxicokinetics *in vivo* (Wenker *et al.* 2001c) and stereochemical metabolism of styrene *in vivo* (Wenker *et al.* 2001a) in 20 male volunteers performing physical exercise did not show a correlation between apparent blood clearance of styrene and individual metabolic capacity (assessed by administering marker substrates for CYP2E1, CYP1A2, CYP2D6,

and total CYP450), and only moderate interindividual differences in the stereochemical metabolism of styrene were observed. Wenker *et al.* (2001c) concluded that the absence of a correlation between clearance and metabolic capacity could be due to dependence of styrene metabolism on liver blood flow.

Carlson (2004a) reported that wild-type mice were more susceptible to styrene-induced hepatotoxicity than C*yp2e1*-knockout mice, but there was no significant difference in the response when styrene-7,8-oxide was administered. These results suggest that Cyp2e1 is important for bioactivation of styrene in the liver. However, Carlson (2003) reported that there was little or no difference in the rate of metabolism of styrene to styrene-7,8-oxide by hepatic microsomes from wild-type and knockout mice. Sumner *et al.* (2001) compared styrene metabolism in *Cyp2e1*-knockout and wild-type mice *in vivo* and reported that the knockout mice excreted more total urinary metabolites than the wild-type mice. Carlson (2003) rejected the conclusion that these data indicated that Cyp2e1 was not important in styrene metabolism because this would contradict findings from many previous studies. Carlson concluded that the data more likely indicate that other enzymes must be contributing to styrene metabolism in knockout mice. Carlson (2004a) also noted the reason for this disconnect was unclear, but thought it might be related to kinetic factors associated with styrene metabolism within the liver of the intact animal.

Carlson (2003), (see Table 2 in Carlson 2003) reported that styrene metabolism by pulmonary microsomes in *Cyp2e1*-knockout mice is about one-half that in wild-type mice. Cyp2f2 was also important for metabolism in mouse lung based on inhibition of styrene metabolism by the Cyp2f2 inhibitor 5-phenyl-1-pentyne. The same inhibitor inhibited the pulmonary cytochrome P450 metabolism of styrene in mice *in vivo* and prevented an increase in cell replication rates (Green *et al.* 2001a). Cyp1a and Cyp2b were considered to play only minor roles in styrene metabolism because of the small inhibitory effect with α-naphthoflavone, an inhibitor of Cyp1a, and α-methylbenzylaminobenzotriazole, an inhibitor of Cyp1b (Carlson *et al.* 1998). Nakajima *et al.* (1994b) reported that metabolism by rat lung microsomes was inhibited only by anti-CYP2B1/2 (which probably corresponds to CYP2B1). In *Cyp2e1*-knockout studies, pulmonary toxicity of styrene was similar in both wild-type and knockout mice, which

supports previous studies that indicated the importance of styrene metabolism by other enzymes such as Cyp2f2 in the lung (Carlson 2004a).

Carlson *et al.* (2001) did not detect 4-vinylphenol when styrene was incubated with hepatic and pulmonary preparations from rats or mice. However, these tissue preparations were shown to have considerable 4-vinylphenol metabolizing ability when incubated with 4-vinylphenol in the presence of NADPH. The rate of metabolic activity in mouse liver microsomes was 3 times faster than in rat liver microsomes, and the rate in mouse lung microsomes was 8 times faster than in rat lung microsomes. Treatment with pyridine, an inducer of Cyp2e1, caused a significant increase in 4-vinylphenol metabolism in the liver but not the lung. Furthermore, 4-vinylphenol metabolism was significantly decreased in mouse liver and lung microsomes treated with diethyldithiocarbamate, an inhibitor of Cyp2e1, or 5-phenyl-1-pentyne, an inhibitor of Cyp2f2. This study also indicated that glutathione conjugation was involved in 4-vinylphenol metabolism, with the highest activity in mouse lung, with or without the addition of NADPH. Carlson (2002) also showed that when rats and mice were pretreated with diethyldithiocarbamate or 5-phenyl-1-pentyne, the hepatotoxicity and pneumotoxicity of 4-vinylphenol were prevented or greatly decreased. These data suggest that the toxicity of 4-vinylphenol in the liver and lungs was due to a metabolite rather than to the parent compound. Vogie *et al.* (2004) examined the microsomal metabolism of 4-vinylphenol in wildtype and *Cyp2e1*-knockout mice and reported no marked differences in the rates of microsomal metabolism prepared from the lung and liver of mice with either genotype. The knockout mice were more susceptible to hepatotoxicity than wild-type mice but there was no significant difference in pneumotoxicity. Thus, in contrast to the findings of Carlson, the animals that were unable to metabolize 4-vinylphenol through the Cyp2e1 pathway were more susceptible. Vogie *et al.* stated that the reason for the discrepancy was unknown, but could be related to inhibition of other cytochrome P450 enzymes by diethyldithiocarbamate, or it could have protected against hepatotoxicity by a mechanism not related to Cyp2e1. The rate of metabolism of 4-vinylphenol metabolism was the same in wild-type and *Cyp2e1*-knockout mice indicating that cytochromes P450 other than Cyp2e1 play an important role (Carlson 2004b). This study also showed that the greatest

inhibition of enzymatic activity occurred with diethyldithiocarbamate, even in knockout mice, and suggests that it must inhibit other P450 cytochromes in addition to Cyp2e1.

5.1.3.6 Detoxification of styrene metabolites

Only a very small fraction of the styrene-7,8-oxide formed from styrene in the liver reaches the systemic circulation. The vast majority is immediately hydrolyzed enzymatically, as shown by epidemiologic studies (Korn *et al.* 1994) and based on theoretical considerations (Arand *et al.* 1999, Oesch *et al.* 2000). The hydrolysis of styrene-7,8-oxide is efficiently carried out by microsomal epoxide hydrolase (mEH). Although the enzyme has a relatively low turnover, it is able to inactivate genotoxic substrates very rapidly via formation of a covalent intermediate, an enzyme-substrate ester, which is subsequently hydrolyzed in a slow, rate-limiting step (Arand *et al.* 1999, Tzeng *et al.* 1998). Overall clearance of styrene-7,8-oxide is efficiently accomplished by liver mEH; however, detoxification outside the liver is usually less efficient, because it depends on local mEH levels. Oesch *et al.* (2000) predicted that the local styrene-7,8-oxide steady-state level will rise sharply as soon as the rate of styrene-7,8-oxide formation exceeds the local capacity for enzymatic hydrolysis of styrene-7,8-oxide. As a result, tissues that produce the relevant CYP and activate styrene to styrene-7,8-oxide in sufficient amounts, but have low mEH activity, may be more susceptible to styrene-mediated genotoxicity than would be predicted from the systemic styrene-7,8-oxide load deduced from the biomarkers measured in blood. The lung, which is the primary entry site for styrene into the human body, contains cell types that produce styrene-activating CYP isoenzymes (e.g., Clara cells), but it has significantly lower styrene-activating activity and mEH activity than the liver.

The second pathway for styrene-7,8-oxide detoxification, the formation of glutathione conjugates and PHEMAs (which constitute less than 1% of styrene metabolites in humans) was reported first in rodents (Delbressine *et al.* 1981) and later in humans (Maestri *et al.* 1997). Although GSH conjugation is a minor detoxification route (Maestri *et al.* 1997), it may become important in extrahepatic tissues with low mEH activity, such as lungs and blood-forming organs, as high levels of GST are found in erythrocytes (Henderson and Speit 2005). Several studies have suggested that polymorphisms in

GSTM1 may influence metabolite excretion; higher levels of mandelic acid and phenylglyoxylic acid urinary metabolites (Teixeira *et al.* 2004) or lower levels of phenylethyl mercapturic acids (De Palma *et al.* 2001, Haufroid *et al.* 2002b) were found in styrene-exposed *GSTM1*-null individuals than in wild-type individuals. Studies on GSTT1 polymorphisms are conflicting (Norppa 2003).

5.1.4 Excretion

The primary route of styrene excretion in both humans and laboratory rodents is the urine (IARC 1994a); however, the metabolic profiles differ among species (as discussed above). Almost all of the absorbed styrene is excreted as urinary metabolites; however, a small fraction (< 5%) may be eliminated as unchanged styrene in exhaled air or urine (ATSDR 1992, IARC 2002). Mandelic acid and phenylglyoxylic acid (see Table 1-3) are the primary urinary metabolites, accounting for as much as 95% to 98% of the total (Manini *et al.* 2002b). Glutathione conjugates generally account for 1% or less of the absorbed dose (IARC 1994a).

5.1.4.1 Humans

Elimination of styrene from blood was biphasic in human volunteers, indicating a two-compartment pharmacokinetic process; the half-lives were 0.58 hours for the rapid phase and 13.0 hours for the slow phase (Ramsey *et al.* 1980). Urinary elimination of mandelic acid and phenylglyoxylic acid also were reported to be biphasic in styrene-exposed workers (IARC 1994a); the half-lives for both were 2.5 hours for the rapid phase and 30 hours for the slow phase (Wieczorek and Piotrowski 1988). Guillemin and Berode (1988) reviewed data on clearance of these metabolites and also reported that clearances were biphasic. Half-lives for mandelic acid ranged from 3.9 to 9.4 hours during the first 20 hours post-exposure and from 16.6 to 26.5 hours after 20 hours post-exposure. Half-lives for phenylglyoxylic acid averaged 10.5 ± 1.4 hours during the first 50 hours post-exposure in one study reviewed by Guilleman and Berode and ranged from 21.5 to 26.7 during the period from 20 to 200 hours post-exposure in a second study.

IARC (1994a) reported that 0.7% to 4.4% of inhaled styrene was eliminated unchanged in exhaled air. Unchanged styrene also was reported to be excreted in urine by styrene-

exposed workers, but the concentration in urine was only about one-tenth that in blood (Guillemin and Berode 1988, IARC 1994a).

Pfaffli *et al.* (1981) reported that they could detect 4-vinylphenol in the urine of styrene-exposed workers but not in nonexposed individuals by GC/MS, but the level detected was only 0.3% of the level of mandelic acid in the same individuals. Johanson *et al.* (2000) exposed four healthy male volunteers to 50-ppm styrene for 2 hours. Based on the relationship of mandelic acid and 4-vinylphenol reported by Pfaffli *et al.* (1981), Johanson *et al.* estimated that the maximum level of 4-vinylphenol in their subjects would be about 0.004 mM [below the detection limit]. Manini *et al.* (2003) used liquid chromatography electrospray tandem mass spectrometry to measure 4-vinylphenol conjugates in urine of workers exposed to styrene and in volunteers exposed to 50 mg/m^3 styrene. Urinary 4-vinylphenol conjugates (glucuronates and sulfates) represented about 0.5% to 1% of the total excretion of styrene metabolites and were significantly correlated with airborne styrene ($r = 0.607$, $P < 0.001$) and the sum of mandelic acid and phenylglyoxylic acid ($r = 0.903$; $P < 0.001$) in end-of-shift samples for workers.

5.1.4.2 Experimental animals

In rats, elimination of styrene from blood was reported to be biphasic over a period of 6 hours (ATSDR 1992, IARC 1994a). Sumner *et al*. (1997) reported that after inhalation exposure to styrene at 250 ppm for 1 to 5 days, male F344 rats, male CD-1 mice, and male $B6C3F_1$ mice eliminated most of the absorbed styrene in the urine. Following a single 6-hour exposure, elimination was faster in rats (89% within 12 hours) and CD-1 mice (83% within 12 hours) than in $B6C3F_1$ mice (59% within 12 hours). The slower elimination in $B6C3F_1$ mice was considered to be consistent with the higher liver toxicity in these mice. However, when the animals were exposed for 3 to 5 days, elimination in all three groups was about 88% within 12 hours. The increased excretion in $B6C3F_1$ mice with longer-term exposure was consistent with induction of styrene metabolism and with the absence of ongoing acute necrosis following multiple exposures. When CD-1 mice and male Sprague-Dawley rats were exposed to ^{14}C-labeled styrene by nose-only inhalation, the primary route of excretion was in the urine (75 ± 7% of inhaled styrene retained by rats and 63 ± 9% of that retained by mice), and only a small fraction was

eliminated in the feces of either species (Boogaard *et al.* 2000a). The species differed in exhalation of $^{14}CO_2$, which in two separate experiments accounted for approximately 2% of retained styrene in rats and 6.4% and 8% in mice. Mice also had higher nonspecific binding of radiolabeled styrene in nasal passages and lung than rats.

5.2 Toxicity

The toxicity of styrene has been reviewed (ATSDR 1992, Bond 1989, IARC 1994a, 2002). The acute toxicity of styrene in laboratory animals and in humans is considered low to moderate. The oral LD_{50} for styrene in rats is 5,000 mg/kg and the inhalation LC_{50} is 2,770 ppm (2-hour exposure). The LD_{50} in mice is 320 mg/kg for oral exposure, 660 mg/kg for i.p. injection, and 90 mg/kg for i.v. injection. The inhalation LC_{50} in mice is 4,940 ppm (4-hour exposure). The major acute toxic effects of styrene include irritation of the skin and respiratory tract and effects on the central nervous system (CNS).

5.2.1 Humans

Drowsiness, listlessness, muscular weakness, and unsteadiness are common signs of systemic styrene intoxication in humans (Bond 1989). Skin, eye, throat, and respiratory tract irritation have been reported in styrene-exposed workers (IARC 2002). Direct skin contact with liquid styrene has caused erythema, dermatitis, and blistering. Minamoto *et al.* (2002) conducted patch tests on 29 fiberglass-reinforced-plastics workers. Of the 22 workers who reported skin problems, one had a positive patch test to styrene. In a study where human volunteers were exposed to styrene concentrations of 51 to 376 ppm for 1 to 7 hours, signs of styrene toxicity (including eye and nasal irritation, nausea, and headaches) occurred only in subjects exposed to 376 ppm (Bond 1989). In another study reviewed by Bond, subjects exposed to 800 ppm experienced immediate irritation of the nose and throat and increased nasal secretions. Respiratory tract irritation was reported in humans exposed for short durations and airflow restriction in those exposed for long durations; however, the concentrations and durations were not fully defined. Röder-Stolinski *et al.* (2008) investigated the mechanisms responsible for styrene-induced inflammatory effects using a human alveolar epithelial cell line (A549). Styrene stimulated the expression of inflammatory mediators, including the chemotactic cytokine monocyte chemoattractant protein-1 (MCP-1) in these cells. MCP-1 expression and

glutathione S-transferase [a marker of oxidative stress] was associated with a concentration dependent pattern of NF-κB activation. NF-κB is a pivotal intracellular signaling pathway involved in inflammatory responses. Treatment with an NF-κB inhibitor and an antioxidant were effective in suppressing styrene-induced MCP-1 secretion.

Respiratory effects from occupational exposure to styrene include bronchitis, asthma, and pneumonia. Chronic bronchitis has been reported in workers exposed to styrene concentrations greater than 100 mg/m^3 [23 ppm], and increased mortality from pneumonia was associated with styrene exposure among 40,000 men and women employed in 660 European reinforced-plastics manufacturing factories; however, no increased mortality from bronchitis, emphysema, or asthma occurred (IARC 2002). In a more recent study (reviewed by IARC 2002) of workers in the reinforced-plastics and composites industry in the United States, there was no relationship between exposure to styrene and mortality from non-malignant respiratory disease.

Effects of styrene exposure on the nervous system, either central or peripheral, have been reported mostly for concentrations of 100 ppm or above (IARC 2002). The effects included decreased nerve conduction velocities and electroencephalographic, dopaminergic, functional, and psychiatric anomalies. At concentrations below 100 ppm, reports of effects have been mixed, with some researchers finding no effects and others reporting memory and neurobehavioral disturbances at concentrations in the range of 10 to 30 ppm. In addition to the effects of styrene on reaction time, color vision, and hearing, researchers also have studied the possible effects of styrene exposure on taste. However, Dalton *et al.* (2003) did not find any evidence for an impairment of olfactory function in a group of fiberglass-reinforced-plastics workers.

Benignus *et al.* (2005) conducted a meta-analysis of the relationship of long-term exposure to styrene and two measures of CNS function: reaction time and color vision. There was a statistically significant relationship between cumulative exposure to styrene and an increased choice reaction time as well as an increased color confusion index. These authors estimated that 8 work-years of exposure to 20-ppm styrene (the ACGIH

limit) produces a 6.5% increase in choice reaction time and an increase in the color confusion index equivalent to 1.7 additional years of age in men (the color confusion index in men increases with age at the rate of about 10% of baseline every 13 years).

Color vision was reported to be impaired in several studies reviewed by IARC (2002), and it was proposed that this effect reflects changes in neural functioning along optic pathways. Effects were seen at concentrations of styrene as low as 20 ppm; however, one study reported that the effects of styrene on color vision were reversed after 4 weeks in a styrene-free environment. In a study of 108 workers in Swedish reinforced-plastics plants, Iregren *et al.* (2005a) concluded that there was a "strong indication" that color vision was negatively affected in workers with exposure below the Swedish occupational exposure limit of 90 mg/m^3 [21 ppm]. Confounding effects of age and higher past exposure levels were also considered by the authors, but they did not consider these factors sufficient to explain all of the differences observed.

Significant changes in hearing thresholds at high frequencies have been reported in workers exposed to styrene in some studies (IARC 2002). Although several studies reviewed by IARC did not find effects on hearing threshold at styrene concentrations below 150 mg/m^3 [35 ppm], one study reported disturbances in the central auditory pathways in 7 of 18 workers exposed for 6 to 15 years to styrene at concentrations below 110 mg/m^3 [25 ppm]. Several recent publications have reviewed the relationship between styrene exposure and hearing loss. Hoet and Lison (2008) reported that styrene appears to be ototoxic in rats, but the human data were insufficient to support a clear conclusion. Sliwinska-Kowalska *et al.* (2007) reported the findings of a scientific workshop that reviewed the ototoxic effects of organic solvents. Seven of nine occupational studies of styrene-only exposure (primarily in the glass fiber–reinforced-plastics industry) showed evidence of hearing loss. Measurements varied among the studies but included pure tone audiometry, high-frequency hearing loss, and central hearing tests. Although one of the primary conclusions from the workshop was that styrene is a risk factor for hearing loss, the authors concluded that the data were not sufficient to derive a dose-response relationship. Johnson (2007) also reviewed these nine studies and noted that the reported effects occurred at concentrations below the current TLV values (20 to 50 ppm) but the

authors considered the effects to be negligible. Fuente and McPherson (2006) also reviewed the literature on solvent exposure and hearing loss. They reported that a positive linear relationship was seen between an average working-life exposure to styrene and hearing thresholds at 6,000 and 8,000 Hz. These authors also noted that there was an additive effect on hearing thresholds with exposure to both styrene and noise. Johnson *et al.* (2006) reported audiological findings in 313 workers from fiberglass and metal-product manufacturing plants. Workers exposed to noise and styrene had significantly poorer pure-tone thresholds in the high-frequency range than controls or noise-only exposed workers.

Toppila *et al.* (2006) noted that styrene is both ototoxic and neurotoxic; thus, styrene exposure could affect postural stability. These authors investigated the effects of low concentrations of styrene on postural stability among 252 male Finnish fiberglass-reinforced plastic boat manufacturers. Smoking history, postural stability, and urine mandelic and phenylglyoxylic acid concentrations were determined. Breathing zone measurements of styrene were measured for 148 workers. Mean styrene concentrations for the age-matched workers were 21 mg/m^3 [4.8 ppm] for nonlaminators and 108 mg/m^3 [25 ppm] for laminators. Their analysis included 88 matched pairs and indicated that postural stability among boat laminators was impaired compared with nonlaminators. The impairment occurred among young workers and worsened with age.

Hepatic and renal effects of styrene exposure were mixed or absent in older studies, but more recent reports have found alterations in hepatic clearance of bilirubin and in hepatic alanine and aspartate transaminase activities (IARC 2002). Urinary markers for renal toxicity are reported to be only weakly correlated with styrene exposure.

The early studies that examined the effects of styrene exposure on the hematopoietic and immune systems failed to find consistent functional changes (IARC 2002). One study found no differences in hemoglobin, erythrocyte, and leukocyte concentrations in 84 workers exposed to styrene concentrations of 50 to 500 ppm for 1 to 36 years. Another study found no evidence of hematological abnormalities in 163 workers in a styrene-butadiene synthetic rubber manufacturing plant. More recent studies reviewed by IARC

reported a 30% increase in the number of peripheral blood monocytes in workers exposed to 13-ppm styrene, and an exposure-related decrease in both the mean corpuscular hemoglobin and neutrophil concentrations among 221 workers in the reinforced-plastics industry that were exposed to 1 to 100 ppm for 1 to 20 years. Other studies reported effects on the immune system, including a reduction in total T lymphocytes and T-helper lymphocytes along with an increase in natural killer cells, and alterations in the cell-mediated immune response of T lymphocytes. Changes in lymphocyte subpopulations were observed mainly at concentrations greater than 50 ppm. Biro *et al.* (2002) investigated the immunotoxicity of styrene in 10 styrene-exposed workers compared with 29 healthy controls. The data indicated that changes in the expression of surface antigens on peripheral lymphocytes were correlated with exposure. The styrene-exposed group had a significant decrease in the level of CD25+ CD4+ lymphocytes (activated helper T cells) with a concomitant increase in the level of CD45RO+ CD4+ lymphocytes (memory helper T cells), suggesting a shift from activated to memory helper T cells. The styrene-exposed group also had a slightly higher ratio of CD4+ lymphocytes to CD8+ T lymphocytes, which the authors concluded was caused by smoking as this was seen among all smokers compared with nonsmokers, and the styrene-exposed group had a higher percentage of smokers than the control group,

Plotnick and Weigel (1979) suggested that a relationship may exist between the distribution of styrene and/or its metabolites in the pancreas and the increased glucose tolerance reported in workers. Chmielewski (1976) reported a statistically significant increased glucose tolerance in workers exposed to styrene for 1 year; however, the increase was not significant in workers exposed for 10 years. Some epidemiological studies have reported pancreatic cancer in workers exposed to styrene (see Section 3.8.2). Impaired glucose metabolism (diabetes mellitus) has been observed in patients with pancreatic cancer, although it is not known whether diabetes develops shortly before or after the clinical manifestations of pancreatic cancer. A meta-analysis of 11 case-control studies (including only studies in which diabetes was present at least 1 year before diagnosis of pancreatic cancer) and 9 cohort studies found a relative risk of 2.1 (95% CI = 1.6 to 2.8) for pancreatic cancer in diabetics, compared with non-diabetics (Everhart and Wright 1995). A large prospective cohort study found an association between post-

load plasma glucose concentration and pancreatic cancer in individuals without self-reported diabetes, which the authors considered suggestive that factors associated with abnormal glucose metabolism could play a role in development of pancreatic cancer (Gapstur *et al.* 2000). In a review of pancreatic cancer, Michaud (2004) concluded that chronic pancreatitis and diabetes mellitus are medical conditions that have been consistently related to pancreatic cancer, and the evidence suggesta that they are causually related to pancreatic cancer rather than consequences of the cancer.

Matanoski and Tao (2003) reported an association between cardiovascular disease and occupational exposure to styrene. Their case-cohort study included 498 cases that died from ischemic heart disease and a 15% random sample (N = 997) of all male workers who were employed during 1943 to 1982 in two styrene-butadiene rubber manufacturing plants in the United States. Recent styrene exposure was significantly associated with acute ischemic heart disease death among active workers. The relative hazard of death for exposure during the most recent two years among active workers with 2 or more years of employment was 2.95 (95% CI = 1.02 to 8.57) at a time-weighted styrene concentration of 0.2 to < 0.3 ppm and 4.3 (95% CI = 1.56 to 11.84) at time-weighted exposure concentrations of ≥ 0.3 ppm. Delzell *et al.* (2005) also examined the relationship of styrene exposure and mortality from ischemic heart disease among 16,579 men employed at 6 styrene-butadiene rubber manufacturing plants (including the 2 plants reported by Matanoski and Tao) for at least one year and employed from 1943 until 1990. Men in the highest quintile of exposure (> 5.5 ppm) and in the highest quintile of cumulative exposure (> 60.67 ppm-year) had ischemic heart disease ratios of 1.14 (95% CI = 0.96 to 1.35), and 1.06 (95% CI = 0.90 to 1.27), respectively. Acute disease was not associated with average intensity of exposure within the most recent 2 years. Incidences of chronic disease were elevated in subjects with the highest exposure, but the associations were weak and imprecise, and there was limited evidence of a dose-response relationship. The authors concluded that their study did not provide strong support for a causal association between styrene and mortality from ischemic heart disease.

The potential reproductive and developmental effects of styrene in humans have been reviewed (IARC 2002, NTP 2006). Some earlier studies suggested an association

between occupational exposure to styrene and congenital CNS malformation and spontaneous abortions; however, these associations were not confirmed in later studies (IARC 2002). Other studies have not shown a consistent or statistically significant relationship between styrene exposure and reduced birth weight, sperm abnormalities, time-to-pregnancy, or menstrual cycle effects. NTP (2006) concluded that the human data were insufficient to conclude that styrene is a reproductive or developmental toxicant; however, based on the animal data, the panel expressed negligible concern for effects in humans. There was suggestive evidence that occupational exposure to styrene was associated with increased serum prolactin and depletion of peripheral blood dopamine-metabolizing enzyme activities, but the clinical relevance of these findings was unclear.

Several publications have reported increased serum prolactin levels in workers exposed occupationally to styrene (Arfini *et al.* 1987, Bergamaschi *et al.* 1996, Bergamaschi *et al.* 1997, Mutti *et al.* 1984); a proposed cause is dopaminergic dysfunction resulting from the interaction between styrene metabolites and dopamine (Mutti and Smargiassi 1998). Although the relationship between the possible styrene-related increases in serum prolactin and breast cancer is not known, Harvey (2005) concluded that the evidence for the role of prolactin in human breast cancer is strong and consistent. Several large epidemiology studies have shown that dopamine antagonists increase breast cancer risk. Hyperprolactinemia is associated with human breast cancer growth and poor prognosis, and prolactin is a mitogen in human breast cancer cells that suppresses apoptosis and upregulates BRCA1. An increased risk of breast cancer was not observed in the cohort studies of styrene-exposed workers, which consisted predominantly of men; however, two studies of the general population (a case-control study and an ecological study) reported an association between breast cancer and styrene exposure (see Sections 3.5.2 and 3.8.6).

5.2.2 Experimental animals

The toxic effects of styrene in experimental animals have been reviewed (ATSDR 1992, Bond 1989, IARC 2002). Acute exposures were associated with eye and nose irritation, CNS depression, and death at high concentrations. Subacute to subchronic exposures have been associated with adverse effects on the liver, pancreas, kidney, nervous system,

respiratory system, immune system, and hematopoietic system. This section briefly reviews the overall toxicity in experimental animals (5.2.2.1) and then describes in greater detail studies of respiratory toxicity (5.2.2.2), toxicity of the stereoisomers of styrene-7,8-oxide (5.2.2.3), and glutathione depletion (5.2.2.4), because these factors have been suggested to play a role in the development of lung tumors in mice (see Sections 5.3 and 5.5).

5.2.2.1 Overall toxicity findings

Acute exposures of rats and guinea-pigs to styrene at a concentration of 650 ppm resulted in eye and nose irritation (IARC 2002). Higher concentrations resulted in weakness, unsteadiness, and other CNS effects (1,300 ppm), unconsciousness (2,500 ppm) and death (5,000 to 10,000 ppm). Eye and nose irritation was also reported in rats exposed to styrene concentrations of 1,300 or 2,000 ppm for 7 to 8 hours per day, 5 days per week for about 6 months (Spencer *et al.* 1942, Wolf *et al.* 1956).

Permanent hearing loss, neurotoxic effects, hematopoietic and immune system effects, and damage to the pancreas, lung, liver, and kidney have been reported in rats, mice, or guinea-pigs (IARC 2002). Sliwinska-Kowalska *et al.* (2007) reported that the lowest concentration of styrene known to cause hearing loss in rats is 300 ppm. Styrene damages the outer hair cells in the cochlea. The neurotoxic effects included decreased monoamine oxidase activity, depletion of dopamine, weakness, and brain damage. Gagnaire *et al.* (2006) investigated the effects of styrene on the extracellular and tissue levels of dopamine, serotonin, and their metabolites in male rats exposed to 750- or 1,000-ppm styrene for 4 weeks. Rats exposed to the high dose had a significant decrease in extracellular acid metabolite concentrations, while tissue levels of these metabolites were decreased to a lesser extent. The effects were reversed after 17 days. Umemura *et al.* (2005) investigated the neuroendocrinological effects in rats exposed to 150-ppm styrene for 10 days. The styrene concentration in the blood was higher in female rats than in male rats, and the prolactin level was significantly increased in female rats. Levels of neurotransmitters were not affected in either sex; therefore, the mechanism enhancing prolactin secretion was unclear. Mouse splenic T-lymphocyte activity was suppressed by *in vitro* exposure to styrene, and oral dosing (20 to 50 mg/kg b.w. styrene daily for five

days) impaired humoral and cell-mediated immunity in male Swiss mice (IARC 2002). The number of erythropoietic cells was increased in male Sprague-Dawley rats exposed to styrene by inhalation or i.p. injection. Nano *et al.* (2000) exposed groups of 6 male Sprague-Dawley rats to i.p. injections of styrene at 40 or 400 mg/kg b.w. or corn oil for 3 consecutive days or by inhalation of styrene vapor (purity 99%) at 0 or 300 ppm 6 hours/day, 5 days/week for 2 weeks. Some of the rats (inhalation exposure) were killed immediately after the last treatment while the others were killed 3 weeks later. Rats injected with 400 mg/kg styrene showed hyperactivity of the erythropoietic series while the granulocytopoietic series was normal. There was a statistically significant increase in basophilic, polychromatophilic, and orthochromatic erythroblasts in rats that inhaled styrene for 2 weeks. There also was a temporary block of immature cells of the granulocytopoietic series.

Khanna *et al.* (1994) exposed mice, rats, and guinea-pigs to styrene orally in groundnut oil on 5 days per week for 4 weeks at 25 or 50 mg/kg b.w. per day for mice and 160 or 320 mg/kg b.w. per day for rats and guinea-pigs. Mice exhibited moderate inflammatory reaction of pancreatic islet cells, congestion of pancreatic blood vessels, moderate congestion of pancreatic lobules, and increased serum insulin levels. Guinea-pigs showed congestion of pancreatic acinar parenchyma, marked degranulation of beta cells of large pancreatic islets, and decreased serum insulin levels. No changes in the pancreas were noted in rats other than decreased serum insulin levels, and no significant changes in blood glucose levels were noted in any of the species studied.

Subacute to subchronic exposure to styrene by i.p. injection or inhalation has caused kidney and liver damage in rodents (IARC 2002). These effects were often associated with glutathione depletion. B6C3F$_1$ mice exposed for 14 days to styrene by inhalation at a concentration of 0, 125, 250, or 500 ppm developed severe centrilobular hepatic necrosis (Morgan *et al.* 1993c, Morgan *et al.* 1993b). Mortality was higher in the 250-ppm group of each sex than in the 500-ppm group. The differences in mortality could not be explained on the basis of styrene-7,8-oxide production, GSH depletion, or hepatotoxicity. Sprague-Dawley rats given repeated i.p. injections of 2.9 to 5.8 mg/kg b.w. for 6 weeks had morphological changes in the kidney (IARC 2002). Mild tubular

damage occurred in Sprague-Dawley rats given daily i.p. injections of 1 g/kg b.w. for 10 days, and inhalation exposure to 300 ppm for 12 weeks resulted in a slight increase in the urinary excretion of plasma proteins and minor changes in kidney histopathology. Hepatotoxic effects included focal necrosis in male albino rats exposed orally to 400 mg/kg b.w. styrene for 100 days. Centrilobular necrosis was reported in several studies in mice exposed to 125 to 500 ppm for 2 weeks. Sex and strain differences in sensitivity have not generally correlated with differences in glutathione depletion or concentrations of styrene or styrene-7,8-oxide in blood. Single i.p. injections of 250 to 1,000 mg/kg b.w. of styrene or styrene-7,8-oxide produced a dose-dependent increase in serum sorbitol dehydrogenase activity [an indicator of hepatotoxicity]. One study indicated that liver toxicity was greater when styrene was administered by i.p. injection compared with inhalation of styrene vapor (De Piceis Polver *et al.* 2003). This may be explained by the fact that the intraperitoneal route results in direct exposure of the liver. Several studies have shown that hepatotoxicity may be enhanced in mice pretreated with CYP enzyme inducers. In one study, the hepatotoxic effects of styrene-7,8-oxide were increased in mice by administration of trichloropropene oxide, an inhibitor of epoxide hydrolase.

The developmental and reproductive toxicity of styrene in experimental animals have been reviewed (Brown *et al.* 2000, IARC 2002, NTP 2006). The available studies have not shown an increased incidence of malformations, but there have been reports of increased embryonic, fetal, and neonatal deaths; skeletal and kidney abnormalities; decreased birth weight, postnatal developmental delays (e.g., incisor eruption, eye opening), and neurobehavioral and neurochemical abnormalities. The reported effects were seen mostly at high doses that were associated with maternal toxicity, but at least one study indicated that styrene might affect the developing brain and postnatal development. Beliles *et al.* (1985) conducted a three-generation study of the reproductive effects of styrene exposure in Sprague-Dawley rats (see Section 4.2.1). The animals were exposed to styrene indirectly *in utero* and as neonates, and directly in drinking water while maturing to become breeders. Fertility, litter size, pup viability, pup survival, sex ratio, pup body weight, weanling kidney and liver weight, and marrow cytogenetics were evaluated. The only reported effects included an apparent reduction in 21-day survival of high-dose F_1 pups, and a loss of breeding efficiency in F_3 parents; however, the authors

noted that there were mitigating factors. These effects were not consistent and were traced to a single or only two individual animals or litters. The authors concluded that styrene exposure produced no deleterious dose-related effects or decrements in reproductive function through three generations.

5.2.2.2 Respiratory toxicity

Respiratory toxicity, including nasal toxicity and pneumotoxicity, has been observed in mice exposed to styrene or styrene-7,8-oxide. The Clara cell is the main site of both bioactivation and toxicity of styrene in the lung (Harvilchuck and Carlson 2006, Hynes *et al.* 1999).

Green *et al.* (2001b, 2001a) exposed CD-1 mice to styrene at 40 or 160 ppm for 3 days. Styrene exposure caused degenerative changes in the nasal cells (including atrophy of the olfactory mucosa and loss of normal cellular organization) and pneumotoxicity (characterized by focal loss of cytoplasm and focal crowding of nonciliated Clara cells, particularly in the terminal bronchiolar region). In mice exposed for 3 days or longer, cell replication rates were increased in the terminal and large bronchioles (Green *et al.* 2001a). Similar effects occurred in mice given oral doses of 100 or 200 mg/kg for 5 days, but not in rats exposed to 500 ppm for up to 10 days. There were no morphological or cell proliferation effects in the alveolar region of the mouse lung. Female CD-1 mice exposed to styrene at 40 or 160 ppm for 1 to 20 consecutive days had decreased levels of Clara-cell–specific protein (CC16) in lung lavage fluid and blood serum, suggesting destruction of Clara cells (Gamer *et al.* 2004). Swiss-Albino mice given i.p. injections of styrene at 800 mg/kg b.w. or styrene-7,8-oxide at 300 mg/kg b.w. had increased levels of gamma-glutamyltranspeptidase (GGT) and lactate dehydrogenase (LDH) in the bronchioalveolar lavage fluid [these are markers of pneumotoxicity] (Gadberry *et al.* 1996).

Cruzan *et al.* (1997) reported nasal toxicity (atrophy of the olfactory epithelium and olfactory nerve fibers, with or without focal respiratory metaplasia) and lung toxicity in CD-1 mice exposed to styrene at a concentration of 100, 150, or 200 ppm for 13 weeks; at 50 ppm, only nasal toxicity was seen. Changes in the lung included decreased eosinophilia of the bronchial epithelium, focal crowding of nonciliated cells in

bronchioles, and focal bronchiolar epithelial proliferation. An increased labeling index in Clara cells was observed after two weeks, but no increase was observed in type II pneumocytes; however, the authors reported that the labeling index in the cell proliferation studies was highly variable among rodents in the same exposure group.

In the chronic inhalation study (see Section 4.1.2), styrene exposure (20, 40, 80, or 160 ppm) resulted in toxic effects in the nasal passages and lung of CD-1 mice (Cruzan *et al.* 2001). Histological effects in the nasal passages included respiratory metaplasia of the olfactory epithelium with changes in the underlying Bowman's glands and loss of olfactory nerve fibers. The effects increased in severity with increasing styrene concentration and duration of exposure, and most changes were observed in all exposure groups by 78 weeks. In the lung, styrene exposure resulted in decreased eosinophilic staining of Clara cells at 12, 18, and 24 months. Bronchiolar epithelial hyperplasia was observed at 12 months (at concentrations > 40 ppm) or 18 months (at 20 ppm); the hyperplasia extended into the alveolar ducts in the high-dose animals. (Lung tumors were observed after 24 months, see Section 4.1.2)

Respiratory toxicity has also been reported in rats exposed to styrene. Exposure at 150 or 1,000 ppm caused a dose-related decrease in tracheal and nasal ciliary activity, but at 12 weeks post exposure, ciliary activity had returned to near control values in the low-dose group and to 50% to 75% of control values in the high-dose group (Ohashi *et al.* 1986). Epithelial changes in the nose and trachea (vacuolation of epithelial cells, nuclear pyknosis, and exfoliation of epithelial cells) were observed in rats exposed to styrene at 800 ppm (IARC 2002). Cruzan *et al.* (1997) also reported histological changes in the olfactory epithelium (focal disorganization, focal hyperplasia of basal cells, single-cell necrosis, and cell loss) in CD (Sprague-Dawley-derived) rats exposed to styrene by inhalation at 500 to 1,500 ppm for 13 weeks. Coccini *et al*. (1997) reported cytoplasmic changes involving bronchiolar and alveolar type II cells (similar to those observed in mice) in Sprague-Dawley rats exposed to styrene by either i.p. injection (40 or 400 mg/kg b.w. daily) or inhalation (300 ppm for 2 weeks); damage was more severe following i.p. injection.

In contrast, several studies have not detected pneumotoxicity in rats. Gamer *et al.* (2004) reported no signs of lung toxicity in female CD rats exposed to styrene at up to 2,150 mg/m^3 [490 ppm] for up to 21 days, and Green *et al.* (2001a) did not observe morphological or cell-proliferative changes in the lungs of Sprague-Dawley rats exposed at 500 ppm for up to 10 days; however, lung toxicity was observed in mice in these studies (see above). Cruzan *et al.* (1997) also did not observe lung toxicity or increased cell proliferation in CD rats [although there was a high variability in the percentage of cells labeled with bromodeoxyuridine].

Some studies have suggested that styrene metabolites other than styrene-7,8-oxide also cause cytotoxicity in the lung. Cruzan *et al*. (2002) reported that styrene metabolism in mice produced 4- to 10-fold more metabolites via ring-oxidation and the phenylacetaldehyde pathways than observed in rats. In another study, the toxicity of 4-vinylphenol, a ring-oxidized metabolite of styrene, was evaluated in lungs of CD-1 mice and female Sprague-Dawley rats exposed by i.p. injection in 3 daily divided doses (2, 6, 20, or 60 mg/kg b.w. per day) for 14 consecutive days (Cruzan *et al.* 2005a). Multifocal hyperplasia was present in the medium bronchi and terminal bronchioles in some of the mice exposed to 6 or 20 mg/kg b.w. and in all of the mice in the high-dose group. However, no evidence of toxicity was found in the lungs of Sprague-Dawley rats. Several studies have investigated the metabolism of the styrene metabolite 4-vinylphenol in rat and mouse liver and lung. Carlson *et al.* (2002) concluded that 4-vinylphenol is a more potent hepatotoxicant and pneumotoxicant than either styrene or styrene-7,8-oxide based on increases in SDH (a marker for hepatic toxicity) in serum and increases in cell numbers and LDH levels in bronchoalveolar lavage fluid from adult male CD-1 mice injected i.p. with 50 mg/kg 4-vinylphenol compared with doses of 500 to 1,000 mg/kg for styrene and 300 mg/kg styrene oxide to induce significant effects in separate experiments from their laboratory in another strain of mice (non-Swiss Albino) (Gadsberry *et al*. 1996).

Kaufmann *et al.* (2005) investigated the effects of styrene and its metabolites on the mouse lung. CD-1 mice were injected i.p. with styrene, styrene-7,8-oxide, 4-vinylphenol, 1-phenylethanol, 2-phenylethanol, phenylacetaldehyde, phenylacetic acid, or

acetophenone. Of the compounds tested, only styrene-7,8-oxide (at 100 mg/kg b.w. 3 times per day) and 4-vinylphenol (5, 20, or 35 mg/kg b.w. 3 times per day) caused increases in cell proliferation in large/medium bronchi (up to 15.1 fold for 4-vinylphenol and 7.5 fold for styrene-7,8-oxide) and terminal bronchioles (up to 19.7 fold for 4-vinylphenol and 10.5 fold for styrene-7,8-oxide). Both compounds also caused glutathione depletion and histomorphological changes in the bronchiolar epithelium. These two molecules also caused histopathological changes in the terminal bronchioles that included the appearance of flattened cells and the loss of the typical bulging of the apical cytoplasm of Clara cells (which the authors describe as "dome-shaped") into the bronchial lumina. Styrene-7,8-oxide, but not 4-vinylphenol, also caused marginal increases in alveolar cell proliferation and an increased number of apoptotic cells in large/medium bronchi. Kaufmann *et al.* concluded that the metabolites of the side-chain hydroxylation pathway (phenylethanols, acetophenone, phenylacetaldehyde, and phenylacetic acid) were of minor relevance for the pneumotoxic effects in the terminal bronchioles, and they proposed that ring-oxidized metabolites could be the cause of styrene-induced oncogenicity based on the cytotoxicity of the ring-oxidized metabolite 4-vinylphenol for Clara cells and the resulting proliferative response in the terminal bronchioles.

Chung *et al.* (2006) compared the cytotoxicity of styrene and styrene-7,8-oxide in a transgenic cell line expressing CYP2E1 (h2E1) and the wild-type cell line (cHol, human B-lymphoblastoid). Cell viability assays demonstrated that styrene was toxic to h2E1 cells (IC_{50} = 121.8 μM) but no significant increase in cell death was observed in wild-type cells at concentrations as high as 1,000 μM. However, there was no significant difference in susceptibility of h2E1 and wild-type cells exposed to styrene-7,8-oxide. These data indicate that CYP2E1 and styrene-7,8-oxide have an important role in the cytotoxic effects of styrene in these cell lines. Inhibition of epoxide hydrolases enhanced cytotoxicity while glutathione conjugation reduced cytotoxicity.

5.2.2.3 Toxicity of styrene stereoisomers

Gadberry *et al*. (1996) examined the pneumotoxicity and hepatotoxicity of styrene and styrene-7,8-oxide (the racemic mixture and *R*- and *S*-enantiomers) in adult male non-

Swiss albino mice. GGT and LDH activity in bronchioalveolar lavage fluid and the activity of the hepatic enzyme serum sorbitol dehydrogenase (SDH) were measured. Groups of 8 to 10 mice were sacrificed 24 hours after i.p. injection with 300 mg/kg b.w. of either racemic, *R*-, or *S*-styrene-7,8-oxide or 800 mg/kg b.w. of styrene, and another group of mice was sacrificed 6 hours after i.p. injection with 300 mg/kg b.w. of either racemic, *R*-, or *S*-styrene-7,8-oxide. Data for the 24-hour sacrifice are shown in Figure 5-2. The *R*-isomer of styrene-7,8-oxide was more hepatotoxic than the *S*-isomer at both the 6-hour (data not shown) and 24-hour time points, based on a significant ($P < 0.05$) increase in SDH activity. In the tests for pneumotoxicity (GGT and LDH), enzyme activity was higher at both time points in the lungs of mice administered the *R*-isomer than in those administered the *S*-isomer. The results for the racemic mixture were variable, being sometimes higher than for either individual isomer, sometimes lower, and sometimes intermediate. However, the authors reported that none of the differences were statistically significant.

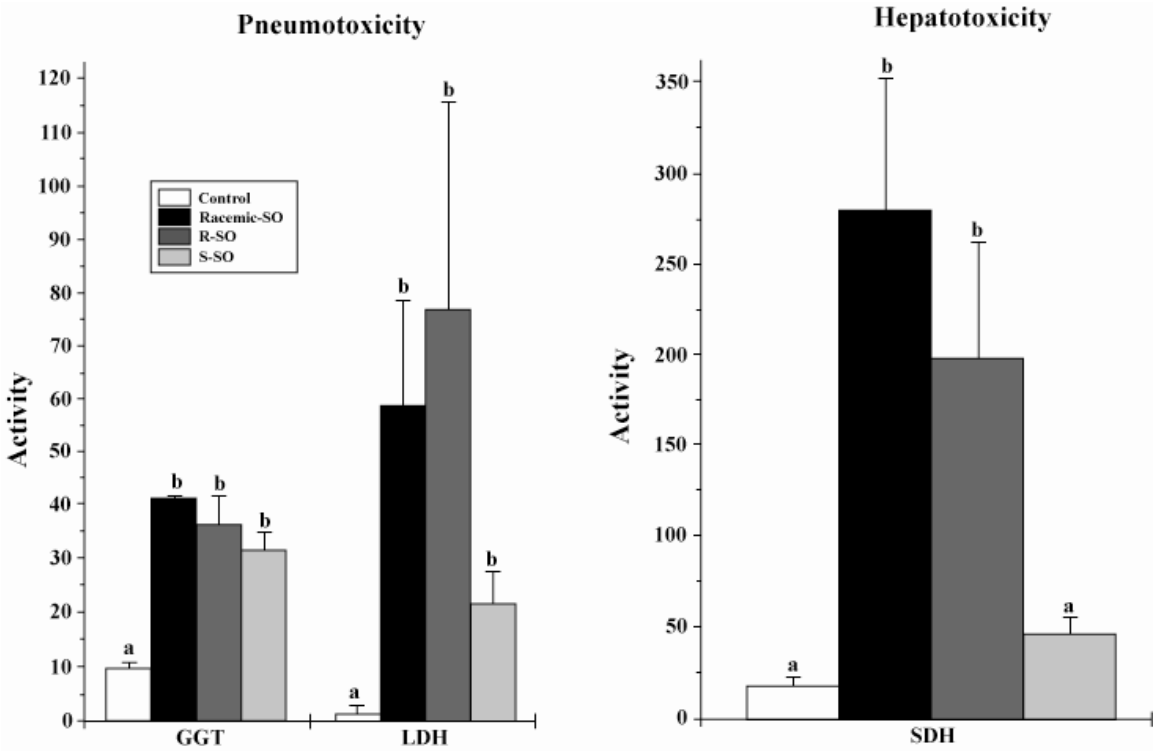


Figure 5-2. Pneumotoxicity and hepatotoxicity of styrene-7,8-oxide enantiomers in male non-Swiss albino mice at 24 hours after i.p. administration

Source: adapted from Gadberry *et al.* 1996.

Results for exposure groups with different letters (a, b) differed significantly from each other at $P < 0.05$.

In an *in vitro* study using isolated Clara-cell (~90% of total cells) preparations prepared from lungs of adult male CD-1 mice, Harvilchuck and Carlson (2006) reported that styrene (LC_{50} = 1.721 mM) was the most toxic substance tested, followed by racemic styrene-7,8-oxide (2.344 mM), *R*-styrene-7,8-oxide (3.243 mM), 4-vinylphenol (3.500 mM), and *S*-styrene-7,8-oxide (4.842 mM); [however, no statistical comparisons were reported and the toxicity data for the various compounds appeared to overlap in the published graph]. Clara cells isolated from Sprague-Dawley rats were 4-fold less susceptible to cytotoxicity of styrene and its metabolites than mouse Clara cells. Styrene also was the most toxic compound tested in isolated rat Clara cells with 4-vinylphenol and *S*-styrene-7,8-oxide being the least toxic. Incubation of mouse Clara cells with test agents at 0.1, 0.5, and 1.0 mM concentrations resulted in significantly ($P < 0.05$, Student-Newman-Keul's test) decreased glutathione levels after a 3-hour incubation with the following substances and concentrations: styrene (1.0 mM), racemic styrene-7,8-oxide (0.5 or 1.0 mM), *R*-styrene-7,8-oxide (1.0 mM), *S*-styrene-7,8-oxide (1.0 mM), and 4-vinylphenol (0.5 or 1.0 mM). In *in vivo* experiments, racemic and *R*-styrene-7,8-oxide (300 mg/kg b.w. doses for each) significantly ($P < 0.05$, Student-Newman-Keul's test) decreased Clara-cell glutathione concentrations at 3 hours after intraperitoneal injection of test agents compared with corn-oil controls, while neither styrene (600 mg/kg b.w.), *S*-styrene-7,8-oxide (300 mg/kg b.w.), nor 4-vinylphenol (100 mg/kg b.w.) differed significantly from controls at this time point.

Several studies have shown differential genotoxicity of the styrene-7,8-oxide enantiomers, in the following order of mutagenicity: *R*-enantiomer > racemic mixture > *S*-enantiomer (Pagano *et al.* 1982, Seiler 1990). However, chromosomal aberrations and sister chromatid exchange in mouse bone marrow cells were increased significantly following *in vivo* exposure to the *S*-isomer but not the *R*-isomer, and the mitotic index was decreased significantly for both isomers (Sinsheimer *et al.* 1993). (See Section 5.5.2.4 for a more detailed discussion of these studies).

5.2.2.4 Gluthathione depletion

Depletion of GSH in the lung has been reported to be associated with an increased risk of lung damage and disease (Rahman *et al.* 1999), and GSH depletion generally has been

shown to correlate with chromosomal DNA fragmentation associated with apoptosis and necrosis (Higuchi 2004). Although Cohen *et al.* (2002)[6] reported that both measured GSH concentrations and those predicted by a physiologically based pharmacokinetic (PBPK) model indicated a greater decrease in GSH in the lungs of mice than rats, these effects were seen only at styrene concentrations of 40 ppm or greater, which exceeded the concentration of 20 ppm at which hyperplasia in the mouse lung was reported by Cruzan *et al.* (2001). Also, Gamer *et al*. (2004) reported that 20 exposures (6 hours per day, 5 consecutive days per week, for 4 weeks) of female CD-1 mice to styrene at 160 ppm, which produced cellular crowding in the epithelial lining of the lung, indicative of very early hyperplasia, did not increase concentrations of 8-hydroxy-deoxyguanosine. No evidence of oxidative stress was seen despite depletion of GSH in homogenates from the styrene-exposed mouse lungs. Similarly exposed female Crl:CD rats did not show any signs of lung toxicity. Turner *et al*. (2005) also found a decrease in GSH in the lungs of mice exposed by i.p. injection to styrene (600 mg/kg b.w., or 5.8 mmol/kg) and styrene-7,8-oxide (300 mg/kg b.w., or 2.5 mmol/kg). However, administration of 4-vinylphenol (100 mg/kg, or 0.83 mmol/kg), which is a more potent hepatotoxin and pneumotoxin than styrene or styrene-7,8-oxide, caused less depletion of GSH.

5.2.3 Estrogenicity studies

Several studies have been published on the potential estrogenicity of polystyrene oligomers, which can leach from polystyrene food containers. Polystyrene dimer and trimer extracts from food containers were tested *in vitro* for estrogen-like effects using estrogen-responsive element reporter, estrogen receptor binding, and cell-proliferation assays, and *in vivo* using a rat uterotrophic assay. Bachman *et al.* (1998) measured the effect of extracts from 23 polystyrenes in a rat uterotrophic assay at concentrations up to 0.75 mg/L [equivalent to 15 microgram/kg-b.w. per day]. None of the polystyrene extracts were positive in this assay. Fail *et al.* (1998) measured the estrogenicity of a polystyrene extract equivalent in dose per body weight to human consumption [amount not specified]. It was negative in a rat uterotrophic assay and in an estrogen-responsive element reporter assay [3 mM, approximate maximum concentration of polystyrene

[6] The expert panel evaluation conducted by the Harvard Center for Risk Analysis and funded by the Styrene Information and Research Center (SIRC).

extract tested]. Azuma *et al.* (2000) and Date *et al*. (2002) reported a lack of estrogenicity of styrene monomers, dimers and trimers using *in vivo* and *in vitro* assay systems. Ohno *et al.* (2001) used high concentrations [up to 10^{-3} M *in vitro*] of specific oligomers in uterotrophic, estrogen-responsive element reporter, and estrogen receptor binding assays and also obtained negative results. In this study, styrene monomer, three styrene dimers, and seven styrene trimers known to dissolve in small amounts from polystyrene cup noodle containers were tested. However, Ohyama *et al.* (2001) tested the same styrene dimers and trimers and obtained positive results (2 positives out of 4 dimers tested and 4 positives out of 7 trimers tested) at concentrations of 10^{-6} and 10^{-5} M [highest concentration tested] in a cell-proliferation assay and in a binding affinity assay for human estrogen receptor alpha (9 oligomers were positive and 2 trimers were negative in this assay). These results were refuted by Ohno *et al.* (2003), whose laboratory tested the same oligomers from the Ohyama report using three different estrogen receptor binding assays. The results for all oligomers were negative in these assays. Further, the results of a rat uterotrophic assay and estrogen response element reporter assay were also negative using the same styrene oligomers. In a letter to the journal *Environmental Health Perspectives*, Ohno and colleagues (Ohno *et al.* 2002) noted that in the assay system of Ohyama, solubility was a problem at high concentrations leading to false positive results, and the validity of the MCF-7 E-Screen assay was also questioned. Ohyama and Nagi replied that their results were valid, because they believed the insolubility of styrene oligomers observed in Ohno and colleagues' studies appeared to be due to using water rather than DMSO (as was used in the Ohyama studies) to dissolve the compounds. Ohyama also defended the use of the MCF-7 E-screen method as a well recognized method for estrogenic screening.

It is possible that metabolic activation of styrene oligomers may affect the estrogenicity of these compounds. Kitamura *et al.* (2003), using rat liver microsomes as a metabolic activating system, found the activated form of *trans*-1,2 diphenylcyclobutane, a styrene dimer, to be estrogenic using a yeast estrogen screening assay and an estrogen-responsive element reporter assay. The active metabolite was a hydroxylated form called *trans*-1(4-hydroxyphenyl)2-phenylcyclobutane [activity at 10^{-5}M]. According to the authors, *cis*-1,2-diphenylcyclobutane, 1,3-diphenylpropane, and 2, 4-diphenyl-1-butene also exhibited

estrogenic activity after metabolic activation, but the activity was lower than with *cis*-1,2-diphenylcyclobutane.

5.3 Interspecies differences in metabolism, toxicity, and toxicokinetics

In its summary of styrene exposure studies in humans (volunteers or workers) and experimental animals, IARC (2002) stated that toxicokinetic pathways are qualitatively similar in humans and animals, but differ quantitatively. This section reviews studies and toxicokinetic models of interspecies differences in styrene-7,8-oxide formation, stereochemistry, and metabolism of styrene-7,8-oxide.

5.3.1 Styrene-7,8-oxide formation in the lung

Styrene-7,8-oxide, a primary metabolite of styrene, is considered to cause many of the toxic and genotoxic effects resulting from styrene exposure, including those in the lung. Clara cells are primarily responsible for the metabolism of styrene to styrene-7,8-oxide in the lung (see Section 5.1.3), and metabolism results in formation of two optically active enantiomers (*R*- and *S*-forms) because of the chiral carbon in styrene-7,8-oxide. Plopper *et al.* (1980a, 1980b) identified interspecies morphological differences in Clara cells that are consistent with the observed differences in styrene metabolism in rodent and human lung. Mouse and rat Clara cells contain an abundance of agranular endoplasmic reticulum, which is associated with metabolism of pulmonary toxins via cytochromes P450. Human Clara cells contain abundant granular endoplasmic reticulum but no agranular endoplasmic reticulum.

Cohen *et al.* (2002) reviewed studies measuring conversion of styrene to styrene-7,8-oxide by cytochrome P450 monooxygenase in pulmonary tissues in rats, mice, and humans. Most studies showed styrene-7,8-oxide production to be highest in mice (0.95 to 4.5 nmol/min per mg protein), followed by rats (0.32 to 11.7 nmol/min per mg protein), and humans (0.006 to 0.014 nmol/min per mg protein). Cohen *et al.* also noted that metabolic conversion rates varied according to cell type or tissue. Mouse Clara cells produced styrene-7,8-oxide (193 pmol/10^6 Clara cells per minute) at 3 times the rate of rat Clara cells (59 pmol/10^6 cells per minute); however, when more aggregate pulmonary tissue fractions (pulmonary microsomes) were compared, the rates differed by a factor of 1.5 (2.13 nmol/min per mg protein in mice vs. 1.44 in rats) (Hynes *et al.* 1999). However,

Cohen *et al.* concluded that differences in styrene-7,8-oxide concentrations in the lung do not sufficiently explain the differences in susceptibility to the carcinogenic effects of styrene between rats and mice. Rats did not develop lung tumors in groups that had similar predicted styrene-7,8-oxide concentrations in the lungs compared with groups of mice that developed lung tumors.

Hofmann *et al.* (2006) investigated the styrene-7,8-oxide levels formed in isolated lungs of male Sprague-Dawley rats and *in-situ* prepared lungs from male $B6C3F_1$ mice. Styrene vapor concentrations were measured in air samples collected in the immediate vicinity of the trachea and were almost constant during each experiment. Styrene vapor concentrations ranged from 50 to 980 ppm for rats and 40 to 410 ppm for mice. Both species metabolized styrene to styrene-7,8-oxide; however, mean styrene-7,8-oxide levels in mouse lungs were about 2 times higher than in rat lungs at equal exposure conditions.

5.3.2 Detoxification of styrene-7,8-oxide in respiratory tissue

Styrene-7,8-oxide is detoxified through hydrolysis mediated by mEH or conjugation with glutathione mediated by GST. Cohen *et al.* (2002) summarized studies in rodents and humans measuring the capacity of mEH in pulmonary tissue to detoxify styrene-7,8-oxide. The metabolic conversion rates (in nanomoles per minute per milligram of protein) for hydration of styrene-7,8-oxide by mEH to form styrene glycol varied among studies, ranging from 0.4 to 2.1 in mice, 0.6 to 2.6 in rats (one study reported < 0.1), and 2.0 to 3.4 in humans. The estimated K_ms for hydrolysis from a previous study were 0.013 mM in mice, 0.0047 mM in rats, and 0.0156 mM in humans. The metabolic conversion rates for conjugation of styrene-7,8-oxide mediated by GST in pulmonary tissues varied among studies (as summarized in Cohen *et al.* 2002); the one study in humans reported a rate similar to that in one study in mice but lower than the rates from other studies in mice or rats. The estimated ratio of V_{max} to K_m (which Cohen stated was an indication of GST metabolic activity) was much lower in humans (19) compared with mice (171) or rats (1,982).

Green *et al*. (2001b) investigated the cytochrome P450-mediated metabolism of styrene to styrene-7,8-oxide and subsequent metabolism of styrene-7,8-oxide by either mEH or GST in nasal and liver microsomal fractions from mice, rats, and humans. P450

metabolism of styrene to styrene-7,8-oxide was similar in rat and mouse olfactory and respiratory fractions but was not detected in human nasal samples. Rates in rodent olfactory fractions were higher than those measured in respiratory or liver fractions. The rates of metabolism of *R* and *S* styrene-7,8-oxides via mEH in rat respiratory fractions were up to 3.5-fold higher while rates in olfactory fractions were up to 10-fold higher than in mice. Rates of mEH-mediated metabolism of styrene-7,8-oxide in human nasal fractions were comparable with mouse olfactory and respiratory tissues and rat respiratory tissues. Rodent nasal and respiratory tissues also metabolized styrene-7,8-oxide via GST at rates significantly higher than those for mEH. Olfactory fractions from rats had 3- to 4-fold greater rates of glutathione conjugation than observed in mice. In contrast, metabolism of styrene-7,8-oxide by glutathione conjugation was undetectable in 5 of 6 samples of human nasal tissues, and the sixth sample metabolized styrene-7,8-oxide at a much lower rate than did mouse or rat tissues.

5.3.3 Stereochemistry considerations

The metabolic activation of styrene to styrene-7,8-oxide enantiomers has been reported to depend on tissue and species, and some authors have suggested that the *R*-enantiomer is more toxic. Cohen *et al.* (2002) summarized the results of studies evaluating the ratio of the *R*- to *S*-enantiomers of styrene-7,8-oxide produced through pulmonary and hepatic metabolism. The results from these studies showed that mouse lung microsomes produced greater amounts of the *R*-enantiomer than did microsomes from rat or human lung. In mouse lung, the *R*/*S* ratio was usually between 2.4 and 2.6, although one study reported a ratio of 1.7; in rat lung, the ratio was 0.52, based on one available study, and in human lung, it was 1.15, based on 1 sample from one study. In hepatic microsomes, the *R*/*S* ratio ranged from 1.18 to 1.78 in mice and was 0.57 in rats (one study) and 0.72 in humans (one study). Cohen *et al.* reported that their PBPK model predicted that the *R*/*S* ratio in mouse and rat lungs would be approximately twice as high as the ratio for total styrene-7,8-oxide between the two species; nevertheless, these differences were insufficient to explain the differences in susceptibility. In studies in mice, the *R*/*S* ratio was 4.0 in isolated Clara cells, but 3.6 in type II cells. In rats, on the other hand, Clara cells produced a nearly racemic mixture of enantiomers, and the *S*-isomer predominated

in type II cells. Green *et al.* (2001b) reported an *R*/*S* ratio of approximately 3 in nasal tissue of rats and mice and liver tissue of mice, and a ratio of 0.72 in liver tissue of rats.

Linhart (2001) reported that human liver microsomes produced a nearly racemic mixture of enantiomers; however, 2 samples showed a predominance of the *S*-isomer. Wenker *et al.* (2001b) reported variable enantioselectivity in human liver microsomes, which produced a moderate excess of the *S*-isomer at a low styrene concentration (16 μM) (mean ± SD = 14.7% ± 6.9%) but an excess of the *R*-isomer at a high styrene concentration (1,100 μM) (7.0% ± 8.9%). When Wenker *et al.* (2000) compared the metabolism of *R*- and *S*-styrene-7,8-oxide by 20 human liver microsomal preparations, they found among the samples a 3- to 5-fold variation in the V_{max}, K_m, and V_{max}/K_m values for the two enantiomers. They were able to demonstrate that the mEH-mediated hydrolysis of styrene-7,8-oxide favored hydrolysis of the reportedly more toxic *R*-enantiomer, because the *S*-isomer had a higher K_m (by a factor of ~6) and V_{max} (by a factor of ~5) than the *R*-isomer. The authors found no association between enzyme kinetics and mEH polymorphisms at exons 3 and 4.

Because of the differences in enantiomeric excess found for each metabolite, Wenker *et al.* (2001a) concluded that the individual enzymes responsible for the biotransformation and excretion of styrene-7,8-oxide differed in their enantiomeric selectivity and/or specificity. Hallier *et al.* (1995) determined that the *R*/*S* ratio ranged from 0.7 to 2.2 in 20 male German workers in the polyester industry exposed to styrene by inhalation at concentrations ranging from 29 to 41 ppm; the differences in the *R*/*S* ratio could not be explained by differences in individual exposure or in urinary metabolite concentrations. The authors proposed that interindividual differences in metabolism of styrene to *R*- and *S*-enantiomers were likely related to enzyme polymorphisms. Drummond *et al.* (1989) measured excretion of *R*- and *S*-enantiomers of mandelic acid in three workers occupationally exposed to 8-hour time-weighted average styrene concentrations of up to 420 mg/m^3 [100 ppm]. The *R/S* ratios for the three individuals were 1.16, 1.27, and 1.14.

Linhart (2001) reviewed the stereochemistry of styrene biotransformation and concluded that the ratio of the enantiomers in a target tissue or cell will depend on both the

stereoselectivity of the formation of styrene-7,8-oxide and the stereoselectivity of the metabolism of styrene-7,8-oxide. In rats, the formation reaction favors the *S*-enantiomer, and detoxification of the *R*-enantiomer is faster. The formation reaction in mouse liver and lungs favors the *R*-enantiomer, but detoxification moves the ratio closer to a racemic mixture. Linhart concluded that the stereochemistry of styrene biotransformation might contribute to species differences in toxicity between mice and rats but that it could not be interpreted as a crucial factor. In addition, the author concluded that the relationship of styrene stereochemistry to toxic effects in humans could not be interpreted, because relevant data were lacking.

5.3.4 Kinetics of styrene metabolism and toxicokinetic models

In addition to the cell-specific metabolism of styrene discussed above, several studies have focused on the kinetics of styrene and styrene-7,8-oxide metabolism in the whole animal for several species (IARC 2002). In one study, the rate of metabolism of styrene to styrene-7,8-oxide was compared among species: the order was guinea-pig > rabbit > mouse > rat. However, for metabolism of styrene-7,8-oxide to styrene glycol by mEH, the order was rat > rabbit > guinea-pig > mouse. In another study, the rate depended on the styrene concentration, decreasing from mouse to rat to human at a low concentration, but from rat to mouse to human at a high concentration (IARC 2002). Cruzan *et al.* (2001, 1998) reported that styrene-7,8-oxide concentration in the blood was lower in mice exposed to a concentration of 160 ppm [an exposure level associated with lung cancer] than in rats exposed at 1,000 ppm [an exposure level at which no tumors were observed].

Several pharmacokinetic models have been developed that compared styrene distribution and metabolism in mice, rats, and humans. Sarangapani *et al*. (2002) reported that the earlier models (Csanady *et al.* 1994, Ramsey and Andersen 1984) did not treat the respiratory tract as a target organ and did not incorporate metabolic production and clearance of styrene-7,8-oxide in the respiratory tract. Therefore, the Sarangapani *et al.* PBPK model incorporated a multicompartmental description of the respiratory tract and specifically added a compartment to represent the terminal bronchiolar region. This model was based on metabolism of styrene in the liver and the terminal bronchiolar

region of the lung, which is richest in the metabolically active Clara cells and in which the authors considered styrene-mediated toxicity to occur.

Filser *et al*. (2002) also developed a PBPK model for styrene in mice, rats, and humans based on metabolism in both the liver and lung. This model divided the lung into two compartments: the gas conducting zone and the gas exchange zone. The enzymatic capacities of the two compartments were based on their shares of the total lung volume because the kinetics of styrene and styrene-7,8-oxide metabolizing enzymes were determined in microsomes and cytosol from whole lung tissue. They tested the validity of their model by comparing the predicted area under the curve for blood styrene-7,8-oxide concentration with reported values from published studies in Sprague-Dawley and F344 rats and $B6C3F_1$ mice.

Both of these models predicted that the order of styrene concentration in the lung (Filser *et al.*) or terminal bronchioles of the lung (Sarangapani *et al.*) would be mouse > rat > human. The Harvard Center for Risk Analysis also developed a PBPK model that predicted the concentrations of styrene-7,8-oxide and *R*-styrene-7,8-oxide in the tissues of humans, rats, and mice exposed to styrene at different concentrations (Cohen *et al.* 2002). This model used Csanády *et al.* (1994) as a starting point but included several modifications (e.g., equations to account for styrene metabolism in the lung and to estimate *R*- and *S*-styrene-7,8-oxide concentrations in various tissues). Results from this model were inconclusive because of inconsistencies among studies in the measured levels of styrene-7,8-oxide in the blood. Depending on the data used for calibration, the model sometimes predicted higher concentrations of styrene-7,8-oxide in the lungs of rats, while in other cases, higher concentrations were predicted for the mouse. However, Csanády *et al.* (2003) reported that there was a typographical error in an equation described in Csanády *et al.* 1994 that was overlooked by Cohen *et al.* (2002) and could explain their inability to copy the Csanády *et al.* model.

Although the available data suggested that the mouse has a greater metabolic capacity for converting styrene to styrene-7,8-oxide, and a greater pharmacokinetic response (particularly with respect to lung tumors), the data were insufficient to explain why mice

were more susceptible than rats. These authors concluded that the existing pharmacokinetic data failed to explain the observed differences in metabolite levels in mice, rats, and humans. These authors further noted that the current inability to explain species differences makes it difficult to determine whether the rat or the mouse is the better model for the human response to styrene.

5.4 Genetic and related effects

Biotransformation of styrene to the genotoxic styrene-7,8-oxide seems to be responsible for the majority of the genotoxic effects associated with styrene exposure (Cohen *et al.* 2002). Furthermore, during the manufacture of reinforced plastics, styrene and trace amounts of styrene-7,8-oxide are released, thus, direct occupational exposure of workers to styrene-7,8-oxide has been shown (see Section 2.5.1.6) (Nylander-French *et al.* 1999, Rappaport *et al.* 1996, Tornero-Velez and Rappaport 2001). This section summarizes the publicly-available peer-reviewed literature on the genetic and related effects of styrene. Styrene genotoxicity has been investigated in many *in vitro* and *in vivo* studies and reviewed in several publications (Barale 1991, Cohen *et al.* 2002, IARC 1994a, 2002, Scott and Preston 1994a, Speit and Henderson 2005, Vodicka *et al.* 2006b). The genetic and related effects discussed below include studies of DNA adducts, alkali-labile lesions, DNA strand breaks, cytogenetic damage, and mutations, with a focus on mammalian systems, especially human cells and studies of styrene-exposed workers.

5.4.1 DNA adduct formation

This section discusses formation and chemistry of styrene-7,8-oxide DNA adducts. Specific studies of DNA adduct formation in cell cultures, experimental animals, and styrene-exposed workers are discussed in the following sections.

DNA adduct detection methods are important tools for determining the etiology of human cancer and for measuring metabolic enzyme and DNA repair system genotypes (Collins 1998, Hemminki *et al.* 2000, Perera and Weinstein 2000). The major metabolite of styrene *in vivo* is styrene-7,8-oxide, which is expected to bind covalently to biological macromolecules. The binding of styrene-7,8-oxide to nucleic acid constituents has been studied extensively during the last 20 years; however, no studies on other styrene metabolites with the potential to bind DNA (e.g., styrene 3,4-oxide) were identified.

Styrene-7,8-oxide possesses two sites (the α- and β-carbons of the epoxide moiety; see Figure 1-2) that are electrophilic and able to react at nucleophilic sites in DNA. Either carbon in the epoxide of styrene-7,8-oxide can react with nucleic acid, and because the carbon atom at the 1-position is a chiral center, there are four possible diastereomers (*R*- and *S*-isomers of the alpha form and *R*- and *S*- isomers of the beta form) (Phillips and Farmer 1994).

The reaction mechanisms of styrene-7,8-oxide alkylation have been intensively studied (Barlow and Dipple 1998, 1999, Latif *et al.* 1988, Qian and Dipple 1995). In this document, binding sites for adducts are identified by the position of the atom as part of a ring (the atom to which the adduct is bound is followed by its position in the ring [e.g., N3 of deoxyguanosine]) or as an exocyclic group on the ring (the atom to which the adduct is bound is followed by the position of the ring atom to which it is bound, superscripted [e.g., N^2 of deoxyguanosine]). The primary target of styrene-7,8-oxide alkylation in DNA is a guanine residue (Hemminki and Hesso 1984, Koskinen *et al.* 2000b, Koskinen *et al.* 2000a, Latif *et al.* 1988, Savela *et al.* 1986). Styrene-7,8-oxide forms adducts at the N7-, N^2-, and O^6-positions of guanine, the N1-, N3-, and N^6-positions of adenine, the N3-, N^4-, and O^2-positions of cytosine, and the N3-position of thymine (Figure 5-3). *In vitro* studies indicated that N7- and N^2-alkylguanine and O^6-adducts of guanine adducts were the most abundant, followed by adducts with deoxycytidine (N3, N^4, and O^2), deoxyadenosine (N1, N3, and N^6), and thymidine (N3) (IARC 2002). The relative reactivity of the nucleosides with styrene-7,8-oxide are dG > dC > dA > T, while the alkylation rates of guanine by styrene-7,8-oxide are deoxyguanosine > single-stranded DNA > double-stranded DNA (Phillips and Farmer 1994, Savela *et al.* 1986). Vodička and Hemminki (1988) reacted radioactive styrene-7,8-oxide with double- and single-stranded DNA. The N7-, N^2-, and O^6-guanine adducts accounted for at least 95% of the total in single stranded DNA and formed in the proportions 54:33:12. The proportions were 74:23:3.7 in double-stranded DNA, indicating suppression at atoms that take part in hydrogen bonding in double-stranded DNA (N^2 and O^6). At neutral pH, styrene-7,8-oxide in solution with guanosine alkylated the nucleoside mainly at the N7-position (57% of identified products), followed by the

N^2- (28%) and O^6-positions (15%) (Hemminki and Hesso 1984). When styrene-7,8-oxide was incubated *in vitro* with double-stranded DNA, the α and β forms of the N7-guanine adduct together constituted up to 74% of total adducts formed, while the α form of the N^2-guanine adduct constituted about 3% and the O^6-guanine adduct about 1% (Koskinen *et al.* 2001b, Vodicka *et al.* 2002a). The exact proportion of O^6-guanine adducts has been difficult to determine because of their chemical instability; however, the half-life of the α isomer of O^6-guanine adducts in double-stranded DNA has been estimated to be 1,320 hours. Thymidine is a poor substrate for styrene-7,8-oxide, with only minor alkylation occurring at pH 7.4 and 37°C at the N3-position (Koskinen *et al.* 1999).

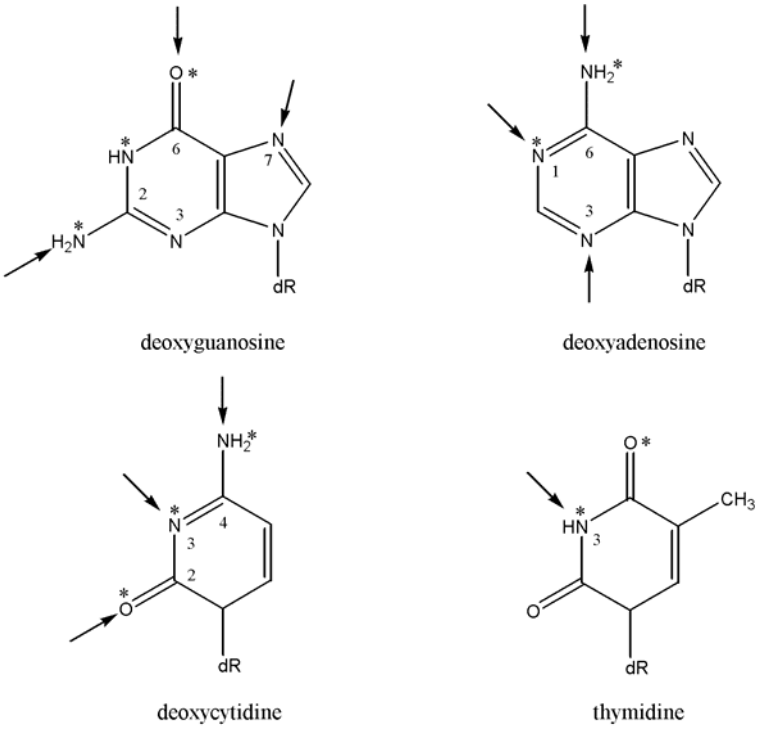


Figure 5-3. Styrene-7,8-oxide binding sites in DNA (from Vodicka *et al.* 2002a)
Styrene-7,8-oxide-binding sites are indicated by arrows; base-pairing sites in DNA are labeled with asterisks.

A number of interconversions may occur with styrene-7,8-oxide nucleotide adducts. N1-adenine adducts can deaminate to form the corresponding hypoxanthine adduct, and N3-deoxycytidine adducts are rapidly deaminated to the corresponding deoxyuridine. N1-adenine adducts also may undergo rearrangement to the N^6-adduct via the Dimroth

rearrangement, in which the adducted molecule moves from the ring nitrogen atom to the exocyclic nitrogen atom (Barlow *et al.* 1998). O^2-cytosine adducts also are unstable, being prone to depyrimidation and interconversion between the α- and β-isomers (Koskinen *et al.* 2000b). The chemically stable DNA adducts identified *in vitro* in the highest proportions are α-N^6–adenine, α-N^2–guanine, and β-N3–uracil (Koskinen *et al.* 2001b); however, these adducts have not yet been identified *in vivo* in experimental animals (Vodicka *et al.* 2006b). O^6-guanine adducts account for about 1% of total adducts (Vodicka *et al.* 1994). In the case of O^6-guanine adducts, the α-isomer can convert to the β-isomer in a base-catalyzed rearrangement (Moschel *et al.* 1986).

Structural data for both α- and β-N^6–dA adducts of styrene-7,8-oxide in DNA is available. The α-N^6–dA adducts locate in the major groove, with their orientation being dependent upon stereochemistry. Adducts with *R*-stereochemistry orient in the 5' direction; whereas, those with *S*-stereochemistry orient in the 3' direction (Feng *et al.* 1995, 1996, Stone and Feng 1996). While the adducts with *S*-tereochemistry induce a slight bend in the duplex, those with *R*-stereochemistry do not (Le *et al.* 2000). The structures of the diastereomeric α-N^6–*dA* adducts mispaired with dC, have also been examined, both in the 5'-CXA-3' sequence and the 5'-AXG-3' sequence. This represents the putative intermediate leading to A to G transitions. In the former sequence, the adduct with *S*-stereochemistry remains in the major groove and oriented in the 3'-direction, as observed for the corresponding adduct paired correctly with thymine. A shift of the modified adenine toward the minor groove results in the styrenyl ring stacking with the 5'-neighboring cytosine, which shifts toward the major groove. A wobble A•C base pair is not observed. In this mismatched duplex, the adduct of *R*-stereochemistry is disordered (Painter *et al.* 1999). In the 5'-CXA-3' sequence, the thermodynamic stability of both the mismatched *R*- and *S*-adducts is dependent upon pH. At neutral pH, both exhibit significant structural perturbations and lower T_m values, as compared with the 5'-CXA-3' sequence. This is attributed to reorientation about the adenine C6-N^6 bond. For the adduct of *R*-stereochemistry, the styrenyl moiety remains oriented in the major groove but now orients in the 3'-direction. For the adduct with *S*-stereochemistry, the styrene ring inserted into the duplex, approximately perpendicular to the helical axis of the DNA, but now in

the 5'-direction (Simeonov *et al.* 2000). The increased tether length of the β-styrene-7,8-oxide N^6-dA adducts results in two changes in structure as compared with the α-styrene adducts. First, less distortion is introduced into the duplex. For both the *R*- and *S*-β-N^6-dA adducts, the styrenyl moiety is accommodated within the major groove of the duplex with little steric hindrance. Second, it mutes the influence of stereochemistry, such that in contrast to the α-N^6-dA adducts, either the *R*- or *S*-stereoisomeric β-N6-dA adducts exhibit similar conformations within the major groove (Hennard *et al.* 2001).

5.4.2 In vitro studies

This section reviews *in vitro* studies of DNA adduct formation (5.4.2.1), DNA damage and repair (5.4.2.2), mutagenicity (5.4.2.3), and cytogenetic markers (5.4.2.4) for styrene and styrene-7,8-oxide.

5.4.2.1 DNA adducts

The various types of DNA adducts associated with exposure to styrene or styrene-7,8-oxide (based on the binding site on the nucleotide) are shown in Figure 5-3.

Styrene

No *in vitro* DNA adduct studies with styrene were identified.

Styrene-7,8-oxide

IARC (1994b, 2002) and Philips and Farmer (1994) reviewed several *in vitro* studies on DNA adduct formation in nucleosides, calf thymus or fish testis DNA, and in DNA in mammalian and human cells exposed to styrene-7,8-oxide. Studies in cellular systems are summarized in Table 5-2. Exposure of 9L cells [rat brain gliosarcoma cells] to 1 mM styrene-7,8-oxide, [a concentration that has resulted in increased chromosome aberrations in human lymphocytes *in vitro*], for 24 hours resulted in formation of several DNA adducts (Liu *et al.* 1988a). DNA adduct formation in human cells exposed to styrene-7,8-oxide has been studied in cultured peripheral blood lymphocytes (PBLs), human embryonic lung fibroblasts (HEL), and keratinocytes. DNA adducts (O^6-guanine, N7-guanine, or N^2-guanine) were induced in all cell lines. These studies indicated a dose-related increase in DNA adducts, persistence of the O^6-guanine adducts, and a correlation with single-strand breaks (see Section 5.4.2.2). In HEL cells, lower levels of N7-guanine

adducts were observed after 18-hour than 3-hour exposures; this finding could be due to the conversion of N7-guanine adducts into abasic sites either spontaneously or through the DNA repair process (Vodicka *et al.* 1996). Pauwels and Veulemans (1998) also reported N7-guanine adducts of styrene-7,8-oxide with human DNA when whole blood was incubated with styrene-7,8-oxide at 9.4 to 460 mM; however, they did not report the numbers of adducts formed.

Adduct persistence varies by binding site, and the time of exposure to the alkylating agent largely determines the relative proportions of the various DNA adducts. The available data indicate that the N7-adducts are lost, with an estimated half-life of about 19 hours in one study (Vodicka *et al.* 1996). Some data indicate that O^6 adducts are stable and can build up over time from chronic low-level exposures (Bastlová *et al.* 1995, Vodicka *et al.* 1999, Vodicka *et al.* 1994). An apparent saturation level is reached considerably faster for the N7-guanine and N3-adenine adducts, because these nucleotides depurinate more readily than adducts formed at positions involved in base-pairing (see Figure 5-3) (Vodicka *et al.* 2002a).

Table 5-2. Styrene-7,8-oxide DNA adducts formed in mammalian cells *in vitro*

	Treatment		Adducts		
Cell type	**Conc. (μM)**	**Duration**	**Type**	**No. per 10^8 dNp**	**Reference**
Rat 9L (gliosarcoma cells)	1,000	24 h	NR	1,950	Liu *et al.* 1988a
Human whole blood	9,400 49,000 96,000 240,000 460,000	2 h	N7-guanine	NR	Pauwels and Veulemans 1998
Human lymphocytes	200 400 600	24 h	O^6-guanine	0.98–2.04 2.46 2.59–4.05	Bastlová *et al.* 1995
	200	6 d	O^6-guanine	1.2	
	600	24 h	N7-guanine N^2-guanine	580 7	Vodicka *et al.* 2002a
Human embryonic lung fibroblasts	10 50 100	3 h	N7-guanine	10[a] 20[a] 35[a]	Vodicka *et al.* 1996
	10 50 100	18 h	N7-guanine	4[a] 8[a] 9[a]	
Human keratinocytes	100 300	NR	N7-guanine	140 470	Vodicka *et al.* 2002a

Cell type	Treatment		Adducts		Reference
	Conc. (μM)	Duration	Type	No. per 10^8 dNp	
	100 300	NR	N^2-guanine	0.8 1.4	

dNp = dinucleotide pair; NR = not reported.
[a] Levels were estimated from figures.

5.4.2.2 DNA damage and repair

Assays for DNA damage may detect double-strand and single-strand breaks, alkali-labile sites, oxidative DNA base damage, or crosslinks (DNA-DNA or DNA-protein). In addition, base and nucleotide excision repair processes also induce transient breaks; therefore, a high level of breaks may indicate high levels of DNA damage or repair (Collins *et al.* 1997). Collins *et al.* noted that single-strand breaks are quickly repaired and are not generally regarded as significantly lethal or mutagenic lesions. Genotoxic agents may directly induce single-strand breaks or may create apurinic/apyrimidinic sites, which are converted to strand breaks in the alkaline electrophoresis solution used in the comet assay.

Styrene

Two studies in *Escherichia coli* strain PQ37 gave somewhat conflicting results for DNA repair as measured by the SOS chromotest. One study gave negative results, and the other was inconclusive, showing positive results but no dose-response relationship (IARC 1994a)

Only one study of single-strand breaks was identified. Sina *et al.* (1983) developed an alkaline elution/rat hepatocyte assay to measure DNA single-strand breaks and tested the method on 91 compounds, including styrene and styrene-7,8-oxide. Rat hepatocytes were treated with styrene at concentrations of 0.03, 0.3, and 3 mM for 3 hours. Single-strand breaks were significantly increased at the highest concentration compared with controls.

Styrene-7,8-oxide

IARC (1994b) reviewed four studies of SOS induction in bacteria (one in *S. typhimurium* and three in *E. coli*). Three studies resulted in a positive response without metabolic activation. One study in *E. coli* was negative with or without metabolic activation.

Styrene-7,8-oxide induced single-strand breaks and DNA damage in rat hepatocytes (Sina *et al.* 1983), neuroadrenergic (pheochromocytoma) rat PC-12 cells (Dypbukt *et al.* 1992), human PBLs and HEL cells (Bastlová *et al.* 1995, Laffon *et al.* 2001b, 2002b, Laffon *et al.* 2003b, Vodicka *et al.* 1996), rat and human testicular cells (Bjørge *et al.* 1996), and Chinese hamster V79 lung fibroblast cells (Herrero *et al.* 1997). The results are summarized in Table 5-3. These studies indicated that single-strand breaks increased in a dose-related manner and were correlated with formation of DNA adducts. Additionally, Bastlová *et al.* (1995) and Vodicka *et al.* (1996) showed that single-strand breaks in DNA in human PBLs and HEL cells were repaired rapidly, with approximate half-lives of 40 to 80 minutes. Vodicka *et al*. (1996) concluded that N7-guanine adducts were important in the formation of single-strand breaks, because of their strong correlation in HEL cells. Higher concentrations of styrene-7,8-oxide were required to induce single-strand breaks in Chinese V79 hamster cells engineered to express human mEH than in cells lacking this enzyme, suggesting that mEH might have protective effects (Herrero *et al.* 1997) (see Sections 5.1.3.6 and 5.3.2 regarding the role of mEH in detoxification). Marczynski *et al.* (1997b) exposed human whole blood to styrene-7,8-oxide for 1.5 to 4 hours and reported that the observed degradation of high molecular weight-DNA fragments in white blood cells was likely due to oxidative stress.

Table 5-3. DNA damage in mammalian cells exposed to styrene-7,8-oxide

Cell type	Treatment concentration (time)	Assay method	LEC/HIC	Results	Reference
Rat hepatocytes	30–3,000 μM (3 h)	Alkaline filter elution	300 μM	+	Sina *et al.* 1983
Rat PC 12 (neuroadrenergic cells)	30–1,000 μM (1 h)	Alkaline filter elution	30 μM	+ Repair after 3 h in fresh medium: 30 μM = 100%, 100 μM = 40% No double-strand breaks or cross-links	Dypbukt *et al.* 1992
Chinese hamster V79 (lung fibroblast cells)	10–1,000 μM (1 h)	Alkaline filter elution	50 μM (mock-transfected cells) 200 μM (hmEH-transfected cells)	+	Herrero *et al.* 1997
Human lymphocytes	10–100 μM (1 h)	Comet assay	Concentration-dependent increase	+ Levels reduced after 1-2 h in fresh medium and restored to control levels after 24 h	Bastlová *et al.* 1995
	0.06–0.18 μmol[a] (1.5–4 h)	Pulsed-field and conventional gel electrophoresis	0.06 μmol[a]	+ No clear association with length of exposure	Marczynski *et al.* 1997b
	10–200 μM (0.5 h)	Comet assay	50 μM	+ DNA damage was correlated with SO concentration	Laffon *et al.* 2001b
	10–200 μM (0.5 h)	Comet assay	50 μM	+ Levels reduced after 30 min in fresh medium and restored to control levels (high-dose group) after 4 h	Laffon *et al.* 2002b
	50–200 μM (0.5 h)	Comet assay	50 μM	+ Increased damage was associated with decreasing EH activity	Laffon *et al.* 2003b
Human embryonic lung fibroblasts	10–100 μM (3–18 h)	Alkaline DNA unwinding and separation of ds and ss DNA by hydroxyapatite chromatography	Concentration-dependent increase at 3 h(P = 0.003)	+ Levels were ~3-fold higher after treatment for 3 h compared with 18 h at 100 μM	Vodicka *et al.* 1996
Human and rat testicular cells	10–300 μM (0.5 h)	Alkaline filter elution	100 μM	+	Bjørge *et al.* 1996

ds = double-stranded; hmEH = human microsomal epoxide hydrolase; HIC = highest ineffective concentration; LEC = lowest effective concentration; SO = styrene-7,8-oxide, ss = single-stranded; SSB = single-strand breaks.
[a]Reported by Marczynski *et al.* (1997b) as dose in μmol.

5.4.2.3 Mutagenicity

The mutagenicity of styrene and styrene-7,8-oxide has been investigated in a number of *in vitro* systems and is discussed below and summarized in Table 5-4.

Styrene

Most of the studies on styrene mutagenicity in bacterial systems were conducted two or three decades ago, and the results were reviewed by IARC (1994a, 2002). Briefly, in *Salmonella typhimurium* strains, the majority of studies on reverse mutation gave negative results without metabolic activation. A few studies reported positive results with metabolic activation in TA100, TA1530, and TA1535, which detect base-pair substitutions. In eukaryotes, positive results were reported for *Saccharomyces cerevisiae* (reverse mutation and gene conversion), *Drosophila melanogaster* (sex-linked recessive mutation in one study), and *hprt* mutations in Chinese hamster V79 cells with metabolic activation (one study). Negative results were reported in two studies of forward mutations in *Schizosaccharomyces pombe*, one study of *w*/*w*+ somatic mutations in *D. melanogaster*, and two studies of *hprt* mutations in Chinese hamster V79 cells without metabolic activation (IARC 1994a, 2002).

Styrene-7,8-oxide

Styrene-7,8-oxide was mutagenic in the majority of *in vitro* systems, primarily without metabolic activation. Positive results were found in *S. typhimurium*, *E. coli* (SOS chromotest), *Klebsiella pneumoniae*, *S. cerevisiae*, *S. pombe*, and *D. melanogaster* (mixed results) and at the *tk* locus in mouse lymphocytes, the *hprt* locus in Chinese hamster V79 cells, and the *HPRT* locus in human T lymphocytes and B lymphoblastoid cells (weakly positive). Negative results were found in the *D. melanogaster w*/*w*+ somatic mutation assay (Rodriguez-Arnaiz 1998). Bastlová and Podlutsky (1996) characterized *HRPT* mutations induced by styrene-7,8-oxide in T lymphocytes. They found that the dominating base substitution in the *HPRT* gene was an A·T→G·C transition, followed by G·C→T·A and A·T→T·A transversions. The DNA adducts resulting in some of these base substitutions were tentatively identified as N^6-alkyladenine (A·T→G·C transition) and N7-alkylguanine (G·C→T·A and A·T→T·A transversions).

Several studies also have compared the mutagenicity of styrene-7,8-oxide enantiomers (Pagano *et al.* 1982, Seiler 1990, Sinsheimer *et al.* 1993). Pagano *et al*. (1982) investigated the mutagenic properties of the *R*-enantiomer, the *S*-enantiomer, and a racemic mixture of *R*- and *S*-enantiomers in *S. typhimurium* TA100. The order of mutagenicity was *R*-enantiomer > racemic mixture > *S*-enantiomer. Seiler (1990) reported on similar studies with styrene-7,8-oxide enantiomers in *S. typhimurium* TA100; an intrinsic difference in the mutagenic activity of the enantiomers was strongly suggested by evidence of qualitative differences in their binding to DNA. Sinsheimer *et al*. (1993) also found the *R*-enantiomer to be a more potent mutagen in *S. typhimurium* than the *S*-enantiomer; however, these results were not predictive of *in vivo* genotoxicity in mice where the *S*-enantiomer rather than the *R*-enantiomer was associated with an increase in chromosomal aberrations and sister chromatid exchange (SCE).

Table 5-4. Mutagenicity of styrene and styrene-7,8-oxide *in vitro*

	Styrene		Styrene-7,8-oxide	
Test system	**–S9**	**+S9**	**–S9**	**+S9**
S. typhimurium (reverse mutation)	–	±	+	±
E. coli (SOS chromotest)	–[a]	NT	+	NT
K. pneumoniae (forward mutation)	NT	NT	+	NT
S. cerevisiae (reverse mutation & gene conversion)	+	NT	+[b]	NT
S. pombe (forward mutation)	–	–	+	NT
D. melanogaster (sex-linked recessive)	+	NT	+	NT
D. melanogaster (somatic *w*/*w*+)	–[c]	NT	NT	NT
Chinese hamster V79 cells (*hprt*)	–	+	+	–
mouse lymphoma L5178Y (*tk*)	NT	NT	+	–
human lymphocytes (*HPRT*)	NT	NT	(+)	NT
human T lymphocytes (*HPRT*)	NT	NT	+	NT
human B lymphoblastoid cells (*HPRT*)	NT	NT	(+)	NT

Source: IARC 1994a, 1994b, 2002.
+ = positive results or generally positive results in multiple studies; (+) = weakly positive results; ± = mixed results; – = negative results or generally negative results in multiple studies; *hprt* = hypoxanthine phosphoribosyl transferase gene (mouse); *HPRT* = hypoxanthine phosphoribosyl transferase gene (human); NT = not tested; *tk* = thymidine kinase gene (mouse).
[a]Negative or inconclusive results in the SOS chromotest for DNA repair.
[b]Positive results for gene conversion only.
[c]A positive result was reported by IARC (2002) for insecticide-resistant strains, which have high bioactivation capacities.

5.4.2.4 Cytogenetic markers

The cytogenetic effects of styrene and styrene-7,8-oxide have been extensively reviewed (Barale 1991, Cohen *et al.* 2002, IARC 1994a, 1994b, 2002, Scott and Preston 1994b) and are summarized below. Both styrene and styrene-7,8-oxide cause cytogenetic damage in various cell types tested *in vitro*. End points investigated include SCE, chromosomal aberrations, micronuclei, and aneuploidy.

Styrene

Results of *in vitro* cytogenetic studies with styrene are summarized in Table 5-5. All studies with human lymphocytes gave positive results. Scott and Preston (1994a) noted that chromosomal aberrations and SCEs in human lymphocytes increased in the presence of erythrocytes (i.e., in whole-blood cultures). Erythrocytes have the capacity to oxidize styrene to styrene-7,8-oxide (see Section 5.1.3.5), while lymphocytes have the potential to inactivate styrene-7,8-oxide through metabolism by mEH.

SCE were observed in rat and human lymphocytes and in Chinese hamster ovary (CHO) cells under certain test conditions (IARC 1994a, 2002, Scott and Preston 1994a). In one study (de Raat 1978), SCE were induced in CHO cells only when metabolic activation (S9 fraction) was combined with incubation with cyclohexene oxide, an mEH inhibitor, suggesting that styrene is metabolically activated to styrene-7,8-oxide but rapidly inactivated by mEH. In another paper reporting six experiments with CHO cells (Norppa and Tursi 1984), styrene at high concentrations (8 to 12 mM) caused SCE in one of three experiments without metabolic activation and in two experiments in the presence of human erythrocytes, but did not cause SCE in one experiment in the presence of S9. [The high concentrations of styrene used in these experiments limit the interpretation of these studies.]

Chromosomal aberrations were reported in studies with *Allium cepa* root-tip cells and human lymphocytes exposed to styrene and in two of three studies in Chinese hamster lung cells (weakly positive results). Micronucleus formation occurred in human lymphocytes and *A. cepa* root-tip cells (IARC 1994a, 2002, Scott and Preston 1994a). A strong c-mitotic effect and disordered anaphases were reported in *A. cepa* root-tip cells,

and aneuploidy occurred in human lymphocytes (Linnainmaa *et al.* 1978a, 1978b) but not in *D. melanogaster* (Penttila *et al.* 1980).

Table 5-5. Cytogenetic effects of styrene *in vitro*

End point	Test system	Metabolic activation[a]	LEC/HIC (mM)[b]	Results	References
SCE	Human lymphocytes (isolated cultures or whole blood)[c]	–	2.0	+	Norppa *et al.* 1983a
		–	0.7	+	Norppa *et al.* 1980a
		–	0.5	+	Norppa *et al.* 1983a
		–	1.0	+	Norppa and Vainio 1983
		–	0.01	+	Chakrabarti *et al.* 1993
		–	0.5	+	Lee and Norppa 1995
	CHO cells	–	8.7	–	de Raat 1978
		S9	8.7	–	de Raat 1978
		S9 + CO	4.4	+	de Raat 1978
		–	15	–	Norppa and Tursi 1984
		–	12	–	Norppa and Tursi 1984
		–	12	+	Norppa and Tursi 1984
		S9	20	–	Norppa and Tursi 1984
		HE	8	+	Norppa and Tursi 1984
		HE	12	+	Norppa and Tursi 1984
	Rat lymphocytes (whole blood)	–	0.5	+	Norppa *et al.* 1983b
Chromosomal aberrations	Human lymphocytes (isolated cultures or whole blood)[c]	–	1.0	+	Jantunen *et al.* 1986
		–	2.6	+	Linnainmaa *et al.* 1978a, 1978b
		–	0.5	+	Pohlova and Sram 1985
		–	2.0	+	Jantunen *et al.* 1986
	Chinese hamster lung cells	–	2.4	–	Matsuoka *et al.* 1979
		S9	2.4	(+)	Matsuoka *et al.* 1979
		–	1.0	(+)	Ishidate and Yoshikawa 1980
	A. cepa	–	0.87	+	Linnainmaa *et al.* 1978a, 1978b
Micronuclei	Human lymphocytes (whole blood)	–	2.6	+	Linnainmaa *et al.* 1978a, 1978b
	A. cepa	–	1.7	+	Linnainmaa *et al.* 1978a, 1978b
Aneuploidy	Human lymphocytes (whole blood)	–	2.6	+	Linnainmaa *et al.* 1978b
	D. melanogaster	–	5	–	Penttila *et al.* 1980
C-mitosis	*A. cepa*	–	0.87	+	Linnainmaa *et al.* 1978a, 1978b

Source: adapted from Scott and Preston 1994a and IARC 1994a, 1994b, 2002.
+ = positive response; (+) = weak positive response; – = negative response.
[a] CO = cyclohexene oxide, an inhibitor of epoxide hydrolase; HE = human erythrocytes; S9 = phenobarbital or 3-methylcholanthrene-induced post-mitochondrial supernatant fraction of rat liver homogenate.
[b] Lowest effective concentration or highest ineffective concentration.
[c] Erythrocytes in whole-blood preparations can act as a metabolic activation system (Norppa *et al.* 1983b) (see Section 5.1.3.4).

Styrene-7,8-oxide

Styrene-7,8-oxide induced cytogenetic effects at lower concentrations than did styrene, and metabolic activation was not necessary. Results are summarized in Table 5-6. SCEs occurred in human lymphocytes, CHO cells, and Chinese hamster V79 cells. Chromosomal aberrations occurred in human lymphocytes and Chinese hamster V79 cells but not in *A. cepa.* Micronuclei were induced in human lymphocytes, Chinese hamster V79 cells, and *A. cepa*. Linnainmaa *et al*. (1978a, 1978b) also reported anaphase bridges in *A. cepa* cells, which induced micronuclei in successive telophases and interphases. It was not possible to assess incidences of aneuploidy in human lymphocytes exposed to styrene-7,8-oxide because of severe chromosome destruction.

5.4.3 In vivo *studies in experimental animals*

5.4.3.1 DNA adducts

As mentioned above, styrene does not bind to DNA unless metabolically activated to styrene-7,8-oxide. The potential of styrene or styrene-7,8-oxide exposure to induce DNA adducts in experimental animals was studied earlier through the use of radiolabeled compounds, as reviewed by Phillips and Farmer (1994) and Cohen *et al.* (2002). These studies generally showed low levels of DNA adducts in rats and mice following exposure to styrene or styrene-7,8-oxide by various routes of administration. However, the reported levels of DNA binding varied by factors of 20 to 50 among studies, for reasons that were not completely understood. According to Phillips and Farmer, differences in route of administration, methods of measurement, and losses from depurination should be considered.

Table 5-6. Cytogenetic effects of styrene-7,8-oxide *in vitro*, without metabolic activation

End point	Test system	LEC/HIC[a] (mM)	Results	References
SCE	Human lymphocytes	0.07 0.15 0.008 0.1 0.05 0.05 0.1 0.05 0.05	+ + + + + + + + +	Norppa *et al.* 1980a Norppa *et al.* 1983a Pohlova and Sram 1985 Zhang *et al.* 1993 Lee and Norppa 1995 Uüskula *et al.* 1995 Chakrabarti *et al.* 1997 Ollikainen *et al.* 1998 Laffon *et al.* 2001b
	CHO cells	0.18	+	de Raat 1978
	Chinese hamster V79 cells	0.17 0.12	+ +	Nishi *et al.* 1984 von der Hude *et al.* 1991
Chromosomal aberrations	Human lymphocytes	0.59 0.1 0.2 0.024	+ + + +	Linnainmaa *et al.* 1978a, 1978b Fabry *et al.* 1978 Norppa *et al.* 1981b Pohlova and Sram 1985
	Chinese hamster V79 cells	0.75	+	Turchi *et al.* 1981
	A. cepa	3.7	–	Linnainmaa *et al.* 1978a, 1978b
Micronuclei	Human lymphocytes	0.59 0.1	+ +	Linnainmaa *et al.* 1978a, 1978b Laffon *et al.* 2001b
	Chinese hamster V79 cells	0.75	+	Turchi *et al.* 1981
	A. cepa	3.7	+	Linnainmaa *et al.* 1978a, 1978b
Anaphase bridges	*A. cepa*	0.74	+	Linnainmaa *et al.* 1978a, 1978b

Source: adapted from Scott and Preston 1994a; IARC 1994a, 1994b, 2002.
+ = positive response; – = negative response.
[a]Lowest effective concentration or highest ineffective concentration.

Styrene

Adducts resulting from exposure to tritiated styrene were detectable in 2 of 4 lung samples from female rats and in mouse liver but not in rat liver; however, no lung tissue was collected from mice in this study (Cantoreggi and Lutz 1993). The earlier study by Byfält-Nordqvist *et al.* (1985) with ^{14}C-labeled styrene in NMRI mice reported adduct values 20 to 50 times those reported by Cantoreggi and Lutz. Philips and Farmer (1994) were not able to identify a reason for the difference although they noted that the methods differed for route of adimistration (inhalation, ingestion, and i.p. injection) and in the

quantitation of radioactivity (coelution with adduct standards vs. measurement of total radioactivity, but they did not consider these differences sufficient to explain the widely differing results.

More recent studies have focused on the quantitative and qualitative determination of specific styrene-induced DNA adducts (Boogaard *et al.* 2000b, Gamer *et al.* 2004, Otteneder *et al.* 2002, Pauwels *et al.* 1996, Vodicka *et al.* 2001b, Vodicka *et al.* 2006a). The studies are reviewed below and summarized in Table 5-7.

DNA adducts resulting from exposure to styrene were detected in tissues from male NMRI mice in several studies by Vodicka and co-workers (Pauwels *et al.* 1996, Vodicka *et al.* 2001b, Vodicka *et al.* 2006a). In the only study using i.p. injection (Pauwels *et al.* 1996), styrene was administered at 0 to 4.35 mmol/kg b.w., and tissues were collected 3 hours later. N7- and O^6-guanine adducts were present in the lungs, liver, and spleen, but N7 adducts were more abundant in all three tissues, and the lungs contained approximately 30% more of these adducts than did the liver or spleen. The authors pointed out that the liver would be expected to be exposed to styrene as the first-pass organ, but they suggested that the balance between formation and detoxification of styrene-7,8-oxide in the organs could explain the higher adduct levels in lung. DNA adduct levels correlated with exposure level and formation of hemoglobin adducts.

In the inhalation studies, β-N7–guanine adducts were detected in the lungs but not liver, and β-N1–adenine adducts were detected in both lungs and liver of male NMRI mice exposed to styrene at 750 or 1,500 mg/m^3 [175 or 350 ppm] 6 hours per day, 7 days per week, for 1, 3, 7, or 21 days (Vodicka *et al.* 2001b, 2006a). Levels of both types of DNA adducts in the lungs correlated significantly with styrene concentrations in blood as a measure of styrene exposure. Levels of N7-guanine adducts were compared between the lungs and the urine (with correction for depurination); the total N7-guanine adducts (23.0 adducts/10^8 nucleotides) in the lungs of mice in the highest exposure group (1,500 mg/m^3 [350 ppm] for 21 days) accounted for approximately 0.5% of the total N7-guanine adducts measured by cumulative urinary excretion.

DNA adducts also were detected in liver, lungs, and isolated lung cells of male CD-1 mice and male Sprague-Dawley rats exposed to [ring-U-^{14}C]styrene at 160 ppm by nose-only inhalation for 6 hours (Boogaard *et al.* 2000b). Tissues were collected either immediately or 42 hours after exposure. Low levels of N7-guanine adducts were detected in both liver and lung; however, unidentified adducts [authors' term] were present in liver at higher levels than the N7-guanine adducts. The level of N7 adducts and of two of the three unidentified adducts increased from 0 to 42 hours. N7 adducts were the major adduct type in the lungs, at a level of about 1 per 10^8 nucleotides immediately after exposure and at about half this level 42 hours after exposure. Adducts also were measured in Clara cells and non-Clara cells. Adducts were analyzed in lung tissue from 2 mice and lung cells from 24 mice. N7-guanine adduct levels were similar in Clara cells, non-Clara cells, and whole lung. After 42 hours, an unidentified adduct was detected at levels of 6 per 10^8 nucleotides in Clara cells and at 80 per 10^8 nucleotides in non-Clara cells. The authors stated that because of the small amount of DNA isolated from non-Clara cells, this value had a large relative error (approximately 30%). The authors used styrene metabolite standards to identify this adduct (which was the same for both lung-cell types) and found that benzoic acid co-eluted with the compound. They speculated that the most likely source was benzaldehyde, which is a putative intermediate in the metabolism of styrene from mandelic acid to hippuric acid; however, they also suggested that this adduct could be an artifact resulting from the radioactive styrene used for the exposure.

N7-guanine adducts were detected in both rat liver and lung; however, the unidentified adducts that were present in mouse liver at higher levels than the N7-guanine adducts (see above) were not detected in rat liver (Boogaard *et al.* 2000b). One rat was used for the adduct analysis from lung tissue and five rats were used for the cell-type analysis. N7-adduct levels were approximately 1 adduct per 10^8 nucleotides immediately after exposure, and about half this level at 42 hours after exposure. Type II cells isolated from lungs of styrene-exposed rats contained higher levels of N7-guanine adducts (2 adducts per 10^8 nucleotides) than whole lung.

Otteneder *et al.* (2002) did not detect O^6-guanine adducts in the lungs or liver of CD-1 mice exposed to styrene at 40 or 160 ppm for 2 weeks. Gamer *et al.* (2004) also reported that no changes were found in 8-hydroxy-deoxyguanosine as an indicator of oxidative stress after either a single 6-hour exposure or multiple exposures of female CD-1 mice to styrene by inhalation; however, they did find that glutathione was depleted in lung homogenates.

In CD-1 rats exposed for 2 years to styrene at 1,000 ppm (the highest level tested), both α-and β-O^6–guanine adducts were detectable in the liver at levels of 90 per 10^8 nucleotides in males and 80 per 10^8 nucleotides in females (Otteneder *et al.* 2002). An isomer-enriched analysis was used for the 1,000-ppm samples only, and all of the lung tissue was used for histopathological analysis. However, no O^6-guanine adducts were detected in the lungs or liver of CD-1 rats exposed to styrene at 500 ppm for 2 weeks.

Overall, the N7- and O^6-guanine adducts were found most often in these studies. As noted in Section 5.4, the N7-guanine adducts are the most common form resulting from exposure to styrene, but the O^6-guanine adducts are more persistent, which may explain their detection along with the N7-adducts.

Table 5-7. Formation of styrene-7,8-oxide DNA adducts in animals exposed to styrene

Species	Exposure[a]	Adducts		Reference
		Type	No./10^8 nucleotides[b]	
Male NMRI mice	0–4.35 mmol/kg b.w measured at 3 h	N7-guanine[c]	lung: [63.5] liver: [47.6] spleen: [36.7]	Pauwels *et al.* 1996
		O^6-guanine[c]	lung: [37.8] liver: [24.7] spleen: [25.7]	
Male NMRI mice	[175 or 350 ppm], 6 h, 7 days/wk, for 1, 3, 7, or 21 d	βN7-guanine	lung: 23 liver: ND	Vodicka *et al.* 2001b Vodicka *et al.* 2006a
		βN1-adenine	lung: 0.6 liver: 0.2	

Species	Exposure[a]	Adducts		Reference
		Type	No./10^8 nucleotides[b]	
Male CD-1 mice	160 ppm for 6 h, measured at 0 and 42 h post-exposure	N7-guanine	42 h lung: ~ 0.5 Clara: ~< 1 non-Clara: ~4 liver: 3.2	Boogaard *et al.* 2000b
		unidentified[d]	42 h lung: < 1.0 Clara: 6 non-Clara: 80[e] liver: 8–11	
Female CD-1 mice	40 or 160 ppm, 6 h, 5 d/wk for 2 wk	O^6-guanine	lung: < detection limit of 1–5/10^7)	Otteneder *et al.* 2002
Female CD-1 mice	40 or 160 ppm, 6 h: single exposure or 5 or 20 d	8-OH-deoxyguanosine	lungs: no evidence of oxidative stress	Gamer *et al.* 2004
Male Sprague-Dawley rats	160 ppm, 6 h, measured at 0 and 42 h post-exposure	N7-guanine	42 h lung: ~0.5 type II cells: 2 non-type II cells: NR[f] liver: 1.9	Boogaard *et al.* 2000b
		unidentified	lung: < 0.5 liver: < 0.5	
Female CD rats	500 ppm (rats), 6 h/d for 2 wk	O^6-guanine	lung: < detection limit of 1–2/10^7	Otteneder *et al.* 2002
Male and female CD rats	1,000 ppm, 6 h, 5 d/wk for 2 yr	O^6-guanine	liver: 90 (males) 80 (females)	Otteneder *et al.* 2002
Female Crl:CD rats	40 or 160 ppm, 6 h: single exposure or 5 d	8-OH-deoxyguanosine	lung: no evidence of oxidative stress	Gamer *et al.* 2004

[a] Styrene exposure was by inhalation in all studies except that of Pauwels *et al.*, which used i.p. injection.
[b] Adduct levels are the highest reported for each study unless otherwise indicated.
[c] Adduct levels were converted from femtomoles per milligram of DNA based on the assumption that 1 mg DNA = 3.24 μmol of nucleotides.
[d] Not the same adduct for all values; in the liver, the peaks eluted at 9 and 37 minutes, and in the lung cells, the major peak was at 9 minutes.
[e] Based on only 170 μg of DNA; the authors stated that the error may be approximately 30%.
[f] N7 adducts were present in non-type II cells immediately after exposure, but the concentration of adducts at 42 hours could not be accurately determined because of the low yield of DNA.

Styrene-7,8-oxide

Philips and Farmer (1994) reported that very low levels of DNA adducts were formed in the forestomach [the target tissue for styrene-7,8-oxide–induced tumors] and liver when

tritiated styrene-7,8-oxide was administered by gavage to rats and by i.p. injection to mice (Cantoreggi and Lutz 1992, Lutz *et al.* 1993). An earlier report by Byfält-Nordqvist *et al.* (1985) in which tritiated styrene-7,8-oxide or styrene was administered by i.p. injection to NMRI mice reported that alkylation of DNA in liver, brain, and lung exceeded that in spleen and testis, but the forestomach was not examined.

5.4.3.2 DNA damage and repair

Results from studies of DNA damage in experimental animals exposed to styrene or styrene-7,8-oxide are summarized in Table 5-8 and discussed below. Most of the studies used the alkaline single-cell gel electrophoresis (comet) assay. The comet assay involves embedding single cells in agarose gel followed by lysis in alkali and electrophoresis (Vaghef and Hellman 1998). This assay can detect strand breaks, alkali-labile sites (converted to single-strand breaks under the alkaline conditions of the assay), oxidative base damage, crosslinks, and DNA repair.

Styrene

DNA damage was observed in two studies in mice exposed to styrene by i.p. injection (Vaghef and Hellman 1998, Walles and Orsen 1983). Walles and Orsen (1983) administered styrene at 1.7 to 10.1 mmol/kg b.w. [177 to 1,052 mg/kg b.w.] to male NMRI mice and determined DNA damage in kidney, liver, lung, testis, and brain at 1 to 24 hours after injection. DNA damage was increased in all tissues examined at 1 hour, and the levels were still elevated at 24 hours in all tissues but the liver. Vaghef and Hellman (1998) determined DNA damage in peripheral blood lymphocytes, bone marrow, liver, and kidney cells at 4 and 16 hours after i.p. injection of 100 to 500 mg/kg b.w. of styrene to female C57BL/6 mice. Significant increases in DNA damage were found in all tissues at both 4 and 16 hours.

Inhalation studies are usually conducted over a long period, to ensure that equilibrium between DNA damage and repair is reached; however, only subacute inhalation studies of styrene have been conducted. Vodicka *et al.* (2001b) exposed male NMRI mice to styrene by inhalation at a concentration of 750 or 1,500 mg/m^3 [175 or 350 ppm] for 7 or 21 days; they found significant increases in DNA damage only in lymphocytes at 7 days, and not in bone marrow or liver cells. Endonuclease III–sensitive sites in bone marrow

were increased significantly at 21 days at both exposure levels, suggesting an increase in accumulation of abasic sites.

Only one study that examined DNA damage in styrene-exposed rats was identified. Kligerman *et al.* (1993) exposed female F344 rats to styrene by inhalation at 125, 250, or 500 ppm, 6 hours per day for 14 consecutive days. No significant increase in DNA damage was detected.

Clay (2004) reported that styrene induced DNA damage and repair in female CD-1 mice in an assay for unscheduled DNA synthesis (UDS). Groups of 6 mice were exposed to styrene by inhalation at either 125 or 250 ppm for 6 hours. A positive control group was administered *N*-nitrosodimethylamine (NDMA) at 10 mg/kg b.w. by gavage. Groups of 3 mice were killed for tissue collection at 2 and 16 hours after exposure to styrene or NDMA. No increase in UDS was observed for any of the animals exposed to styrene, but the positive control induced increases in UDS that the author characterized as appropriate.

Styrene-7,8-oxide

After a single i.p. injection of styrene-7,8-oxide, single-strand breaks or alkali-labile sites in DNA were increased in male NMRI mice (in kidney, liver, lung, testis, and brain) (Walles and Orsen 1983), male CD-1 mice (in liver, lung, kidney, spleen, and bone marrow) (Sasaki *et al.* 1997), female C57BL/6 mice (in liver, lymphocytes, bone marrow, and kidney) (Vaghef and Hellman 1998), and male ddY mice (in stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow) (Tsuda *et al.* 2000). Tsuda *et al.* (2000) and Sasaki *et al.* (1997) took measurements at multiple time points (3 to 24 hours) and found that DNA damage decreased with time.

Table 5-8. DNA damage in experimental animals exposed to styrene or styrene-7,8-oxide.

Species (organs)	Exposure (administration)	Assay method	Result	Reference
Styrene				
Male NMRI mice (kidney, liver, lung, testis, and brain)	1.7–10.1 mmol/kg b.w. [177–1,052 mg/kg b.w.] (single i.p. injection)	DNA unwinding; hydroxylapatite separation	+	Walles and Orsen 1983
Female C57BL/6 mice (liver, PBLs, bone marrow, and kidney)	100–500 mg/kg b.w. (single i.p. injection)	comet assay	+	Vaghef and Hellman 1998
Male NMRI mice (PBLs, bone marrow, and liver)	[175–350 ppm], 6 h/d, 7 d/wk, for 1, 3, 7, or 21 d (inhalation)	comet assay	±	Vodicka *et al.* 2001b
Female Fischer rats (PBLs)	125–500 ppm, 6 h/d, for 2 wk (inhalation)	comet assay	–	Kligerman *et al.* 1993
Styrene-7,8-oxide				
Male NMRI mice (kidney, liver, lung, testis, and brain)	1.8–7 mmol/kg b.w. [216–841 mg/kg] (single i.p. injection)	DNA unwinding; hydroxylapatite separation	+	Walles and Orsen 1983
Male CD-1 mice (liver, lung, kidney, spleen, and bone marrow)	400 mg/kg b.w. (i.p.)	comet assay	+	Sasaki *et al.* 1997
Female C57BL/6 mice (liver, lymphocytes, bone marrow, and kidney)	50–200 mg/kg b.w. (i.p.)	comet assay	+	Vaghef and Hellman 1998
Male ddY mice (stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow)	400 mg/kg b.w. (i.p.)	comet assay	+	Tsuda *et al.* 2000

+ = positive; ± = equivocal – = negative.

5.4.3.3 Mutations

No studies evaluating specific gene mutations in experimental animals exposed to styrene or styrene-7,8-oxide were identified.

5.4.3.4 Cytogenetic studies

Cytogenetic effects include SCE, chromosomal aberrations, micronuclei, aneuploidy, and polyploidy. *In vivo* cytogenetic studies of styrene and styrene-7,8-oxide exposure have been conducted in mice, rats, and hamsters and are reviewed below. Cohen *et al.* (2002) reviewed the cytogenetic effects of styrene and styrene-7,8-oxide in experimental animals

and reported that positive effects were seen only at high exposure levels that are not likely relevant for human exposure; however, because huyman exposures are usually of much longer duration, the authors suggested that lower exposure levels over longer exposure periods could have clastogenic effects in animals.

Styrene

IARC (1994a) and Scott and Preston (1994a) reviewed three to eight studies each for SCE, chromosomal aberrations, and micronuclei in experimental animals (rats and mice) exposed to styrene by inhalation, i.p. injection, or gavage. All of the studies gave positive or weakly positive results for SCE; SCE were detected in liver, alveolar macrophages, lungs, bone marrow, splenocytes (weakly positive), and lymphocytes of mice and splenocytes and lymphocytes (weakly positive) of rats. In contrast, all the studies except one (for each end point) gave negative results for chromosomal aberrations and micronuclei. Polyploidy was observed in Wistar rat bone-marrow cells following administration of styrene by inhalation at 300 ppm for 11 weeks. Most studies were of short duration (≤ 2 weeks). One inhalation study lasted for 12 months but did not report increased incidences of chromosomal aberrations in rat bone marrow following exposure to concentrations up to 1,000 ppm. Results from the studies reviewed by IARC (1994a, 2002) and Scott and Preston (1994a) are summarized in Table 5-9.

Only a few studies were identified that examined cytogenetic effects in experimental animals exposed to styrene and were published after the IARC (1994a) review. IARC (2002) reviewed one additional study of SCE and chromosomal aberrations in F344 rats exposed to styrene at 4,260 mg/m^3 [1,000 ppm] for 4 weeks. The results were negative. The genotoxicity of styrene and 1,3-butadiene was evaluated in B6C3F$_1$ mice exposed for 8 hours by inhalation (Leavens *et al.* 1997). Butadiene-exposed mice exhibited increased micronuclei in bone marrow, while styrene-exposed mice did not. In another study, male NMRI mice exposed to styrene at 1,500 mg/m^3 [350 ppm] had significantly increased micronuclei in bone marrow after 7 days of exposure but not after 21 days of exposure (Vodicka *et al.* 2001b). However, when this study was repeated, there was no evidence of clastogenicity (micronuclei or chromosomal aberrations) in male NMRI mice exposed to styrene at 750 or 1,500 mg/m^3 [175 or 350 ppm] for 1, 3, 7, 14, or 21 days [micronuclei

were evaluated independently by two laboratories] (Engelhardt *et al.* 2003). The authors suggested that the positive result in the first experiment might have been the result of some unidentified experimental variation, because the results were inconsistent between time points.

Styrene-7,8-oxide

Fewer studies have examined the cytogenetic toxicity of styrene-7,8-oxide (IARC 1994b, Scott and Preston 1994a). Results are summarized in Table 5-9. IARC (2002) did not review any additional studies of clastogenic effects in experimental animals exposed to styrene-7,8-oxide. SCEs were not increased in Chinese hamster bone-marrow cells following inhalation or i.p. injection of styrene-7,8-oxide or in mouse bone-marrow cells following inhalation exposure. Positive results for SCE were found in mouse bone-marrow cells following a single i.p. injection of 100 mg/kg b.w., and weakly positive results in mouse liver cells and alveolar macrophages following inhalation exposure. Chromosomal aberrations were increased in mouse bone-marrow cells in two of three studies, but not in Chinese hamster bone-marrow cells. Only two studies were available that examined micronuclei in rodents exposed to styrene-7,8-oxide. In both studies, one in BALB/c mice and the other in Chinese hamsters, micronucleus formation was not increased in bone-marrow cells following a single i.p. injection of styrene-7,8-oxide at 250 mg/kg b.w.

One of these studies, Sinsheimer *et al.* (1993), investigated the effects of both the *R*- and *S*-styrene-7,8-oxide isomers when administered by i.p. injection at 100 mg/kg b.w. to male CD-1 mice. No effect on chromosomal aberrations per cell was reported with either isomer, but the percentage of cells with SCE increased significantly ($P < 0.01$) following exposure to the *S*-isomer (2.75 ± 0.50, mean ± SD) but not the *R*-isomer (1.75 ± 0.96), compared with dimethylsulfoxide (DMSO) solvent controls (1.00 ± 0.82). The number of SCE per cell was also significantly higher for the *S*-isomer than for the *R*-isomer or DMSO. The mitotic index was significantly lower for both the *R*-isomer (2.74 ± 0.28) and the *S*-isomer (2.58 ± 0.22), compared with controls (3.51 ± 0.30).

Table 5-9. Cytogenetic effects of styrene and styrene-7,8-oxide in experimental animals

End point	Species and cell type	Styrene		Styrene-7,8-oxide	
		Results	LED/HID[a] (mg/kg)	Results	LED/HID[a] (mg/kg)
SCE	mouse bone marrow	+	500	±[b]	100
	mouse liver	+	580	(+)	72
	mouse alveolar macrophages	+	580	(+)	72
	mouse lymphocytes	+	450	NT	NT
	mouse lung cells	+	450	NT	NT
	mouse splenocytes	(+)	450	NT	NT
	rat splenocytes	+	750	NT	NT
	rat lymphocytes	±	225	NT	NT
	Chinese hamster bone marrow	NT	NT	–	500
Chromosomal aberrations	mouse bone marrow	–	1,000	+[c]	50
	mouse lung cells	–	900	NT	NT
	mouse lymphocytes	–	900	NT	NT
	mouse splenocytes	–	900	NT	NT
	mouse spermatocytes	NT	NT	–	250
	rat bone marrow	±	270	NT	NT
	rat lymphocytes	–	450	NT	NT
	Chinese hamster bone marrow	–	225	–	500
Micronuclei	mouse bone marrow	±	250	–	250
	mouse splenocytes	–	900	NT	NT
	mouse erythrocytes	–	900	NT	NT
	rat bone marrow	–	3,000	NT	NT
	rat lymphocytes	–	450	NT	NT
	Chinese hamster bone marrow	–	1,000	–	250
Polyploidy	rat bone marrow	+	270	NT	NT
Aneuploidy	rat bone marrow	–	270	NT	NT

Sources: IARC 1994a,b, 2002, Scott and Preston 1994a.
NT = not tested; + = positive in most studies; (+) = weakly positive; ± = Similar number of positive and negative studies; – = negative in most studies.
[a]LED = lowest effective dose in positive studies; HID = highest ineffective dose in negative studies; LED given for studies with mixed results.
[b] One study was positive for the *S*-isomer but not the *R*-isomer.
[c] Positive in 2 of 3 studies.

5.4.4 Studies in styrene-exposed workers

Many studies have examined the genetic effects of styrene in human populations; however, interpretation of these studies is complicated by a number of factors that increase the likelihood of both false positive and false negative results. These include less control over study design details than in *in vitro* studies or animal bioassays, lack of appropriate exposure data, and the need to control for possible confounding factors, such as smoking or co-exposure to other chemicals used in the plastics industry (e.g., organic peroxides, dichloromethane, hydroquinone, dimethylaniline, and maleic anhydride).

Other important study limitations include relatively small control groups, low sensitivity, and high interindividual variability. [These facts have an impact on any human biomonitoring study and, together with interlaboratory differences, may be responsible for much of the ambiguity and inconsistency apparent in styrene population studies.]

Exposure to other chemicals that may also cause genetic damage is often correlated with exposure to styrene. The following factors were considered by Cohen *et al.* (2002) to increase the probability that an observed relationship is causal: (1) adequate statistical control of confounders, (2) a positive dose-response relationship among exposed subjects, and (3) a positive association across studies between a central measure of exposure and the average magnitude of the increased frequency of the effect in each study. Results of studies in styrene-exposed workers are summarized below for DNA adducts, DNA damage, mutations, and cytogenetic markers.

5.4.4.1 DNA adducts

Results from studies in Bohemia (Czech Republic), the United States, and Germany are summarized in Table 5-10. Very few studies were available on the detection of styrene-specific DNA adducts in humans before 1994 (IARC 1994a). Liu *et al.* (1988b) reported unidentified adducts in 1 styrene-exposed worker, and Vodicka *et al.* (1993) and Vodicka and Hemminki (1993) reported O^6-guanine adducts in lamination workers.

The two reports of DNA adducts in lamination workers by Vodicka and coworkers were part of a series of studies (Koskinen *et al.* 2000a, 1995, Vodicka and Hemminki 1993, 1999, Vodicka *et al.* 1994) using samples collected from workers at a group of factories in the same geographic area of Bohemia. [In many cases, the same individuals were sampled repeatedly, and although the same individuals could be identified across some of the studies, this was not possible in all cases.] Up to six samples were collected from each individual between December 1992 and March 1995 (Vodicka *et al.* 1999). These six occasions were (I) in December 1992, (II) in July 1993 one day before summer vacation, (III) in August 1993 on the first day of work after two weeks of vacation, (IV) in September 1993 after an additional month of work, (V) in February 1994, and (VI) in March 1995. Data from these samplings are reported in Table 5-10. Two groups of controls were used in this study: 7 factory controls and 8 laboratory controls (increased to

13 for sampling VI). The factory controls were sampled on occasions II through V, and the laboratory controls were sampled on occasions V (N = 8) and VI (N = 13); sampling VI was reported in the study by Vodicka *et al.* (1999) and included data for laboratory controls only. These studies also included measurements of single-strand breaks in DNA (see Section 5.4.4.2) and *HPRT* mutations in the same groups of workers (see Section 5.4.4.3).

The results for O^6-guanine adducts from sampling I were reported in Vodicka *et al.* (1993) for 23 hand-lamination workers. The workers were divided into 2 groups that differed by styrene exposure duration and levels, and adduct levels did not differ between the controls and either group. The results for samplings II, III, and IV for samples from 7 or 9 workers (see Vodicka *et al.* 1999) were reported as part of a study of the persistence of O^6-guanine adducts (Vodicka *et al.* 1994). Vodicka *et al.* (1995) also reported the results for O^6-guanine adducts from samplings II, III, and IV and added results for sampling V. [In the Vodicka et al. (1995) publication, the December 1992 results reported by Vodicka *et al.* (1993) were not included, and the other samplings were numbered I through IV.] Levels of styrene-specific DNA adducts were significantly higher in workers than in controls at all sampling times before and after vacations for samplings II, III, and IV, but there was no significant difference between samplings for the exposed workers. Vodicka *et al.* (1994) therefore concluded that removal of specific O^6-styrene adducts from DNA was very slow. The results of the final sampling (VI) from this group of workers was reported by Vodicka *et al.* (1999) together with occupational exposure data from the earlier samplings. Separate values were reported for 11 workers and 10 controls (of a total of 13 sampled in each group) and for the 8 workers and an unspecified number of controls studied in previous samplings.

In the series of six consecutive samplings over 3 years described above, no tendency of O^6-guanine adducts to accumulate was reported, suggesting a well-established equilibrium between DNA adduct formation and removal in chronically and highly exposed hand-lamination workers (Vodicka *et al.* 1999). Although this study did not find continued accumulation of O^6-guanine adducts, Vodicka *et al.* (1994) interpreted the

relatively constant levels of these adducts over time, including time away from work for vacations, as evidence for their persistence.

In addition to the O^6-guanine adducts reported in the studies summarized above, other types of adducts (e.g., β-N1-adenine, N^2-guanine, and 8-hydroxy-2'-deoxyguanosine) have been measured in styrene-exposed workers (Horvath *et al.* 1994, Koskinen *et al.* 2001a, Marczynski *et al.* 1997a, Rappaport *et al.* 1996, Vodicka *et al.* 2003). In studies in Bohemia, low levels of β-N1-adenine adducts were detected in styrene hand-lamination workers by a high-performance liquid chromatography–based method, but the adduct levels were not significantly higher in workers than in controls (Koskinen *et al.* 2001a, Vodicka *et al.* 2003). In studies of U.S. workers, N^2-guanine adducts and a second unidentified adduct were detected in 48 workers of both sexes employed in a boat-manufacturing facility where mean styrene exposure was 64 mg/m^3 [15 ppm] (range = 1 to 235 mg/m^3 [0.2 to 55 ppm]) (Horvath *et al.* 1994, Rappaport *et al.* 1996). [However, these studies included no controls.] Marcynski *et al.* (1997a) reported on 17 styrene-exposed boat builders in Germany (aged 23 to 60) and 67 age-matched healthy volunteers without prior exposure to styrene. Levels of 8-hydroxy-2'-deoxyguanosine adducts [an indicator of oxidatively damaged DNA] were significantly higher in the workers than in the controls.

Levels of β-N1-adenine adducts were significantly correlated with measures of styrene exposure, including styrene in exhaled air ($r = 0.613$, $P = 0.007$), styrene in blood ($r = 0.558$, $P = 0.003$), and urinary mandelic acid ($r = 0.836$, $P = 0.0003$) (Koskinen *et al.* 2001a), and styrene at the workplace ($r = 0.730$, $P < 0.001$), styrene in blood ($r = 0.605$, $P < 0.001$), and urinary mandelic acid ($r = 0.670$, $P = 0.001$) (Vodicka *et al.* 2003). Significant correlations between adducts and styrene exposure were also reported for the population studied by Horvath *et al.* (1994) and Rappaport *et al.* (1996) (for styrene in workplace air and N^2-guanine adducts, $r = 0.244$, $P = 0.049$; for unidentified adducts, $r = 0.330$, $P = 0.012$).

Table 5-10. Studies of DNA adducts in white blood cells of workers occupationally exposed to styrene in Bohemia, the United States, and Germany

No. of subjects (exposed/ control)	Mean years employed (exposed subjects)	Exposure indicators (mean)		Adduct type	Adducts/10^8 nucleotides (mean ± SD)		Reference
		Styrene in air [ppm][a]	Urinary mandelic acid (mg/g of creatinine)		Exposed	Controls	
Bohemia[b]							
					Sampling		
10/8 13/10	12 6	[86] [50]	380 330	O^6-guanine	(I) 4.7 ± 1.9[c] 7.3 ± 4.9[c]	0.3 ± 0.3 1.1 ± 1.3	Vodicka *et al.* 1993
9/7 7[d]/7 9/7	6.7	[28]	157	O^6-guanine	(II) 4.9 ± 2.4** (III) 5.1 ± 1.9** (IV) 6.0 ± 1.8**	1.4 ± 0.8 0.7 ± 0.3 0.9 ± 0.6	Vodicka *et al.* 1994
8/7 (factory) 8/8 (laboratory)	9	[21]	146	O^6-guanine	(V) [4.8 ± 2.5][e]***	[0.8 ± 0.4][e] [0.2 ± 0.5][e]	Vodicka *et al.* 1995
11/10 8/?	7.2	[16]	187	O^6-guanine	(VI) 5.9 ± 4.9*** 7.2 ± 4.9***	0.7 ± 0.8 0.8 ± 0.8	Vodicka *et al.* 1999
9/11	7.8	[18]	106	β-N1-adenine	0.08 ± 0.01	≤ 0.04[f]	Koskinen *et al.* 2001a
19/7	14.1	[40]	NR	β-N1-adenine	0.3 ± 0.05	≤ 0.04[f]	Vodicka *et al.* 2003
United States							
47/0	at least 1	[15]	NR	N^2-guanine unidentified	15.8 ± 3.2[g] 14.2 ± 2.3	no controls no controls	Horvath *et al.* 1994 Rappaport *et al.* 1996
Germany							
17/67	1– > 10	NR	NR	8-OH-2α-deoxyguanosine	2,230 ± 540***	1,520 ± 450	Marczynski *et al.* 1997a

**Significantly different from the controls at $P < 0.01$ by Student's *t*-test.
***Significantly different from the controls at $P \leq 0.001$ by the Mann-Whitney U test (Vodicka *et al.* 1995, 1999) or Student's *t*-test (Marczynski *et al.* 1997a).
[a] Values converted from mg/m^3 to ppm by multiplying by 0.233 and rounding to 2 significant figures.
[b] The populations overlapped between studies to some extent, as noted in the text; see text for description of samplings.

[c] The authors reported that the true differences were larger than calculated because of an unusually high adduct level in one of the controls, no statistical analysis was reported.
[d] Per Vodicka *et al.* (1995).
[e] Estimated from Figure 2 of Vodicka *et al.* (1995).
[f] Detection limit; no adducts were detected in controls; no statistical analysis reported.
[g] Mean ± SE.

5.4.4.2 DNA damage and repair

Pero *et al.* (1982) tested the sensitivity of human lymphocytes to stimulation of UDS by *N*-acetoxy-2-acetylaminofluorene (NA-AAF) after exposure to styrene *in vivo* or *in vitro*. UDS resulting from exposure to NA-AAF *in vitro* was significantly greater in lymphocytes obtained from workers in a Swedish fiberglass-reinforced polyester plastic factory exposed to styrene at 1 to 40 ppm than in lymphocytes from workers in a mechanical industry in the same town. In lymphocyte cultures exposed to styrene at 0 to 750 μM, NA-AAF–induced UDS was increased significantly ($P < 0.001$) compared with the mean level of unexposed controls, and there was a significant ($P < 0.001$) linear correlation with styrene concentration up to 100 μM, above which the effect remained elevated. The authors concluded that styrene could make lymphocytes more sensitive to other genotoxic exposures and suggested that one potential mechanism could be induction of mixed-function oxygenase activity by styrene, leading to increased metabolism and activation of genotoxins such as NA-AAF.

DNA repair capacity was measured in lymphocytes from 14 styrene-exposed boat builders and 7 controls from the wood manufacturing industry in an X-ray challenge assay (Oberheitmann *et al.* 2001). Lymphocytes obtained were exposed to X-rays, and the rate of exchange-type chromosomal aberrations per 100 metaphases was determined. However, the duration of radiation exposure was different for the exposed and control cultures, so the results could not be used for comparison. The authors compared the results with those for 2 historical control subjects, who were individuals from the research institute (the authors noted that the comparison could only be exploratory). Significantly more chromosomal aberrations were found in the lymphocytes from styrene-exposed workers than in the historical controls. In the exposed group, the challenge response was significantly correlated with cumulative lifetime exposure to styrene (years of exposure), but not with the current exposure (measured as styrene in the blood). The authors concluded that their results were consistent with the hypothesis that long-term exposure to styrene affects DNA repair activities in humans.

A significant positive correlation was observed between exposure parameters and rates of base-excision repair (irradiation-specific repair and the repair of oxidatively damaged

DNA) (Vodicka *et al.* 2004a). Peripheral lymphocytes from styrene-exposed workers at three plants or from controls working in a regional hygienic station were compared for their ability to repair single-strand breaks induced by γ-rays *in vitro*. Repair rates (reported as SSB/10^9 Da) were significantly higher for Plant A (mean ± SD = 0.94 ± 0.32, $P = 0.023$), Plant B (0.96 ± 0.44, $P = 0.016$), and Plant C (1.63 ± 0.41, $P = 0.001$) than for controls (0.55 ± 0.64). Across the three plants, the rate of DNA repair correlated significantly ($r = 0.308$, $P = 0.031$) with styrene concentration in the blood. DNA repair increased with increasing styrene air concentration, but differed significantly from the controls only for the high-exposure group (exposed to styrene at > 50 mg/m^3 [12 ppm]; $P = 0.034$). The authors suggested that particular DNA repair pathways might be induced by styrene exposure.

Slyskova *et al.* (2007) compared the capacity to repair oxidatively damaged DNA in mononuclear leukocytes obtained from 24 lamination workers occupationally exposed to styrene for an average of 14.6 years and 15 unexposed controls. The DNA-repair capacity was moderately higher in the exposed group compared with the controls, but the difference was not significant. There was no significant correlation between the DNA-repair capacity and styrene exposure or biomarkers of genotoxic effects (strand breaks, DNA adducts, chromosomal aberrations, or *HPRT* mutant frequencies). The authors suggested that the lack of a significant difference was most likely related to inter-individual variability in DNA-repair rates (significant differences were noted for sex and polymorphisms in *GSTM1*, *XRCC1*, and *XPC* genotypes), differences in the levels and duration of exposure, and the small sample size.

The results of 13 studies evaluating DNA damage in workers with high levels of styrene exposure from fiberglass-reinforced-plastics production, boat building, or hand lamination are summarized in Table 5-11. Twelve studies used peripheral blood lymphocytes, and one study (Migliore *et al.* 2002) used sperm cells. All studies included exposure measures — either styrene in air, mandelic acid (or mandelic acid plus phenylglyoxylic acid) in urine, or both. In three studies (Godderis *et al.* 2004, Laffon *et al.* 2002a, Maki-Paakkanen *et al.* 1991), the authors estimated styrene concentrations in air from urinary mandelic acid levels.

Higher levels of DNA damage were found in styrene-exposed individuals than in controls in all of the studies using the DNA unwinding assay (Brenner *et al.* 1991, Maki-Paakkanen *et al.* 1991, Shamy *et al.* 2002, Walles *et al.* 1993) and in 6 of the 8 studies using the comet assay (Buschini *et al.* 2003, Laffon *et al.* 2002a, Migliore *et al.* 2002, Somorovská *et al.* 1999, Vodicka *et al.* 1995, Vodicka *et al.* 1999). No increase in single-strand breaks was reported in a study that used nick translation (Holz *et al.* 1995).

Some studies found a significant correlation between DNA damage and markers of styrene exposure ($r = 0.753$, $P < 0.01$ for urinary mandelic acid; $r = 0.601$, $P < 0.01$ for urinary phenylglyoxylic acid (Shamy *et al.* 2002); $r = 0.470$, $P = 0.031$ for styrene concentration at the workplace, and $r = 0.545$, $P = 0.036$ for styrene concentration in the blood (Vodicka *et al.* 1999); see Section 5.5.3.1 for a description of the studies by Vodicka *et al.*). Significant correlations also were found between DNA damage and *N*-terminal hemoglobin adducts (partial $r = 0.23$, $P = 0.010$) (Godderis *et al.* 2004) or O^6-guanine DNA adducts ($r = 0.719$, $P = 0.001$) (Vodicka *et al.* 1999). Walles *et al*. (1993) reported that single-strand breaks correlated significantly with increasing exposure when measured at the end of a shift but not at the beginning of a shift. [This study did not use controls.] Single-strand breaks in sperm cells did not correlate with urinary markers in a study of hand laminators in Italy, but the urinary samples were taken on a different day than the semen samples. In contrast to these findings, (Vodicka *et al.* 2004a) found that single-strand breaks correlated negatively with most markers of styrene exposure ($r = –0.350$, $P = 0.007$ for styrene in blood; $r = –0.402$, $P = 0.01$ for urinary mandelic acid; $r = –0.403$, $P = 0.001$ for urinary phenylglyoxylic acid; and $r = –0.375$, $P = 0.003$ for urinary 4-vinylphenol conjugates). As discussed above, Vodicka *et al.* suggested that styrene exposure might induce more efficient repair of single-strand breaks because of a positive correlation between DNA repair capacities and markers of styrene exposure.

All of the studies of DNA damage obtained information on the smoking history of the subjects, and two studies (Shamy *et al.* 2002, Vodicka *et al.* 1995) noted that no smokers were included in their exposed or control groups. Of the remaining 11 studies, 3 found that smoking had potentially confounding effects on levels of DNA damage. Brenner *et al.* (1991) observed that the number of cigarettes smoked per day significantly increased

the number of single-strand breaks in the exposed group [no smokers were included in the control group]. Walles *et al.* (1993) reported that smoking increased single-strand breaks in samples taken at the end of a shift, and Laffon *et al.* (2002a) found an increase in DNA tail length in the comet assay for smokers in the exposed group. No other potential confounders were reported to have a significant effect; however, not all studies included potential confounders in their statistical analyses.

Table 5-11. DNA damage (single-strand breaks or alkali-labile sites) in workers occupationally exposed to styrene

Reference Location	Styrene in air (ppm)[a] Urinary mandelic acid (mg/g of creatinine)[a]	Study population Exposure duration (mean or range) No. exposed & controls	Method and results	Comments
Peripheral blood lymphocytes				
Brenner *et al.* 1991 U.S.	air: 0.6–44 urine: 244	fiberglass-reinforced boat building 2.7 yr 14 exposed 9 controls	*DNA unwinding, hydroxylapatite sep.* negative log of fraction of double-stranded DNA exposed 0.025 ± 0.02** control 0.15 ± 0.01	More smokers (43%) among workers than controls (0%), but reverse for ex-smokers (21% exposed; 55% controls) Workers also exposed to acetone [nongenotoxic] and methylene chloride
Maki-Paakkanen *et al.* 1991 NR	air: [70][b] urine: 9.4 mmol/L	reinforced-plastics production 6.7 yr 9 exposed 8 controls	*DNA unwinding, hydroxylapatite sep.* negative log of fraction of double-stranded DNA exposed 0.13 ± 0.04* control 0.09 ± 0.02	Authors reported that other variables were considered to exclude their effects on the results: age, sex, health status, recent viral infections, vaccinations, exposure to possible mutagenic chemicals, alcohol consumption, and drug intake; however, statistical analyses do not appear to include these variables
Walles *et al.* 1993 Sweden	air: 0.4–20 urine: ND–261	plastics factory 0–25 yr 17 exposed 0 controls	*Alkaline elution* normalized area above elution curve *Time relative to shift* before 33×10^{-3}/h* end 41×10^{-3}/h correlated significantly with increasing exposure at end of shift but not before	Highest levels seen in one man who had taken paracetamol, which has increased single-strand breaks in mice

Reference Location	Styrene in air (ppm)[a] Urinary mandelic acid (mg/g of creatinine)[a]	Study population Exposure duration (mean or range) No. exposed & controls	Method and results	Comments
Holz *et al.* 1995 Former German Democratic Republic	air: [0.017–0.82] urine: 43.9 ± 31.5	styrene production plant 18 yr 25 exposed 25 controls	*Nick translation* cpm of radioactivity incorporated exposed 2,370 ± 1,358 control 2,550 ± 988	Significantly ($P < 0.01$) higher styrene, ethylbenzene, benzene, and toluene in exhaled air of exposed workers than controls Subjects and controls matched for age and sex; similar smoking habits confirmed by plasma cotinine; higher self-reported alcohol consumption in controls
Vodicka *et al.* 1995 Bohemia	air: [21–28] urine: 146 ± 77	hand laminators 9 yr 9 exposed 15 controls	*Comet:* tail moment *Abnormal cells* exposed 5.50 ± 3.04* control 1.00 ± 3.41 tail length and % DNA in tail also significant *Total cells:* NS	All subjects were nonsmokers
Vodicka *et al.* 1999 Bohemia	air: [16–38] urine: 161–351	hand laminators 7.2 yr 13 exposed 13 controls	*Comet*: tail moment exposed 1.9 ± 0.8*** control 0.6 ± 0.2 tail length and % DNA in tail also significant except for exposed vs. control smokers	Difference between exposed and controls also significant when smokers ($P < 0.019$; 4 exposed/5 control) and nonsmokers ($P < 0.005$; 9 exposed/8 control) were considered separately
Somorovská *et al.* 1999 Bohemia	air: high: [46 ± 24] med: [13 ± 5.3] urine: NR	hand laminators and sprayers[c] 14.0 yr 17 high exposure (hand laminators) 12 med. exposure (sprayers) 19 controls	*Comet*: % DNA in tail[d] high-exposure [30 ± 9]*** medium-exposure [27 ± 8]*** control [14 ± 5]	Styrene levels almost 4 times as high in the high-exposure than medium-exposure group, but no difference in SSBs

Reference Location	Styrene in air (ppm)[a] Urinary mandelic acid (mg/g of creatinine)[a]	Study population Exposure duration (mean or range) No. exposed & controls	Method and results	Comments
Laffon *et al.* 2002a Spain	air: 17–19[b] urine: 313–353	fiberglass-reinforced-plastics factory 17 yr 14 exposed 30 controls	*Comet*: tail length exposed 48.68 ± 0.33** control 43.34 ± 0.18	Smoking significantly increased tail length in exposed but not controls; smoking time related to age and styrene exposure duration Influence of exposure duration, age, smoking, and *GSTM1* and *GSTT1* genotype included in analysis of variance; other possible confounders considered in interviews (alcohol consumption, medication, medical diagnostic tests, previous occupational exposure to chemicals); however, they do not appear to have been included in the statistical analysis Exposure to other possible genotoxins [organic peroxides, acetone, and dichloromethane] possible, but not evaluated
Shamy *et al.* 2002 Egypt	air: NR urine: 90–170	reinforced plastic plant 20 yr 26 exposed 26 controls	*DNA unwinding assay hydroxyapatite separation* *% DNA with SSBs*, median (range) exposed 40 (22–65)** control 10 (6.5–13) *Exposure-response with urinary markers (r)* mandelic acid 0.754*** phenylglyoxylic acid 0.601***	

Reference Location	Styrene in air (ppm)[a] Urinary mandelic acid (mg/g of creatinine)[a]	Study population Exposure duration (mean or range) No. exposed & controls	Method and results	Comments
Buschini *et al.* 2003 Italy	air: 36.8 ± 0.7 urine: 206 ± 2.4[e]	polyester resin production; glass-fiber-reinforced plastic manufacture 8.5 yr 48 exposed 14 controls	*Comet:* tail moment 99th percentile exposed 34.1 ± 14.0*** control 12.4 ± 4.9 median: NS	Controls were of comparable age and sex
Vodicka *et al.* 2004a Bohemia	air: [19 ± 13.1] urine: 206 ± 2.4[e]	fiberglass-reinforced plastic manufacture 4 yr 86 exposed 16 plant controls 26 external controls	*Comet*: SSBs per 10^9 Da exposed 0.29 ± 0.21 plant control 0.57 ± 0.26 external control 0.53 ± 0.26 correlation (*r*), *P*-value *Blood:* styrene: –0.350, 0.007 *Urinary:* mandelic acid: –0.402, 0.01 phenylglyoxylic acid –0.403, 0.001 4-vinylphenol conj. –0.375, 0.003	Exposed subjects almost 9 years younger than controls and included more men (71% vs. 52%) and more smokers (51% vs. 19%)
Godderis *et al.* 2004 Belgium	air: 9.5 ± 9.6[b] urine: 202 ± 148	laminators 14.2 yr 44 exposed 44 controls	*Comet:* % DNA in tail exposed 0.80 ± 0.31 control 0.80 ± 0.34	Controls matched for age and smoking habits and recruited from 2 plants manufacturing electrical wires and telecommunications cables

Reference Location	Styrene in air (ppm)[a] Urinary mandelic acid (mg/g of creatinine)[a]	Study population Exposure duration (mean or range) No. exposed & controls	Method and results	Comments
Sperm cells				
Migliore *et al.* 2002 Italy	air: NR urine: 202 ± 148	hand laminators 9.2 years 46 exposed 27 controls	*Comet:* tail moment exposed 1.5 ± 0.6*** control 0.8 ± 0.4 % DNA in tail: significant*** *Exposure-response with urinary markers*: NS	More smokers (63.0%) in control group than in exposed (48.2%) Semen samples (for SSB analysis) and urine samples taken on different days

C = controls, E = exposed, ext. = external; HA = hydroxylapatite; MA = urinary mandelic acid; med= medium, ND = not detected; NR = not reported, PGA = urinary phenylglyoxylic acid; 4-VPT = urinary 4-vinylphenol conjugates.

[a] Mean ± SD or range; air concentrations in brackets converted from mg/m^3 to ppm (1 mg/m^3 ≈ 0.23 ppm).

[b] Calculated from urine mandelic acid levels by the study authors.

[c] Study population also included workers with low exposure (maintenance workers), but these were not included in the analysis.

[d] Values estimated from graph.

[e] Mandelic acid + phenylglyoxylic acid.

5.4.4.3 Mutations

Studies evaluating mutation frequency (for *HPRT* or glycophorin A [*GPA*] genes) in styrene-exposed workers are summarized in Table 5-12. Mutations at the *HPRT* locus may be associated with a number of other factors (e.g., different types of T cells with different lifespans, host polymorphisms affecting metabolism and DNA repair, and background exposures, such as food intake or smoking) (Vodicka *et al.* 1995).

Tates *et al.* (1994) reported that *HPRT* mutation frequency was higher among 46 workers from the former German Democratic Republic who had been exposed to styrene and dichloromethane than in 5 controls [mutation frequency could be measured for only 5 of 23 controls]. Controls were matched by age, sex, and smoking status [No other information was provided on the control subjects.].

A series of studies measuring *HPRT* mutation frequency in a relatively small number of lamination workers from Bohemian hand-lamination factories (with up to six samplings for each individual) was conducted during a three-year period by Vodicka and co-workers and reported in two publications (Vodicka *et al.* 1995, 1999) (see also the description of this population in Section 5.4.4.1), and another study of workers with differing levels of exposure to styrene in these factories was published by Somorovská *et al.* (1999) and Vodicka *et al.* (2001a). Results for *HPRT* mutation frequency from all of these studies were also summarized and reanalyzed in two reviews of styrene genotoxicity by Vodicka *et al.* (2002a, 2003). The 2003 analysis of all of the samplings from this population (Vodicka *et al.* 1995, 2001a, 1999) found a higher *HPRT* mutation frequency in styrene-exposed workers (19.8 ± 20.1 per 10^6 cells) than in controls (14.9 ± 7.7), but the difference was not significant ($P = 0.656$). Some of the individual samplings did result in significant differences between exposed workers and controls; *HPRT* mutation frequency was significantly higher in workers than external controls but not factory controls in one of four samplings in the 1995 study and in the sixth sampling in the 1999 study ($P = 0.039$) (Vodicka *et al.* 1999). In the 2001 study of these workers, *HPRT* mutations were higher in styrene-exposed workers than controls, though not significantly so. Although Vodicka *et al.* (2003) reported that their analysis of all data from the hand-lamination workers did not show a significant difference between exposed

workers and controls, they did find a significant correlation ($r = 0.588$, $P = 0.001$) between *HPRT* mutation frequency and cumulative exposure [the product of an arbitrary exposure level and years of employment]. Individual studies had also shown significant correlations between *HPRT* mutation frequency and styrene concentration in air, styrene concentration in blood, urinary mandelic acid, hemoglobin adducts, years of employment, age of employees, or heterozygosity in the CYP2E1 and GSTP1 genes (Vodicka *et al.* 2001a, Vodicka *et al.* 1999). None of the studies reviewed reported a significant correlation between DNA adducts and *HPRT* mutation frequency.

Somatic mutations at the *GPA* locus in erythrocytes were measured in 47 workers from 10 reinforced-plastics plants in Finland (Bigbee *et al.* 1996). The controls were 47 unexposed individuals matched for age, gender, and smoking status. All exposed and control subjects had the *GPA* M/N heterozygous genotype. *GPA* variant frequencies reflecting allele loss (ϕ/N) or allele loss and duplication of the remaining allele (N/N) were examined. Styrene exposure did not affect ϕ/N frequency, but N/N frequency was higher among workers than controls ($P = 0.058$). When workers were classified into low- and high-exposure groups, the N/N frequency was significantly higher ($P = 0.036$) in the high-exposure group, based on a multivariate analysis of covariance model. However, the significant difference was seen only when one individual in the high-exposure group with an exceptionally low value was excluded; when that individual was included in the analysis, no difference was found. Significant increases in both ϕ/N and N/N frequencies also were seen among active smokers, but the analysis for styrene exposure was adjusted for smoking. The authors concluded that occupational exposure to styrene in the reinforced-plastics industry resulted in mutagenic effects.

Table 5-12. Mutation frequencies in workers exposed to styrene

Reference, industry, & mutation	Group (no. M/F)	Years exposed[a]	Styrene in air [ppm][a]	Mutation frequency per 10^6 cells ± SD	Comments
Germany					
Tates *et al.* 1994 Production of containers & boards with polyester resin (19% –33% styrene) *HPRT*	5 control (NR) exposed: 24 group I (16/8) 22 group II (9/13) 46 total (25/21)	0 19 (4–31) 20 (8–31) 20 (4–31)	0 [20 (0–140)] [12 (0–34)] [16 (0–140)]	8.6 ± 1.2 15.9 ± 21.1 12.7 ± 6.8 14.3 ± 15.7	Only 5 of 23 control samples could be analyzed because of losses during transport Groups I & II sampled 1 wk apart Workers also exposed to dichloromethane
Bohemia (some overlap in subjects among studies; hand laminators from same plants)					
Vodicka *et al.* 1995 Hand-lamination plant *HPRT*	7 factory control (3/4) 9 exposed (2/7)	NR 9 (1.5–17)	0 [21 (5.8–58)][b]	15.7 ± 8.3 17.5 ± 12.3 4 samplings combined	Factory controls (but not external controls) had measurable levels of styrene-specific DNA adducts, suggesting possible low-level exposure Significant difference seen in only 1 of 4 samplings of the same individuals
	8 external control (0/8) 9 exposed (2/7)	0 9 (1.5–17)	0 [21 (5.8–58)][b]	11.8 ± 6.8 18.0 ± 5.2* sampling IV[c]	
Vodicka *et al.* 1999 Hand-lamination plant *HPRT*	13 external control (3/10) 12 exposed (4/8)	0 7.2 (2–17)	0 [16 (3.5–36)]	14.2 ± 6.5 22.3 ± 10.6* sampling VI	Only external controls used
Somorovská *et al.* 1999, Vodicka *et al.* 2001a Plastics lamination plant (hand-lamination workers) *HPRT*	19 control (8/11) 19 exposed (2/17)	0 14 ± 6.1	0 [23 ± 23.5][d]	13.3 ± 6.3 20.2 ± 25.8	Controls were clerks in the same factory Mutation frequency significantly higher (P = 0.04) in smokers than nonsmokers overall but not among controls or exposed analyzed separately

Reference, industry, & mutation	Group (no. M/F)	Years exposed[a]	Styrene in air [ppm][a]	Mutation frequency per 10^6 cells ± SD	Comments
Finland					
Bigbee *et al.* 1996 Reinforced-plastics workers *GPA ϕ/N*	47 control (23/24) 47 all exposed (23/24) 28 highest exp. (NR) 19 lowest exp. (NR)	0 8.5 ± 6.6 NR NR	0 [36 ± 27] [≥ 20] [0.2–19]	8.1[e] 7.2[e] 7.6[e] 7.5[e]	Multivariate analysis of data adjusted for age, gender, smoking status, and styrene exposure for both ϕ/N and N/N
GPA N/N	47 control (23/24) 47 all exposed (23/24) 28 highest exp. (NR) 19 lowest exp. (NR)	0 8.5 ± 6.6 NR NR	0 [36 ± 27] [≥ 20] [0.2–19]	5.0[e] 6.3[e] 7.2[e]* 6.0[e]	Difference nonsignificant for high-exposure group when 1 subject with an exceptionally low value was included

F = female; M = male; NR = not reported.
*Significantly different from the control group at $P < 0.05$ by the Mann-Whitney U test (Vodicka *et al.* 1995, 1999) or multivariate analysis of variance (Bigbee *et al.* 1996).
[a] [Mean and range; air concentrations in brackets converted from mg/m^3 to ppm (1 $mg/m^3 \approx 0.23$ ppm).]
[b] Air concentrations measured on the day of sampling.
[c] Listed as sampling IV (Vodicka *et al.* 1995), which corresponds to sampling V in Vodicka *et al.* (1999).
[d] Based on mean for all exposed workers; data were not provided for the subset of workers used to study *HPRT* mutations.
[e] Least squares mean of log-transformed data adjusted for age, gender, and smoking status.

5.4.4.4 Cytogenetic markers

Cytogenetic markers include chromosomal aberrations, micronuclei, and SCE. The cytogenetic effects of occupational exposure to styrene have been reviewed (Bonassi *et al.* 1996, Cohen *et al.* 2002, Henderson and Speit 2005, Vodicka *et al.* 2006b). Guidelines for monitoring of genotoxic effects in humans are available in Albertini *et al.* (2000). Many of the studies reviewed in this section evaluated more than one cytogenetic marker; however, results are discussed separately for each marker.

Most of the reviewed studies used questionnaires to gather information on exposed and referent population characteristics such as age, sex, socio-economic status, disease status, smoking habits, vaccinations, and past or current exposures to other clastogenic agents, including X-rays.

Chromosomal aberrations

Structural chromosomal aberrations were measured in lymphocytes from styrene-exposed workers in 31 studies. Details on the study population, exposure levels, study design, and results for structural chromosomal aberrations are summarized in Table 5-13 and the findings are summarized after the tables.

.

Table 5-13. Chromosomal aberrations in lymphocytes from workers occupationally exposed to styrene

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response		Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)			
Meretoja *et al.* 1977 (Finland)	Polyester plastic manufacturing workers — 3 plants (laminators) (0.6–8.5 yr)	Exposed 10 Controls 5	NR	[721 (23–3,257)]	Gaps[e] [0.3] [0.2]	Breaks[e] [16.3]*** [1.6]	Unmatched controls but similar age range; all subjects were male No previous exposure to known clastogenic agents; no recent viral infections or vaccinations Cell harvest at 64–68 h No dose-response analysis Statistics: Student's *t*-test
Meretoja *et al.* 1978a (Finland)	Polyester plastic manufacturing workers — 2 plants (laminators) (1–15 yr)	*Exposed* 1976 16 1977 10 *Controls* 6	NR (≤ 300) NR (≤ 300)	[570 (23–3,257)] [329 (52–1,646)]	Total 15.1*** 16.2*** 2.0		Unmatched controls but similar age range; all subjects were male 10 exposed subjects first samples in 1976 were reanalyzed in 1977 No previous exposure to clastogenic agents No correlation with smoking. Cell harvest at 64–68 h CAs without gaps not reported. Chromosome-type breaks most common among exposed while aneuploidy was most common in controls Statistics: Student's *t*-test
Fleig and Thiess 1978, Thiess and Fleig 1978 (Germany)	Group A: styrene plant (14–25 yr); Group B: poly-styrene plant (3–39 yr); Group 3: three unsaturated polyester resin plants (2–24 yr)	*Exposed* group A 5 group B 12 group C 14 *Controls* 20	NR NR (0.01–0.53)[f] NR (50–300)[g]	(mg/L) NR (19–40) NR (< 5–100) NR (102–> 1,500)	Total 3.8 5.1 9.2* 5.5	w/o Gaps 1.6 1.9 5.3* 2.1	Group B and controls were male, gender not identified for other groups. Mean age similar for all groups Workers also exposed to peroxides, styrene-7,8-oxide, methylene chloride, and acetone Cell harvest at 70–72 h CA reported as including and excluding gaps but types of CAs in these categories were not defined; polyploid cells counted separately

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response		Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)			
							Statistics: Method and *P* values not identified
Hogstedt *et al.* 1979 (Sweden)	Fiberglass-reinforced polyester resin boat manufacture workers (0.5–10 yr)	Exposed 6 Controls 6	NR [11.5–92]	490 (225–2,100)	(CA/100 cells) Total 10.2*** 4.9 Breaks 6.9** 2.5 Gaps alone and the sum of gaps, breaks and hyperdiploidy were also significantly higher in exposed than controls		Controls from nearby paper factory matched on age and smoking; all subjects were male Workers exposed to phthalic acid and maleic acid anhydride, propylene glycol, methylethyl ketone peroxide, acetone and cobalt salt Cell harvest at 72 h Total in this table includes breaks and gaps; hyperdiploidy was also scored. Total aberrations not related to exposure time Statistics: Mann-Whitney U-test, one-tailed
Andersson *et al.* 1980 (Sweden)	Reinforced plastic boat factory workers (0.3–12 yr)	*Exposed* total 36 high 14 low 22 *Controls* 37	(mg/m^3 × yr) 575 (6–1589) 1204 (710–1589) 137 (6–283)	NR	(CA/100 cells) Gaps[h] 6.1 6.6 5.8 3.8 w/o Gaps 7.9*** 7.8*** 8.0*** 3.2 Increase in frequency of all types of CA measured in exposed compared with controls. Exposure response *Cumulative styrene exposure (time vs. air levels)* Low exposure group: (*r* = 0.58): significant High-dose group: not significant		Total population included 39 exposed and 41 controls but results not available for all subjects Age-matched controls included office, assembly shop, and workshop employees; all subjects were male Also exposed to methylethyl ketone peroxide Cell harvest at 68 h Chromosomal aberrations included gaps (not included in total), breaks, minutes, dicentrics, rings, and acentric fragments; chromatid breaks were most frequent Negative correlation with smoking in controls Statistics: *t*-test, multiple regression including CA frequency, employment duration, styrene exposure, smoking, alcohol intake, exposure to X-rays and solvents, and use of a breathing mask

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response		Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)			
Thiess *et al.* 1980 (Germany)	Polyester resin processing workers (4–27 yr)	*Exposed* 24 laboratory pilot plant *Controls* 24	 6 (1–11.5) 58 (0.7–178)	(mg/L) 0–320	Gaps 5.1 NR NR 3.8	w/o Gaps 1.9 NR NR 1.5	Controls: Occupational Health and Protection Department, office staff and plant maintenance workers. Gender not identified Smoking and alcohol habits, virus disease and consumption of drugs were recorded. Cell harvest at 70–72 h Authors defined "w/o gaps" to include breaks, fragments, chromatid interchanges and dicentric chromosomes; "including gaps" to include both chromatid and isochromatid gaps, but it was not clear if this group also included other CA because it was called "including gaps" by study authors Statistics: Fisher-Yates exact test
Watanabe *et al.* 1981 (Japan)	Group 1: Reinforced-plastics boat workers Group 2: Polyester resin board workers (0.6–9.3 yr)	*Group 1* exposed 9 controls 5 *Group 2* exposed 7 controls 8	< 70 (1–211) 36 (NR)	(mg/L) 647 (90–4,300) 32 (5–115) 526 (300–1,360) 32 (5–115)	Total 3.3 3.6 3.6 2.9		Controls matched on age and sex; Group 1 subjects were male; Group 2 subjects included both male and female Exposure varied depending on the work in workshop 1 but was stable in workshop 2 CA scored in M_2 cells (< 50/person), most aberrations were gaps. Mitomycin C treatment did not increase the number of CA in exposed or controls Statistics: *t*-test or Chi-square
(Watanabe *et al.* 1983) (Japan)	Male fiber-reinforced-plastics boat factory workers in 2 workshops	*Exposed* total 18 group A 10 group B 8 *Controls* 6	40–50 (NR)	(mg/L) 332 (0–1,041) 399 (0–1,041) 249 (8–999)	Total 6.5[i] 6.6 6.4 4.7	w/o Gaps 1.1 1.0 1.3 1.1	All subjects were male, controls matched according to age Smokers: 72% exposed and 50% controls Most aberrations were gaps but also included

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response		Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)			
	(1 mo–30 yr)				Exposure response *Styrene air levels, urinary levels and exposure duration:* no relationship		acentric fragments, deletions, and breaks Cell harvest at 50 h No difference in CA between smokers and non-smokers in exposed or control groups Statistics: Mann-Whitney U test and *t*-test (2-tailed)
Dolmierski *et al.* 1983 (NR)	Laminators (1–30 yr)	Exposed 30 Controls 2	NR [< 23]	NR	Gaps 26.9* 14.4	Breaks 6.8 0	Little information on exposed or control subjects. Gender was not identified, only 2 controls (ages 22 and 28); ages in exposed ranged from 22–58 yr Exposure was "haphazard" and measured once a year; repeated on 6 subjects after 1.5 yr Cell harvest at 68 h ~40 metaphases/person examined Gaps most common but were not measured in a group of 6 subjects because "interpretation" was difficult Statistics: Poisson's distribution
					Exposure response *Length of employment:* no correlation		

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response	Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)		
Camurri *et al.* 1983, Camurri *et al.* 1984 (Italy)	Reinforced unsaturated polyester resin manufacturing workers in 9 plants (1–22 yr)			(mg/L)	Total	Data described for 6 plants in 1983 publication, all data described in 1984 publication Controls matched for age, sex, and smoking. No subjects had recent viral infections, vaccinations or exposure to known clastogenic agents; however, processing of unsaturated polyester resins in the reinforced-plastics industry involves exposure to other industrial chemicals (e.g., organic peroxides, solvents, and dyes) Cell harvest at 50 h CA did not correlate with smoking habits 32–360 metaphases/subject (1983 publication) Types of CAs were not reported and it is not clear whether gaps were included in total Statistics: Student's *t*-test, linear regression
		Plant 1 3 Controls 3	NR [7–9]	NR (45–75)	30** 9	
		Plant 2 4 Controls 4	NR [16–23]	NR (65–133)	23*** 8	
		Plant 3 4 Controls 6	NR [23–34.5]	NR (170–694)	24*** 8.4	
		Plant 4 5 Controls 6	NR [34.5–46]	NR (151–786)	26*** 8.4	
		Plant 5 6 Controls 6	NR [46–57.5]	NR (340–671)	32*** 7	
		Plant 6 2 Controls 2	NR [57.5–69]	NR (615–777)	39** 6.5	
		Plant 7 2 Controls 2	NR [69–80.5]	NR (489–828)	37** 8	
		Plant 8 4 Controls 2	NR [80.5–92]	NR (504–909)	25*** 4	
		Plant 9 7 Controls 4	NR (> 92)	NR (389–1,108)	44*** 4.5	
					Exposure response (all subjects) *Styrene air concentrations and urinary metabolites:* Linear increase ($P < 0.01$) in 1983 study but not in 1984 study	
Hansteen *et al.*	Glass-fiber				(CA/100 cells)	Controls matched for sex, age and smoking;

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response		Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)			
1984 (Norway)	reinforced polyester plant workers (NR)	*Exposed* Total 18 group 1 11 group 2 7 *Controls* 9	13.2 (2–44) 7.5 (2–13) 22.3 (14–44)	NR (200–1,200)[j]	Gaps 19.5*** 17.6 22.4*** 13.4	w/o Gaps 1.2 1.1 1.4 1.7	exposed divided into 2 groups based on exposure Cell harvest at 48–53 h CA breaks were significantly higher in smokers vs. non-smokers. The group w/o gaps was labeled as "breaks" by the authors and included breaks, fragments and exchanges No differences between 2 exposed groups Statistics: Significant by Fisher-Irwin test but not by Wilcoxon's 2 sample ranking test; *P* values reported for total group for gaps only
Nordenson and Beckman 1984 (Sweden)	Glass-fiber reinforced polyester plant workers (1–26 yr)	*Total* exposed 15 controls 13 *Smokers* exposed 4 controls 3 *Nonsmokers* exposed 11 controls 10	24 (NR)	(mM) NR (< 2)	(CA/100 cells) Total 2.8 2.7 4.6 2.7 2.1 2.7	 Breaks[k] [0.4] [0.4] [0.6] [0.3] [0.3] [0.4]	All subjects were male; controls were salesmen or office workers with similar smoking habits, and similar age distribution. Exposed to acetone Cell harvest at 64–68 h Total includes gaps, chromatid breaks and chromosome breaks. Most aberrations were gaps. No correlation with exposure time (type of analysis not reported). Statistical methods: Fisher's exact test
van Sittert and	Propylene				(CA/100 cells)		All subjects were male

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response		Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)			
de Jong 1985 (Netherlands)	oxide and styrene manufacturing workers				Gaps 1.33 4.36* 0.84 1.32 0.78	Breaks 0.65 1.47* 0.60 0.33 0.44	and 20 subjects not involved in manufacturing (matched by age and smoking status). Samples taken in workers 1 yr (1979), 2 yr (1980), and 3 yr (1981) after exposure; fewer exposed workers each year due to transfer to other plants Workers exposed to propylene oxide and benzene Authors reported no change in styrene, propylene oxide, and benzene air levels from 1978–1981, thus they did not think the increase in CA in 1980 was due to occupational exposure Statistics: methods not reported
					No significant difference in exchange-type CA was found between exposed and controls		
Pohlova and Sram 1985 (Czech Republic)	Plant A: polystyrene vessels Plant B: sports boats, plastics (1–11 yr)	*Plant A* June 36 November 34 Controls 19 *Plant B* June 22 Controls 22 January 19 Controls 17	[16–35 (1.4–226)] [NR (9–126)]	(µg/L) NR (35–510) NR (88–972) NR (40–1140) NR (200–3,000)	% AB.C 1.38 1.41 1.26 1.72 1.36 2.81 1.88	(CA/100) Gaps 1.36 2.56 1.63 3.23* 1.78 2.59 2.29	Controls matched for sex and age. Smoking and drug intake were similar for all groups. Subjects not exposed to other known mutagens (queried about viral infections, drug intake, X-rays, smoking and alcohol use) CA measured twice: June and November (Plant A) and June and January (Plant B); there was no concurrent control for November sampling in Plant A Cell harvest at 54 h Percent aberrant cells (% AB.C) included cells with breaks and exchanges. Results for gaps only provided per 100 cells. Inter-sampling differences: Plant A – significant increase ($P < 0.01$) in rates of gaps (from 1st sampling to 2nd sampling) in styrene-exposed workers. Plant B – significantly higher rate of

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response		Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)			
							gaps at first sampling and significantly higher rate of % AB.C at 2nd sampling in exposed workers No significant differences found for drug intake, gender, and smoking Statistics: Student's *t*-test
Maki-Paakkanen 1987 (Finland)	Reinforced-plastics workers (mainly laminators) (1–25 yr)	Exposed 21 Controls 21	[23 (8–60)]	(mM) 2.0 (0–7.3)	Total 4.5 4.9	w/o Gaps 3.0 3.7	Controls matched according to sex and smoking. Control and exposed subjects were similar in alcohol and drug intake, vaccinations, recent viral infections, and previous occupational exposure to chemicals Exposed group was mainly laminators Cell harvest at 50 h CA included gaps (most frequent), breaks (mainly chromatid type), and rearrangements (infrequent). CA slightly higher (not significant) in smokers than non-smokers among controls. No correlation with exposure extent or duration Statistics: Student's *t*-test
Forni *et al.* 1988 (Italy)	(A) Reinforced-plastics workers (18.8 yr) (B) Plastic boat manufacturing workers (4.5 yr)	*Factory A* exposed 32 controls 32 *Factory B* exposed 8 controls 8	NR [0.4–57] NR [9.4–45.5]	NR	Total 3.2 2.9 4.3 2.9	w/o Gaps 2.3* 1.6 2.5 1.5	All factory workers were male Controls lived in the same industrial area (gender not identified) matched for age and smoking Subjects not exposed to other known genotoxic agents (radiation, chemicals, drug intake, and

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response		Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)			
					Factory A workers had significantly higher frequency of unstable CA		recent viral infections); workers exposed to low levels of acetone. Cell harvest at 48 h CAs included gaps, breaks, exchanges and unstable chromosome-type aberrations (acentric fragments, dicentrics, and ring chromosomes) Statistics: Wilcoxon matched-pairs test
Jablonicka *et al.* 1988 (Czech Republic)	Laminated plastic shop workers (11 yr)	Exposed 11 Controls 11	[58 (27–134)]	(μL/mM)[m] NR (214–711)	% AB.C 1.3 1.4	(CA/100) Gaps [0.27][n] [0.55]	All subjects were female Controls and exposed subjects had similar age, social habits, living and working environments. Histories of viral infections and other diseases during past 3 mo along with drug and alcohol intake, smoking, and X-ray exposure recorded Subjects with illness or taking medications were excluded; only 2 weak smokers in each group % AB.C included chromosome and chromatid breaks and chromatid exchanges (no exchanges observed), the total number of gaps observed in 1,100 metaphases were reported. Gaps were not considered as aberrations Cell harvest at 50–52 h (approximately 82% first-division cells) Statistics: Student's *t*-test
(Hagmar *et al.* 1989) (Sweden)	Glass reinforced polyester plastic workers	Exposed 11 Controls 14	[13 (0.9–127)]	NR	(CA/100 cells) w/o Gaps 1.2 1.5	 Gaps 0.7* 1.7	Total population included 20 workers and 22 controls; technical difficulty prevented analysis on all subjects

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response		Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)			
	(0.1–25.4 yr)				Exposure response *No association with years of employment*		One exposed subject was female More smokers in controls (64%) than exposed workers (45%). Similar number of exposed and control subjects reported recent exposure to X-rays (65% vs. 55%) and regular prescription drug use (15% vs. 23%) Workers exposed to low levels of acetone and methylene chloride Cell harvest at 48 h The group "w/o gaps" was labeled as "breaks" by the authors, but included chromatid and isochromatid breaks, pericentric inversions, rings, and dicentrics. Statistics: data were adjusted for age and smoking and transformed using the average square root. Multiple linear regression evaluated employment time, smoking, and age.
Maki-Paakkanen *et al.* 1991 (Finland)	Reinforced plastic workers (controls from research institute) (smokers – 6.4 yr, non-smokers – 7.2 yr)	*Total* exposed 17 controls 17 *Smokers* exposed 11 controls 11 *Nonsmokers* exposed 6 controls 6	[~ 70] (NR)[o]	(mM) 9.4 (< 1–21.5) 11.0 (< 1–16.6) 6.5 (< 1–21.5)	Total 5.3 5.7 4.9 6.8 6.0* 3.7 Exposure response *Exposure duration: r = 0.59, P < 0.02*	w/o Gaps 3.0 3.1 3.0 3.4 3.0 2.7	Controls selected from a research institute Age, sex, smoking, health status, alcohol and drug intake, viral infections, vaccinations, and exposure to other chemicals were considered. CA significantly increased in control smokers compared with control non-smokers CA types not defined except to distinguish CA with gaps and CA without gaps Cell harvest at 50 h Statistics: Wilcoxon rank-sum test (one-tailed testing) and *t*-test (one-tailed)

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response	Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)		
Sorsa *et al.* 1991 (Finland)	Reinforced plastic industry workers from 32 enterprises (NR)	*Past exp. (index pt)* *Laminators* low 22 high 28 *Other workers* low 11 high 14 *Controls* plastics plant 12 other factory 42 *Pop with CA data* Exposed 109 Controls 54	43 (5–182) 11 (1–133)	(mM) 2.2 (NR)[j,p]	w/o Gaps 1.8 1.9 2.4 1.4 1.8 1.6 NR NR Exposure response: *Current or past exposure:* no correlation	Total population consisted of 248 exposed workers, including 154 laminators and 63 controls. CA results available for a subset. Inadequate description of the study population. Exposure groups were divided into two subsets based on estimated past exposure index points (calculated from exposure duration, urinary metabolites, and styrene concentrations). Past exposure index not available on all subjects Cell harvest at 50 h Types of CAs not reported except to state that they did not include gaps Age ($P = 0.06$) and smoking ($P = 0.08$) were correlated with CA Statistics: regression analysis, no details provided
Tomanin *et al.* 1992 (Italy)	Polyester resin workers at 2 factories producing fiberglass tanks (1–18 yr) or fiberglass boats (1.5–15 yr)	*Factory 1 (low)* exposed 7 controls 7 *Factory 2 (high)* exposed 11 controls 11	NR [4.8–23] NR [26–100]	186 (46–345) 725 (423–1,325)	w/o Gaps 1.4 1.4 3.0* 0.8 Exposure response *Exposure duration:* no correlation	Controls matched for sex, age, and smoking Cell harvest at 48 h CA including breaks, dicentrics and other exchanges; gaps not reported No significant effects of smoking Statistics: Mann-Whitney U test (2-sided), and linear regression

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response		Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)			
Tates *et al.* 1994 (Germany)	Polyester resin/fiberglass plastic products production workers (4–31 yr)	*Exposed* total 46 group 1 24 group 2 22 *Controls* 23	[16 (0–138)] [20 (0–138)] [12 (0–34)]	NR	Total 2.9 *** 3.0*** 2.8*** 0.9 Exposure response *Styrene air levels (TWA:* Positive correlation with styrene exposure in Group 1 workers only	(CA/100) w/o Gaps 2.0*** 2.1*** 1.9*** 0.4	Controls matched for age, sex and smoking Workers divided into 2 groups with similar working conditions but blood samples collected 1 wk apart Culture time not stated CA consisted of gaps, iso-gaps, chromatid breaks, isochromatid breaks and fragments; chromatid exchanges were rare. Workers also exposed to dichloromethane (a genotoxin), which was associated with CA in group 1 and the total population No significant differences for chromosomal aberrations between smokers and nonsmokers. Statistics: one-tailed Mann-Whitney U test and bivariate regression analysis
Artuso *et al.* 1995 (Italy)	Fiber-reinforced plastic boat building workers (NR)	*Exposed* low 23 high 23 *Controls* 51	NR [0.5–28] NR [20–319]	NR	(CA/100 cells) w/o Gaps 2.8 4.0** 2.1 Regression analysis (RR, 95% CI) *Low:* 1.38 (0.98–1.94) *High:* 1.71 (1.25–2.33)		All subjects were male; controls lived in same geographic area and had similar ages and smoking habits as exposed Carpenters in the low-exposure group also exposed to solvents and wood dust CA analyzed by 2 different labs and 3 slide readers Cell harvest at 72 h CAs included breaks and exchanges; gaps scored but not reported Smoking, alcohol, and exposure to X-rays were not associated with CA Statistics: Mann-Whitney U test, Terpstra-Jonckheere test for trend and Poisson regression

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response	Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)		
Anwar and Shamy 1995 (Egypt)	Reinforced-plastics plant workers (5–22 yr)	Exposed 18 Controls 18	NR	328 (145–1,204) 50 (22–92)	Total / w/o Gaps 6.1* / 4.0* 3.4 / 1.4 Exposure response *Duration of exposure, urinary mandelic acid:* no significant correlation	All subjects were male; no smokers Controls were admin staff matched for age No information on potential confounding exposures Control samples were collected 1 wk after the exposed samples Cell harvest at 48 h 50 to 100 metaphases/person CA included chromatid gaps, chromatid breaks acentric fragments, and dicentrics. Statistics: Chi-square test
Lazutka *et al.* 1999 (Lithuania)	Carpet plant workers (2 mo–21 yr) Plasticware plant workers (2 mo–25 yr)	*Exposed* carpet 79 plastics 97 *Controls* 90	NR [0.03–0.32] NR [1–1.4]	NR NR	(CA/100 cells) w/o Gaps 3.8* 4.2* 1.7 Exposure response *Exposure duration: no association*	Controls matched for age Workers at both facilities were exposed to phenol and formaldehyde (higher concentrations than styrene at the carpet plant) Cell harvest at 72 h CA included breaks (chromatid & chromosome) and exchanges; breaks were most common. Gaps not recorded. CA not affected by smoking, age Statistics: *t*-test on average square root-transformed data, ANOVA
Somorovská *et al.* 1999 (Bohemia)	Plastics hand-lamination plant workers (14 yr)	*Exposed* total 44 low 15 medium 12 high 17 *Controls* 19	[23] (NR) [6] (NR) [13] (NR) [46] (NR)	NR	(CA/100 cells) w/o Gaps 3.3*** 3.3*** 2.5** 3.8*** 1.4	Control group from the same factory matched by age but not gender or smoking No exposure to other chemicals Cell harvest at 48 h Smoking habits not related to CA frequency CA defined as chromatid-type and

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response	Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)		
					Exposure response *Styrene in air:* $r = 0.43$ ($P = 0.001$) *Blood levels:* $r = 0.41$ ($P = 0.001$) *Exhaled air:* $r = 0.5$, ($P < 0.001$) *Exposure duration:* $r = 0.55$ ($P < 0.001$)	chromososome-type; gaps scored but not reported CA frequency was higher in females than males Suppression of the proliferative response of T cells to mitogenic stimulations seen in styrene-exposed workers CA also correlated with single-strand breaks Statistics: Mann-Whitney U test, regression analysis
Oberheitmann *et al.* 2001 (Germany)	Boat building workers (8.7 yr)	*Total* exposed 14 controls 7 *Smokers* exposed 4 controls 3 *Nonsmokers* exposed 10 controls 4	NR [<23]	NR	(CA/100 cells) Exchanges 0.22[q] 0.14 0.25 0.54 0.23 0.10	Controls were from a wood manufacturing training center, not matched for smoking (higher in contols) Cell harvest at 48 h Only reported exchange type CA (analyzed by FISH) Results from X-ray challenge assay indicated that exchange-type aberrations were significantly higher in exposed than historical controls (N = 2) and correlated with lifetime exposure to styrene but not current exposure Statistics: Fisher's exact (right-sided) and regression analysis
Biró *et al.* 2002 (Hungary)	Oil refinery workers (NR)	Exposed 10 Controls 25	NR	NR	w/o Gaps 3.8 1.8	All individuals were asked to provide information on age, medication, smoking, and drinking habits, and medical and work histories. Exposed included more men (80% vs. 20%) and smokers (80% vs. 20%) than controls; ages were similar Cell harvest at 50 h Smoking did not correlate with CA

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response	Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)		
						Types of aberrations not identified but did not include gaps or aneuploidy Exposed subjects had significant decrease in CD25+ CD4+ cells and an increase in CD45RO+ CD4+ T cells Statistics: Student's *t*-test
Vodicka *et al.* 2004a (Czech Republic)	3 Reinforced-plastic lamination plants: (A: 3.4 yr) (B: 5.6 yr) (C: 2.5 yr)	*Exposed* total 86 plant A 35 plant B 31 plant C 20 *Controls* plant 16 external 26	[19] (NR) [26] (NR) [11] (NR) [19] (NR)	497 (NR)[j] 798 (NR) 270 (NR) 308 (NR) 42	w/o Gaps 2.3 2.5 2.3 2.0 1.7 3.2 No significant differences for chromatid-type aberrations (with or without gaps) or chromosomal breaks. Exposure response *No correlation with any marker of styrene exposure*	Internal controls: male maintenance workers plant B; External controls: regional hygienic station employees Control groups not matched on age, sex, or smoking but differences considered in the analysis Cell harvest at 48 h Types of CA not completely defined but included chromatid type without gaps, chromatid type with gaps, and chromosome breaks; it is not clear whether total CA without gaps includes CA other than chromatid type without gaps and chromosome breaks CA correlated with age Statistics: Mann-Whitney U test
Vodicka *et al.* 2004c (Slovak Republic)	3 Groups of tire plant workers (1: 19.4 yrs) (2: 19.1 yrs) (3: 12.1 yrs)	*Exposed* group 1 53 group 2 41 *Controls* 16	NR [1.9–3.0] NR (NR)	NR	Total 2.2 1.3* 2.3	All subjects in control group (group 3) and group 1 were male, group 2 included 9 females. Group 1 were workers from the mixing departments and had a higher risk of xenobiotic exposure. Group 2 were workers from production, pressing, fire brigade, and clerks Control group not matched by age or smoking

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (% cells with CA)[c] Exposure response		Comments[d]
			Air (ppm)[b]	Urinary MA (mg/g creatin.)			
							Confounding factors (such as X-rays, drug use, dietary and life style-factors were recorded in questionnaires Types of CA not defined and it is not clear whether gaps were included. It is also not clear whether data is given as CA/100 cells or % AB.C Workers exposed to 1,3-butadiene, PAHs, and sulfur dioxide Statistics: Mann-Whitney U-test
Migliore *et al.* 2006b (Italy)	Fiber reinforced-plastics or polyester resin workers from 10 plants (< 1–34 yr)	Exposed 72 Controls 89	[8.5 (0.5–123)]	300 (10–1,856)[j]	Total 3.3 3.6	w/o Gaps 2.4 2.5	Total population included 95 exposed workers and 98 controls. CA analysis not conducted in all subjects. Controls were from the same geographic area with comparable age. Controls had fewer smokers (42% vs. 53%) but more women (32% vs. 20%) compared with exposed Subjects interviewed for personal, occupational, and medical history (X-rays, viral infections and inflammatory disease, drug use) CA without gaps were higher in smokers but were not related to gender. CAs defined as chromatid and chromosomal type aberrations. Statistics: Multifactorial ANOVA and first-order regression
					Exposure response *Chromosomal type CA (without gaps)* Airborne styrene: $r = 0.393$, $P = 0.013$ MA + PGA: $r = 0.293$, $P = 0.070$ 4-VPT: $r = 0.399$, $P = 0.012$, PHEMA: $r = 0.306$, $P = 0.058$ *Chromatid type CA* Airborne styrene, MA + PGA, 4-VPT – no correlation PHEMA: $r = 0.342$, $P = 0.033$		

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
CA = chromosomal aberrations, FISH = fluorescence *in situ* hybridization, MA = mandelic acid, NR = not reported, PGA = phenylglyoxylic acid, PHEMA = phenylhydroxyethylmercapturic acids, 4-VPT = 4-vinylphenol.

[a] Study population includes both sexes unless otherwise noted.
[b] [Bracketed data were converted from mg/m^3 to ppm (1 mg/m^3 styrene ≈ 0.23 ppm).]
[c] Types of CA data and units varied but are reported as follows: Total = total of all CA including gaps, w/o Gaps = total CA excluding gaps, % AB.C = % aberrant cells, Breaks = breaks only, Gaps = gaps only, Exchange = exchange type aberrations not otherwise defined.
[d] Potential confounders (e.g., differences in age, sex, smoking, exposures to other chemicals, recent infections, vaccinations, etc.) are noted as identified by the study authors.
[e] Authors only provided CA data for individuals. [Population means were calculated by NTP.]
[f] Range presented for areas of the plant where workers were always present. Concentrations in areas visited for short periods during inspections were below 43 mg/m^3 (10 ppm) except on two occasions where concentrations of 91 and 202 mg/m^3 (~21and 46 ppm) were recorded.
[g] Concentrations reported in Thiess *et al.* 1980.
[h] *P* values not reported.
[i] Marginal increase ($0.05 < P < 0.06$).
[j] Sum of mandelic and phenylglyoxylic acids.
[k] Calculated sum of chromosome and chromatid breaks.
[l] Results reported for Dean and Clare laboratory. A second laboratory also analyzed a subset of samples collected in 1981. No significant differences reported for either lab.
[m] Units as reported by the study authors, [but considered questionable].
[n] Calculated values expressed per 100 cells based on 3 gaps in the exposed and 6 gaps in the controls out of 1,100 metaphases examined.
[o] Air concentration was estimated from urine mandelic acid levels.
[p] Average urinary mandelic acid levels were 2.4 mM in laminators that did not use a respirator and 1.3 mM in those who used a respirator.
[q] Study authors did not explain how the value for the total group was less than recorded for either the exposed smokers or exposed nonsmokers.

[In general the studies (N = 31) of chromosomal aberrations in styrene-exposed workers were limited by a small number of subjects and potential confounding from other workplace exposures.] Most studies included 25 or fewer subjects per group, but some studies (Andersson *et al.* 1980, Dolmierski *et al.* 1983, however there were only 2 controls) (Artuso *et al.* 1995, Forni *et al.* 1988, Pohlova and Sram 1985, Somorovská *et al.* 1999, Tates *et al.* 1994, Vodicka *et al.* 2004c) had somewhat larger populations (between 30 and 50 in the total exposed or controls) and five studies had populations between 75 and 100 (Lazutka *et al.* 1999, Migliore *et al.* 2006b, Sorsa *et al.* 1991, van Sittert and de Jong 1985, Vodicka *et al.* 2004c).

[The exposed and referent populations were usually matched for age, gender, and smoking habits.] Some studies that did not use matched subjects controlled for variables (such as age, gender, and smoking habits) in the analysis or reported that age, smoking, and gender distribution were similar between groups. Studies that did not meet these criteria include Thiess *et al.* (1980) (although the authors stated that smoking information was recorded), Sorsa *et al.* (1991) [not clear whether smoking and age were controlled for in the dose-response regression analysis], Biró *et al* (2002) (smoking and gender differed between exposed and controls but ages were similar), Oberheitmann *et al.* (2001) (similar ages but smoking differed between exposed and controls) and Vodicka *et al.* (2004c). [None of these studies found an association between chromosomal aberrations and styrene exposure, see below.] Several studies evaluated the effects of potential confounders such as smoking, age, and gender on aberration frequency. Of the 13 studies that evaluated smoking, 11 (Andersson *et al.* 1980, Artuso *et al.* 1995, Biró *et al.* 2002, Camurri *et al.* 1983, 1984 [considered as one study], Lazutka *et al.* 1999, Maki-Paakkanen 1987, Meretoja *et al.* 1978a, Pohlova and Sram 1985, Somorovská *et al.* 1999, Tates *et al.* 1994, Tomanin *et al.* 1992) reported that smoking did not affect, or was not correlated with an increase in chromosomal aberrations; one study (Maki-Paakkanen *et al.* 1991) found higher chromosomal aberrations in smokers in the control group than non-smoking controls, and another study (Sorsa *et al.* 1991) reported a positive correlation between chromosomal aberration frequency and smoking. Conflicting results were found for the two studies that evaluated age; Sorsa *et al.* reported that age was correlated with chromosomal aberration frequency; however, Lazutka *et al.* did not find

an effect. One study (Somorovská *et al.* 1999) reported that chromosomal aberration frequency was higher in females compared with males; however, Pohlová and Srám did not find any differences related to gender.

[Common study quality issues, which are related to the measurement of chromosomal aberrations included cells cultured too long for the peak period of chromosomal aberrations, inadequate number of metaphases scored per individual, or incomplete exposure data. These potential quality issues are identified in the "Comments" column in Table 5-13. Most of the studies examined at least 100 metaphases per person; however, current guidelines recommend a minimum of 200 metaphases per subject (Albertini *et al.* 2000). Studies that scored fewer than 100 metaphases per person are identified in the "Comments" column. Several studies used cell-culture times that were longer than ideal (i.e., comprising mostly second division cells). In some cases the authors stated that the longer culture times were chosen so that a larger number of mitotic cells would be available for scoring.]

[It is difficult to compare results across studies because the studies were not consistent in data reporting]. Some studies reported the percentage of cells per subject with chromosomal aberrations, while others reported the mean number of aberrations per 100 cells. Studies also varied in the type and description of the aberrations reported; some only gave total estimates, whereas others reported the frequency of specific types of chromosomal aberrations (e.g., gaps, breaks, exchanges) or general categories of chromosomal aberrations (e.g., with or without gaps). The data in Table 5-13 include the most comprehensive measures of chromosomal aberrations reported by the study authors and are identified in the "Results" column. When available, information is presented for total chromosomal aberrations without gaps.

One study (Oberheitmann *et al.* 2001) measured exchange-type chromosomal aberrations and did not find a significant increase in styrene-exposed workers compared with controls. [The study was limited by small numbers of subjects and unmatched controls (wood manufacturing industry) for smoking (more controls than exposed smoked).] However, after X-ray challenge, the rate of exchange type aberration frequency was

higher in exposed compared with historical controls (N = 2). The response was correlated with cumulative lifetime exposure to styrene but not current exposure (see Section 5.4.4.2).

Of the remaining 30 studies, 17 studies — Meretoja *et al.* (1978a, 1977), Fleig and Thiess (1978) (high exposure subgroup), Högstedt *et al.* (1979), Andersson *et al.* (1980), Dolmierski *et al*. (1983), Camurri *et al.* (1983, 1984), Hansteen *et al.* (1984), Pohlová and Srám (1985) (only for one of two plants, and only for one of two samplings), Forni *et al.* (1988), Mäki-Paakkanen *et al*. (1991) (nonsmokers only), Tomanin *et al*. (1992) (high exposure factory*)*, Tates *et al*. (1994), Artuso *et al.* (1995), Anwar and Shamy (1995), Lazutka *et al.* (1999), and Somorovska *et al.* (1999) — reported a significant increase in the frequency of chromosomal aberrations in the exposed population (or subgroup of exposed workers) compared with the controls. [The studies were not consistent in the types of aberrations found to be elevated (see Table 5-13).] Three of these studies found significant increases in gaps only (Dolmierski *et al.* 1983, Hansteen *et al.* 1984, Pohlova and Sram 1985). [Findings reported by Dolmierski *et al.* were limited by the small numbers of controls (N = 2), and findings in some other studies were limited by potential confounding from other occupational exposures.] Workers in the study reported by Tates *et al.* were also exposed to dichloromethane. A positive correlation between styrene exposure (TWA) and chromosomal aberrations was found in one of two exposed subgroups but not the pooled population; however, positive correlations were also found between dichloromethane exposure (TWA) and chromosomal aberrations in that subgroup as well as the total exposed subjects. Workers in the study reported by Lazutka *et al.* were exposed to higher levels of phenol and formaldehyde than styrene, [but no dose-response analysis was performed]. The authors stated that the literature on chromosome damage by occupational exposure to formaldehyde is not consistent and no literature was available on the genotoxic effects of environmental exposure to phenol. Workers in other studies were often exposed to other agents such as peroxides, methylene chloride, and acetone.

[In general, “positive” results were observed in studies with higher levels of exposure or in the high-exposure subgroup. An exception is the Lazutka *et al.* study (exposure

between 0.03 and 1.4 ppm). This study was also one of the larger studies, and as mentioned above the workers were also exposed to formaldehyde and phenol.] Migliore *et al.* did not report an increase in chromosomal aberration frequency in the exposed workers versus the controls, but did find positive correlations with chromosomal type aberrations (without gaps) with various measures of styrene exposure (styrene air levels and urinary metabolites, and chromatid type aberrations with urinary phenylhydroxyethylmercapturic acids. The average exposure levels were low in this study. In addition to Migliore *et al.*, positive correlations with measures of styrene exposure were also reported by Camurri *et al.* (1983, but not 1984 analysis), Andersson *et al.* (cumulative styrene exposure, increase observed in both low and high subgroup but only significant in the low group), Tates *et al*. (in one subgroup but not in the total population), and Somorovska (air, exhaled air, urinary metabolites, and blood levels). Artuso *et al.* used a multivariate regression model and found a higher RR for chromosomal aberrations for high styrene exposure (RR = 1.71, 95% CI = 1.25 to 2.33) than low styrene exposure (RR = 1.38, 95% CI = 0.98 to 1.94). (Smoking, alcohol drinking, and diagnostic X-rays were not risk factors for chromosomal aberrations in this model). Fleig and Thiess, and Tomanin *et al.* reported higher chromosomal aberrations in the high-exposure subgroup (as assessed by air and urinary metabolite levels) compared with the low-exposure subgroup. Pohlová and Srám measured urinary metabolites and chromosomal aberrations in the same workers (at two different plants) at two different sampling times. Urinary metabolites (see Table 5-13) and chromosomal aberrations increased at the second sampling. At the second sampling, styrene-exposed workers in Plant A had a significant increase ($P < 0.01$) in rates of gaps (from 1st sampling to 2nd sampling), and styrene-exposed workers in Plant B had significantly higher rate of percent aberrant cells (cells with breaks or exchanges).

In contrast, the following studies did not find a positive correlation between styrene exposure (either air levels or urinary metabolite level) and chromosomal aberrations: Watanabe *et al*. (1983), Sorsa *et al.* (1991), Anwar and Shamy (1995), and Vodicka *et al*. (2004a). Several studies did not report a significant correlation with exposure duration (Anwar and Shamy 1995, Hagmar *et al.* 1989, Lazutka *et al.* 1999, Tomanin *et al.* 1992, Watanabe *et al.* 1981), while Somorovska *et al.* (1999) and Mäki-Paakkanen *et al.* (1991)

did report a significant correlation with exposure duration. [The ability to detect dose-response relationships is limited by small numbers in most studies; studies with the larger numbers of exposed subjects include Migliore *et al.* (2006b), Somorovska *et al.* (1999), Sorsa *et al.* (1991), and Vodicka *et al.* (2004c).]

Studies that did not find any significant increases in chromosomal aberrations in workers exposed to styrene include Thiess *et al.* (1980), Watanabe *et al*. (1983, 1981), Nordenson and Beckman (1984), Mäki-Paakkanen (1987), Jablonicka *et al.* (1988), Sorsa *et al.* (1991), Biró *et al.* (2002), and Vodicka *et al.* (2004c, 2004a). van Sittert and de Jong (1985) used pre-exposure measures for the reference group and followed the study population for a couple of years. An increase in chromosomal aberrations was observed for only 1 of the 3 follow-up years. The authors reported that there were no changes in styrene, propylene oxide, and benzene air levels from 1978 to 1981, thus, they did not think the increase in chromosomal aberrations in 1980 was due to occupational exposure, and Migliore *et al.* (2006b) reported a positive dose-response relationship but no significant pair-wise comparison, [which complicates the classification (in terms of positive or negative) of these studies. The study by Sorsa *et al*. had limited documentation on its study population.] Mäki-Paakkanen *et al.* (1991) reported an increase in chromosomal aberration frequency in non-smokers but not in smokers or the total population. Watanabe *et al.* (1983) reported that there was a marginal ($0.05 < P < 0.06$) increase in chromosomal aberration frequency in the exposed group compared with the controls. [Most of the "negative" studies had somewhat lower levels of exposures except for Watanabe *et al*. (1983, 1981) and Jablonicka *et al.* (1988).]

As mentioned above, there have been several reviews or evaluations of the cytogenetic effects of styrene. Early reviews such as IARC (1994a,b) and Scott and Preston (1994a) are not discussed here since they only include a subset of the available literature to date. Bonassi *et al.* (1996) performed a meta-analysis of 25 (22 with results for chromosomal aberrations) biomonitoring studies of occupational exposure to styrene. The review included all studies up to Artuso *et al.* (in Table 5-13), but did not include two earlier studies, Dolmierski *et al.* (1983) and van Sittert and de Jong (1985). The authors found a positive association (weighted frequency ratio = 2.18, 95% CI = 1.52 to 3.13, weight was

assigned to each study depending on its sample variance) between styrene exposure level and chromosomal aberration frequency when exposure levels were dichotomized as greater or less than a threshold value of 30 ppm for an 8-hour time-weighted average (which was the median exposed-group exposure level in the identified studies).

Cohen *et al*. (2002) concurred with the Bonassi *et al.* review and also noted that the finding of dose-response relationships makes confounding unlikely. They concluded that there was "compelling evidence" of a positive association between styrene exposure at occupational levels and the frequency of chromosomal abnormalities. However, a review by Henderson and Speit (2005) concluded that the evidence for chromosomal aberrations was conflicting. This review included 27 studies [it did not include Dolmierski *et al.* (1983), van Sittert and de Jong (1985), Vodicka *et al*. (2004c), and Migiliore *et al.*(2006a) The authors stated that the Bonassi review did not account for study quality or the type of chromosomal aberrations (such as including gaps).

Micronucleus formation

Details on the study population, exposure levels, study design, and results for structural micronuceus formation are summarized in Table 5-14, and the findings are discussed after the tables.

The current guidelines for investigating the frequency of micronucleated blood lymphocytes or epithelial cells in humans are presented by Albertini *et al.* (2000). The cytokinesis-block micronucleus technique is the method of choice [first used in studies on styrene exposure by Mäki-Paakkanen *et al.* 1991]. Cytochalasin B (Cyt-B) is added to the cell culture to block cells from dividing after they have undergone one round of replicative synthesis since mitogen stimulation; such cells are binucleate. Most studies score 1,000 to 2,000 binucleated cells per subject. The results are expressed as the number of micronucleated cells per 1,000 binucleate cells or the percentage of cells with micronuclei (Table 5-14). Some studies also indicated the number of kinetochore-positive/centromere-positive micronuclei and the number of kinetochore-negative/centromere-negative micronuclei.

Table 5-14. Micronuclei in lymphocytes from workers occupationally exposed to styrene

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
Meretoja *et al.* 1977 (Finland)	Polyester plastic manufacturing workers from 3 plants (0.6–8.5 yr)	Exposed 10 Controls 5	NR	[721 (23–3,257)]	MN/1,000 cells [8.8 ± 2.9][d] [0.8 ± 1.1]	All subjects male; unmatched controls selected from outside the factory environment but similar age ranges No exposure to known clastogenic agents or recent viral infections or vaccinations Mainly 2nd division cells scored Statistics: not performed
Hogstedt *et al.* 1983 (Sweden)	Fiberglass-reinforced polyester resin manufacturing workers (1–23 yr)	*Preserved cytoplasm* exposed 38 controls 20 *Hypotonic treatment* exposed 38 controls 20	13 (1–36)	65 (9–316)	MN/1,000 cells 5.9** 3.6 4.3 3.7 Exposure response *Styrene air concentrations, exposure duration, cumulative dose, and urinary mandelic acid:* no correlation	All subjects male controls from nearby mechanical industry groups and matched for age Workers interviewed about potential confounders including occupational and medical history, viral infections, drug use, smoking and alcohol habits and exposure to X-rays and heavy metals. The following differences were found: (1) smokers– exposed 45%, controls 40%; (2) X-rays– exposed 29%, controls 25%; (3) drug use– exposed 11%, controls 15% Workers exposed to phthalic acid anhydride, maleic acid anhydride, propylene and/or ethylene glycol, hydroquinone, methylethyl ketone peroxide, cobalt salt, methylene chloride, solvents, and acetone MN analyzed by 2 methods: (1) preserved cytoplasm, and (2) hypotonic treatment with KCl Statistics: (1) Effect of exposure: multiple regression analysis controlling for smoking and

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
						age using log-transformed values, (2) exposure response– Spearman rank correlation coefficient
Nordenson and Beckman 1984 (Sweden)	Glass-fiber reinforced polyester plant workers (1–26 yr)	*Total* exposed 12 controls 12 *Smokers* exposed 4 controls 3 *Nonsmokers* exposed 8 controls 9	24 (NR)	(mM/L) NR (< 2)	MN/1,000 cells 3.5* 0.8 3.3* 1.0 3.6* 0.7	All subjects male controls were office workers Exposed to acetone No indication if slides were coded Cultures from 4 subjects (3 exposed and 1 control) were unsuitable for analysis MN similar in smokers and non-smokers Exposed also had significantly higher number of subjects with > 3 MN/1,000 cells ($P < 0.001$) Statistics: one-tailed exact test
Maki-Paakkanen 1987 (Finland)	Reinforced-plastics workers (mainly laminators) (1–25 yr)	*Total* exposed 21 controls 21 *Smokers* exposed 15 controls 15 *Nonsmokers* exposed 6 controls 6	[23 (8–60)]	(mM/L) 2.0 (0–7.3)	% cells with MN 1.5 ± 0.1[e] 1.6 ± 0.1 1.4 ± 0.2 1.6 ± 0.1 1.6 ± 0.2 1.6 ± 0.3 Exposure response *Duration, air, or urinary MGA: no correlation*	Controls were office workers matched by sex and smoking. No differences between groups in alcohol and drug intake, vaccinations, recent viral infections, or previous exposure to chemicals Statistics: *t*-test (one sided); analysis for exposure response not reported
Hagmar *et al.* 1989	Glass reinforced polyester plastic	*PHA* exposed 20	[13 (0.9–127)]	NR	MN/1,000 cells 4.3	All but one subject (exposed group) were male Some subjects reported recent exposures to X-rays

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
(Sweden)	workers (0.1–25.4 yr)	controls 22 *PWN* exposed 20 controls 22			4.4 5.9 7.0 Exposure response *Employment length:* no association	(65% exposed, 55% controls) and regular prescription drug use (15% exposed, 23% controls). More smokers in controls (50%) than exposed workers (30%) Workers exposed to low levels of acetone and methylene chloride Parallel cultures were set up and stimulated with pokeweed (PWN) or phytohemagglutinin (PHA) MN frequency (PHA) was correlated with age Statistics: significant testing of difference in means, after average square root transformation, adjusted for age and smoking; multiple linear regression evaluating employment length, smoking, and age
Brenner *et al.* 1991 United States	Reinforced-fiberglass plastic boat workers (2.7 yr)	*Exposed* total 10 high 4 low 6 *Controls* 9	11.2 (1–44) 27.2 (7–44 6.8 (1–18)	[24.3 (9.6–250)] [52 (9.6–250)] [18 (9.6–50)]	MN/1,000 cells 10.3 ± 0.4** 10.8 ± 0.6 10.0 ± 0.5 6.5 ± 0.5 Exposure response *Positive association by ANOVA, no response observed with continuous variables (air, urinary markers, and cumulative exposure)*	All exposed subjects were male; controls included male and female library workers Controls were library workers at a university and differed by sex and current smoking (which were retained in the analysis), education, and medication (which could not be retained in the analysis due to small numbers of subjects). No differences with respect to age, caffeine and alcohol intake, recency of colds or X-rays, other tobacco-related exposures, and exposure to wood smoke or solvents Co-exposure to acetone and methylene chloride Gender, education, and smoking had no effect on MN when analyzed by ANOVA

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
						Statistics: log transformed, Wilcoxon rank-sum test, ANOVA, which included gender, smoking, exposure, and educational status, used to evaluate exposure response in 3 exposure groups
Maki-Paakkanen *et al.* 1991 (Finland)	Reinforced plastic workers (smokers – 6.4 yr, non-smokers – 7.2 yr)	*Total* exposed 17 controls 17 *Smokers* exposed 11 controls 11 *Nonsmokers* exposed 6 controls 6	[~ 70] (NR)[f]	(mM) 9.4 (< 1–21.5) 11.0 (< 1–16.6) 6.5 (< 1–21.5)	% cells with MN 1.4 ± 0.6 1.2 ± 0.8 1.3 ± 0.7 1.1 ± 0.8 1.5 ± 0.4 1.4 ± 0.8	Controls from a research institute Age, sex, smoking status, health status, alcohol and drug intake, viral infections, vaccinations, and exposure to other chemicals were considered First study to use cytokinesis-block technique 500 binucleated cells/subject analyzed Statistics: Chi square test
Sorsa *et al.* 1991 (Finland)	Reinforced-plastics industry workers from 32 enterprises (NR)	*Past exp. (index pts)* *Laminators* low 15 high 13 *Other workers* low 5 high 6 *Controls* other factory 31 plastics factory 6 *All subjects* exposed 50 controls 37	43 (5–182) 11 (1–133)	(mM) 2.2 (NR)[g,h]	% cells with MN 0.6 ± 0.5 0.7 ± 0.4 0.4 ± 0.3 1.0 ± 0.2 0.8 ± 0.5 0.8 ± 0.3 NR NR Exposure response	Total population included 248 exposed workers, including 154 laminators, and 63 controls. MN results available on a subset; past-exposure index not available on all subjects Exposed groups divided into 2 groups based on past-exposure index points derived from exposure duration and concentrations, urinary metabolites, and job type Cytokinesis-block technique 500 binucleated cells/subject analyzed MN was associated with age ($P = 0.03$), but not with smoking Statistics: regression analysis including exposure, smoking and age, details not reported

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
					No association with styrene exposure	
Tomanin *et al.* 1992 (Italy)	Polyester resin workers at 2 factories: fiberglass tanks (1–18 yr) or fiberglass boats (1.5–15 yr)	*Factory 1* exposed 7 controls 7 *Factory 2* exposed 12 controls 12	NR [4.8–23] NR [26–100]	186 (46–345) 725 (423–1,325)	<u>% cells with MN</u> 8.7 ± 4.0 10.2 ± 4.4 12.6 ± 6.6 8.5 ± 3.3 <u>Exposure response</u> *Exposure duration:* no correlation *Urinary MA:* weak correlation R = 0.61 (P value not given)	Controls matched for sex, age, and smoking Subjects questioned about previous exposure to genotoxins, smoking and alcohol habits, recent viral infections or vaccinations, and exposure to X-rays Cytokinesis-block technique Different number of cells scored in exposed and control groups No significant effect with smoking Statistics: Mann-Whitney U test (2-sided), and simple linear regression
Yager *et al.* 1993 (United States)	Boat manufacturing workers (0.5–27 yr)	Exposed 48	[15 (0.2–54)]	NR	<u>MN/1,000 cells</u> 8.9 ± 0.9[e] <u>Exposure response</u> *No association with exposure to styrene after adjusting for gender*	No controls, exposed subjects 54% male, 46% female. Longitudinal study; exposure measured by personal monitors and concentrations in exhaled breath 7 times over a 1-yr period Cytokinesis-block technique MN frequency increased with age and was higher in females Statistics: linear regression analysis including styrene exposure, age, sex, lifestyle variables (such as smoking, alcohol intake, some dietary factors, drug intake, immunizations, infections, exposures from hobbies and home repairs) and occupational history

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
Tates *et al.* 1994 (Germany)	Polyester resin/fiberglass plastic products production workers (4–31 yr)	*Exposed* total 46 group 1 24 group 2 22 *Controls* 23	[16 (0–138)] [20 (0–138)] [12 (0–34)]	NR	MN/1,000 cells 35.1 ± 20*** 32.3 ± 24*** 38.2 ± 13*** 14.3 ± 7.3 Exposure response Duration: positive correlation (P = 0.001) in Group 2 only Concentration: Group 1 only (P = 0.05) Duration × TWA: P = 0.035 for styrene/DCM exposure	Controls matched for age, sex, and smoking Workers divided into 2 groups with similar working conditions, but blood samples were taken 1 wk apart Subjects questioned about health status, exposure to X-rays, drug use, and smoking and alcohol habits; blood samples tested for some viral infections Workers exposed to dichloromethane (genotoxin) Cytokinesis-block technique No significant differences for MN between smokers and nonsmokers Significant difference in MN between the 2 exposure groups (P = 0.04) Statistics: one-tailed Mann-Whitney U test and bivariate regression analysis
Van Hummelen *et al.* 1994 (Belgium)	Fiberglass-reinforced plastic pipes and cisterns workers (2.9 yr)	*Smokers* exposed 32 controls 13 *Nonsmokers* exposed 17 controls 10	[7 (0.5–25)]	102 (11–649)	MN/1,000 cells 3.28 ± 0.28[e] 4.32 ± 0.55 3.50 ± 0.34 4.75 ± 0.71 Exposure response Air, urinary MA no correlation	Study consisted of 52 exposed and 24 non-nonexposed workers, but cytogenetic results were not available on all subjects because of technical problems All subjects were males, controls were from a different factory (pallet production and repair) Subjects were interviewed regarding exposure to potential carcinogens and mutagens, smoking habits, diet, viral infections, vaccinations, chemotherapy, and X-rays. Exposed were significantly older (31 vs. 27), consumed less

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
						alcohol (1 vs 2.6 drinks/d), and had more recent X-rays (0.6 vs. 3.6 yr ago) Cytokinesis-block technique No correlation of MN with age, smoking, medical history, or other lifestyle factors Statistics: two-tailed Mann-Whitney U test, Spearman ranked correlation, ANOVA (using square root of value)
Anwar and Shamy 1995 (Egypt)	Reinforced-plastics plant workers (10–22 yr)	Exposed 18 Controls 18	NR	328 (145–1,204) 50 (22–92)	MN/1,000 cells 6.55 ± 3.47 6.00 ± [2.83] Exposure response *Duration, urinary MA*: no correlation	All subjects were male; controls were administrative staff from the same factory, matched for age, and not exposed to genotoxic agents. No smokers were included Exposure measurements were done on the "pilot study" that included 70 exposed workers and 68 controls No information on potential confounding exposures Control samples were collected 1 wk after the exposed samples Cytokinesis-block technique MN did not correlate with age or urinary thioester (UT) levels (biomarkers of electrophilic compounds). UT levels significantly higher in exposed than controls (pilot study) 500–1,000 binucleated cells/individual Statistical analysis: Chi-square test
Holz *et al.*	Styrene	*Total*			% cells with MN	Controls matched for age and sex and from the

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
1995 Germany	production plant workers (1–34 yr)	exposed 25 controls 25 *Total* exposed 25 controls 25 *Smokers* exposed 17 controls 13 *Nonsmokers* exposed 8 controls 12	NR [0.02–> 0.9] NR [≤ 0.01]	13.3–43.9 (NR)[i] 4.3–5.5 (NR) 13.3–43.9 (NR)[i] 4.3–5.5 (NR) 10–49.4 (NR) 5.6–6.3 (NR) 20.3–32.4 (NR) 2.8–4.6 (NR)	1.90 ± 0.78 1.87 ± 0.71 % K+ MN 39.4 ± 10.2** 31.8 ± 8.2 38.3 ± 11.3* 30.3 ± 7.9 42.1 ± 7.4* 33.3 ± 8.51	same facility Subjects questioned about alcohol consumption, smoking, drug use, and exposure to aromatic hydrocarbons outside the workplace Workers exposed to aromatic hydrocarbons: ethylbenzene (highest exposure), benzene, toluene, and xylene Modified cytokinesis-block methodology CREST staining to detect kinetochore-positive (K+) MN No confounding effect from smoking K+ MN indicates aneuploidy. Authors suggested that increase in K+ MN was consistent with exposure to benzene Statistical analysis: *t*-test
Karakaya *et al.* 1997 (Turkey)	furniture workers (10 yr)	*Total* exposed 50 controls 41 *Smokers* exposed 36 controls 29 *Nonsmokers* exposed 14 controls 12	30 (20–300)	207 (14–1,482)[g] 12 (0–38)[g]	% cells with MN 1.98 ± 0.50 2.09 ± 0.35 1.91 ± 0.46 2.20 ± 0.31 2.18 ± 0.57 1.82 ± 0.30 Exposure response *Duration: nonsignificant trend*	All subjects were male; controls were university employees matched on age and smoking Subjects interviewed about occupational, family, and dietary history No information on other current workplace exposures MN in control smokers were significantly higher than control non-smokers, and was higher in older subjects (> 36 yr) in both controls and exposed groups Urinary thioethers were significantly higher in exposed than controls but did not correlate with MN in the exposed group

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
						Statistics: *t*-test, Spearman rank correlation, average square root transformation
Laffon *et al.* 2002a Spain	Fiberglass-reinforced-plastics production workers (≥ 7 yr)	Exposed 14 Controls 30	< 20 (NR)[e]	313–353 (NR)[j]	MN/1,000 cells 24.6 ± 1.5**[e] 13.9 ± 0.81 Exposure response *Duration: positive correlation (P < 0.001)*	All subjects were male; controls were university employees Subjects interviewed on smoking, alcohol consumption, medication, recent viral infections, vaccinations, X-rays, and previous occupational exposure to chemicals More controls smoked (63%) than exposed (36%), but exposed subjects had smoked longer Workers also exposed to peroxides Cytokinesis-block technique MN non-significant increase with age, but significant increase with smoking (# of cigarettes and years smoked) in exposed group Statistics: ANOVA (one way), Student's *t*-test, Pearson correlation
Teixeira *et al.* 2004 NR	2 small reinforced-plastics plants (12 yr)	*Total* exposed 28 controls 28 *Men* exposed 18 controls 18 *Women* exposed 10 controls 10	27 (2–91)	401 (47–1,490)[g]	MN/1,000 cells 3.68 ± 0.46[e] 2.82 ± 0.47 2.50 ± 0.35 2.00 ± 0.33 5.80 ± 0.77 4.30 ± 1.03	Controls were office workers and were similar in age, sex ratio, and smoking habits as exposed group Subjects queried about lifestyle factors (smoking and alcohol habits, medications, X-rays, and diet), and occupational exposures to chemicals Workers also exposed to low levels of toluene and acetone (< 1% of styrene levels) Cytokinesis-block technique MN significantly ($P < 0.05$) higher in styrene-

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
						exposed women than men, smoking not significantly related to MN Statistics: Student's *t*-test
Godderis *et al.* 2004 Belgium	Fiberglass-reinforced plastic workers from 2 plants (7 mo–38 yr)	*Lymphocytes* MNBC 38 controls 41 MNMC 38 controls 41 *Nasal cells* exposed 23 controls 17	9.5 (0–36.6)[f] 9.5 (0–36.6) 7 (0–24.6)	202 (ND–618) 202 (ND–618) 162 (ND–104)	MN/1,000 cells 3.93 ± 2.8* 2.65 ± 1.94 0.71 ± 0.9*** 0.11 ± 0.20 0.52 ± 0.49* 0.23 ± 0.31 Exposure response *Employment duration*: *positive association with MNBC and MNMC* *Average styrene exposure: positive association with nasal cell MN*	All subjects were male; controls recruited from electrical wire and cable manufacturing companies from the same region and matched on age and smoking. Controls and exposed had similar alcohol intake, blood lead levels. 3 exposed and 7 controls had urinary chromium levels above the reference value but under ACGIH biological exposure index Cytokinesis-block technique Binucleated (MNBC) and mononucleated (MNMC) lymphocytes and nasal epithelial cells analyzed MNBC correlated with age ($P = 0.014$) in the total population, MNBC and MNMC correlated with smoking ($P < 0.05$) in the exposed groups and XRCC1 polymorphism ($P < 0.05$) in the total population Statistics: Mann-Whitney U test for lymphocytes and unpaired *t*-test for nasal cells, bivariate correlation analysis multiple regression analysis (backward, stepwise)
Vodicka *et al.* 2004a (Czech Republic)	3 Reinforced-plastic lamination plants (A: 3.4 yr)	*Exposed* total 86 plant A 35 plant B 31	[19] (NR) [26] (NR) [11] (NR) [19] (NR)	497 (NR)[g] 798 (NR) 270 (NR)	% cells with MN 15.1 ± 6.7 17.9 ± 8.1**[k] 13.4 ± 4.3	Internal controls: male maintenance workers External controls: employees of the regional hygienic station Controls were older (+8.7 yr), had fewer men

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
	(B: 5.6 yr) (C: 2.5 yr)	plant C 20 *Controls* plant 16 external 26		308 (NR) 42	12.5 ± 4.7 11.6 ± 4.9 15.6 ± 6.9 Exposure response (correlations with styrene exposure) *Air levels: P < 0.001* *Blood levels: P < 0.001* *Cumulative exposure P < 0.05*	(52% vs. 71%), and fewer smokers (19% vs. 51%) than exposed but had a similar socioeconomic background. Differences accounted for in the analysis Cytokinesis-block technique MN significantly increased with age, were higher in women and external controls Statistical analysis: Mann-Whitney U test, Spearman correlation analysis
Migliore *et al.* 2006b (Tuscany and Parma, Italy)	Fiberglass reinforced-plastics workers from 13 plants (1–34 yr)	Exposed 92 Controls 98 Exposed 86 Controls 57 Exposed 86 Controls 57	[8.5 (0.5–123)]	300 (10–1856)[f]	% cells with MN 13.8 ± 0.5***[c] 9.2 ± 0.42 % cells C+MN 7.43 ± 0.34*** 4.75 ± 0.37 % cells C–MN 5.76 ± 0.3*** 3.20 ± 0.3 Exposure response *Air levels:* No correlation with total	Controls were from the same geographic area with comparable age. Controls had fewer smokers (42% vs. 53%) but more women (32% vs. 20%) compared with exposed Subjects interviewed for personal, occupational, and medical history (X-rays, viral infections and inflammatory disease, drug use) 4-Vinylphenol conjugate levels were available on the Parma workers (26 males and 19 females) MN measured by FISH, centromere + (C+) and centromere – (C–) cells also scored (2 scorers used) Smoking had no effect on total MN and C+MN frequency but was associated with a decreased C–

Reference (location)	Study population[a] (yrs employed)	Number of subjects	Styrene exposure Mean (range)		Results (mean ± SD) Exposure response	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)		
					MN: C+ or C–MN *MA+PGA metabolites:* Total MN and C–MN, $P > 0.05$, C+MN, $P = 0.011$ *4-VPT metabolites:* total MN and C+MN, $P < 0.01$	MN frequency; MN and C+MN increased with age, and MN (total) were higher in females than males MN also higher in GSTT1-null exposed subjects Statistics: Multifactorial ANOVA including smoking habits, age, and sex MN data on a subset of male workers from the Tuscany cohort (42 exposed workers and 25 controls was reported by Miglore *et al*. 2006a. The exposed in this subset also had increased MN (13.8) compared with the controls (6.2)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
FISH = fluorescence *in situ* hybridization, MN = micronuclei, NR = not reported.
[a] Study population includes both sexes unless otherwise noted.
[b] [Bracketed data were converted from mg/m^3 to ppm (1 mg/m^3 styrene ≈ 0.23 ppm).]
[c] Potential confounders (e.g., differences in age, sex, smoking, exposures to other chemicals, recent infections, vaccinations, etc.) are noted as identified by the study authors.
[d] No *P* value provided but reported as an increase by study authors and identified as a positive study by Scott and Preston (1994a).
[e] Mean ± SE.
[f] Air concentration was estimated from urine mandelic acid levels.
[g] Sum of mandelic and phenylglyoxylic acids.
[h] Average urinary mandelic acid levels were 2.4 mM in laminators that did not use a respirator and 1.3 mM in those who used a respirator.
[i] Values are the range of means reported before and after work shift.
[j] Range of means from three samplings.
[k] Compared with plant controls.

Micronuclei were measured in peripheral blood lymphocytes from workers exposed to styrene in 20 studies and in nasal epithelial cells in one study (Table 5-14). All but three studies (Anwar and Shamy 1995, Maki-Paakkanen *et al.* 1991, Sorsa *et al.* 1991) scored a minimum of 1,000 cells per subject. [As with the chromosomal aberration studies, data quality issues (e.g., small number of subjects, unmatched controls, and exposure to other clastogenic agents) were identified for several of the studies. About half of the studies included fewer than 25 subjects per group. Most of the studies included control groups matched on one or more of the following variables: age, gender, or smoking. Studies that did not report using matched control groups included Meretoja *et al.* (1977), Hagmar *et al.* (1989), Brenner *et al.* (1991), Sorsa *et al.* (1991), and Vodicka *et al.* (2004a). Most of the studies that did not use matched subjects controlled for variables (such as age, gender, and smoking habits) in the analysis or reported that age, smoking, and gender distribution were similar between groups. Only one study (Sorsa *et al.* 1991) did not appear to meet that criterion, although it was not clear whether smoking and age were controlled for in the dose-response regression analysis.]

[Most studies evaluated the effects of potential confounders such as smoking, age, and gender on micronuclei, but the results were mixed]. Only three studies (Godderis *et al.* 2004, Laffon *et al.* 2002a, Migliore *et al.* 2006b) reported that smoking was correlated with an increase in micronuclei, but nine studies (Brenner *et al.* 1991, Hagmar *et al.* 1989, Holz *et al.* 1995, Nordenson and Beckman 1984, Sorsa *et al.* 1991, Tates *et al.* 1994, Teixeira *et al.* 2004, Tomanin *et al.* 1992, Van Hummelen *et al.* 1994) did not show a correlation with smoking. Migliore *et al.* (2006b) reported that smoking was not correlated with total micronuclei but was correlated with a decrease in centromere-negative micronuclei. Six studies reported a significant correlation with age (Godderis *et al.* 2004, Hagmar *et al.* 1989, Migliore *et al.* 2006b, Sorsa *et al.* 1991, Vodicka *et al.* 2004a, Yager *et al.* 1993), but four studies reported no correlation with age (Anwar and Shamy 1995, Brenner *et al.* 1991, Laffon *et al.* 2002a, Van Hummelen *et al.* 1994) [although a non-significant increase with age was reported by Laffon *et al.*]. Four studies (Migliore *et al.* 2006b, Teixeira *et al.* 2004, Vodicka *et al.* 2004a, Yager *et al.* 1993) reported that micronuclei were higher in females compared with males. However, Brenner *et al.* (1991) did not find any differences related to gender.

Yager *et al.* (1993) conducted a longitudinal study (without controls) that compared styrene exposure (measured at several times during a one year period) with micronucleus frequency. No correlation was found between styrene exposure (air levels and exhaled air) and micronucleus frequency.

Micronuclei were significantly increased in styrene-exposed workers in at least one exposed group in 10 (of the remaining 19) studies (Brenner *et al.* 1991, Godderis *et al.* 2004, Hogstedt *et al.* 1983, Holz *et al.* 1995, Laffon *et al.* 2002a, Meretoja *et al.* 1977, Migliore *et al.* 2006b, Nordenson and Beckman 1984, Tates *et al.* 1994, Vodicka *et al.* 2004a). Only kinetochore-positive micronuclei [an indicator of aneuploidy] were increased in the study by Holz *et al.* (1995), and Vodicka *et al.* (2004a) reported significantly more micronuclei in a single subgroup of workers (one of three plants). Of the 12 studies that evaluated dose-response relationships, five reported a significant correlation with styrene exposure (Brenner *et al.* 1991, Tates *et al.* 1994 (in one of the subgroups but not the pooled population), Laffon *et al.* 2002a, Godderis *et al.* 2004, and Vodicka *et al.* 2004a). Workers in the study reported by Tates *et al.* were also exposed to dichloromethane; however, no correlation was found between dichloromethane [which is a genotoxin] and micronuclei. Holz *et al*. (1995) attributed the increase in kinetochore-positive micronuclei to exposure to benzene. Workers in other studies were also exposed to other chemicals such as peroxides, organic solvents, acetone (Brenner *et al.* 1991, Hogstedt *et al.* 1983, Laffon *et al.* 2002a, Nordenson and Beckman 1984), but it was not reported whether these chemicals can cause micronuclei.

Nine studies did not find increased micronuclei frequency in the exposed groups (Anwar and Shamy 1995, Hagmar *et al.* 1989, Karakaya *et al.* 1997, Maki-Paakkanen 1987, Maki-Paakkanen *et al.* 1991, Sorsa *et al.* 1991, Teixeira *et al.* 2004, Tomanin *et al.* 1992, Van Hummelen *et al.* 1994). The exposed populations in these studies ranged from 7 to 50 subjects, and the reported mean styrene concentrations ranged from about 7 to 70 ppm, which were similar to those reported for the positive studies. Bonassi *et al.* (1996) conducted a meta-analysis of 10 studies of micronucleus frequency in styrene-exposed workers (Brenner *et al.* 1991, Hagmar *et al.* 1989, Hogstedt *et al.* 1983, Maki-Paakkanen 1987, Maki-Paakkanen *et al.* 1991, Meretoja *et al.* 1977, Nordenson and Beckman 1984,

Sorsa *et al.* 1991, Tates *et al.* 1994, Tomanin *et al.* 1992) and concluded that the data were inconclusive. Of the 10 studies published since that analysis, five reported positive associations. Cohen *et al.* (2002), noting a general lack of evidence of a significant dose response and inadequate control for potential confounders, concluded that there was no compelling evidence in humans that exposure to styrene was associated with micronucleus formation.

Sister chromatid exchange

Details on the study population, exposure levels, study design, and results for structural sister chromatid exchange (SCE) are summarized in Table 5-15, and the findings are discussed after the tables.

SCE scoring is conducted in second-division metaphases and requires DNA replication in the presence of bromodeoxyuridine (BrdU) for two consecutive cell cycles, or at least the first of two consecutive cell cycles (Albertini *et al.* 2000). It is necessary to score 30 to 50 second-division metaphase cells to obtain a stable estimate of the mean; however, a minimum of 80 cells is recommended to identify a small proportion (~10%) of high-frequency SCE cells (cells with an abnormally high number of SCEs). Seven studies included subjects that had fewer than 30 metaphases scored (Andersson *et al.* 1980, Brenner *et al.* 1991, Holz *et al.* 1995, Meretoja *et al.* 1978a, Teixeira *et al.* 2004, Watanabe *et al.* 1983, Watanabe *et al.* 1981). Data are recorded as the frequency of SCE per cell and also may include the proportion of high-frequency cells (HFCs). Because baseline levels of SCEs show considerable variation among individuals and between studies, it is difficult to classify subjects into high, medium, or low categories (Albertini *et al.* 2000).

Table 5-15. Sister chromatid exchange in lymphocytes from workers occupationally exposed to styrene

Reference (location)	Study population (yrs employed)[a]	Number of subjects	Styrene exposure mean (range)		Results	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)	SCE/cell (mean ± SD) Exposure response	
Meretoja *et al.* 1978a (Finland)	Polyester plastic manufacturing workers (laminators) (1–15 yr)	Exposed 11 Controls 3	NR (≤ 300)	NR (23–3,257)	5.3 ± 1.0 4.4 ± 0.6	Total population included 16 laminators and 6 controls, but results not available for all subjects Controls not matched, but had similar age range; all subjects were male No previous exposure to known clastogenic agents SCE were not correlated with smoking 15–25 metaphases/subject Statistics: Student's *t*-test
Andersson *et al.* 1980 (Sweden)	Reinforced-plastics boat factory workers (0.3–12 yr)	*Exposed* total 20 high 6 low 14 *Controls* 21	(mg/m^3 × yr) 575 (6–1,589) 1,204 (710–1,589 137 (6–283)	NR	8.4 ± 1.3* 8.7 ± 1.3* 8.2 ± 1.3 7.5 ± 1.1	Total population included 39 exposed and 41 controls, but results not available for all subjects Subjects interviewed about health history Controls matched on age and included 3 groups (office, assembly shop, and workshop) from the same factory; all subjects were male 25 metaphases/subject Statistics: Student's *t*-test
Watanabe *et al.* 1981 (Japan)	Group 1: Reinforced-plastics boat factory (workshop 1) Group 2: Polyester resin	*Group 1:* exposed 9 controls 5 *Group 2:* exposed 7 controls 8	< 70 (1–211) 36 (NR)	(mg/L) 647 (90–4,300) 32 (5–115)[d] 526 (300–1,360) 32 (5–115)[d]	7.8 ± 1.6 7.6 ± 1.2 6.7 ± 0.8 7.6 ± 1.2	Controls matched on age and sex, all subjects in group 1 were male; Group 2 included males and females Exposure varied depending on the work in workshop 1 but was stable in workshop 2 17–50 metaphases/subject

Reference (location)	Study population (yrs employed)[a]	Number of subjects	Styrene exposure mean (range)		Results	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)	SCE/cell (mean ± SD) Exposure response	
	board workers (workshop 2) (NR)					Mitomycin C treatment did not increase the number of SCE in exposed or controls Statistics: Student's *t*-test or Chi-square
Watanabe *et al.* 1983 (Japan)	Fiber reinforced-plastics boat factory workers in 2 workshops (groups A & B) (1 mo–30 yr)	*Exposed* total 18 group A 10 group B 8 *Controls* 6	40–50 (NR)	(mg/L) 332 (0–1,041) 399 (0–1,041) 249 (8–999)	8.9 ± 1.4 8.6 ± 1.2 9.1 ± 1.8 8.5 ± 1.0 Exposure response *total urinary metabolites: r = 0.525, P < 0.05*	Controls matched on age; all subjects were male Subjects interviewed about occupational and medical history, and smoking habits; more smokers in exposed group than controls (72% vs. 50%) Workers not exposed to other industrial chemicals SCE significantly higher in exposed smokers than exposed non-smokers; no difference in controls 9–30 metaphases/subject Statistics: Mann-Whitney U test, *t*-test (two-tailed)
Camurri *et al.* 1983, 1984 (Italy)	Reinforced unsaturated polyester resin manufacturing workers in 9 plants (1–22 yr)	Plant 1 3 Control 3 Plant 2 4 Control 4 Plant 3 4 Control 6 Plant 4 5 Control 6 Plant 5 6 Control 6	NR [7–9] NR [16–23] NR [23–34.5] NR [34.5–46] NR [46–57.5]	(mg/L) NR (45–75) NR (65–133) NR (170–694) NR (151–786) NR (340–671)	12.7 ± 0.7 12.1 ± 1.3 12.7 ± 0.4* 11.7 ± 0.6 10.9 ± 1.0 9.7 ± 1.5 10.3 ± 0.9 9.7 ± 1.5 11.8 ± 0.5** 10.8 ± 0.3	Data described for 6 plants in 1983 publication; all data described in 1984 publication Controls matched for age, sex, and smoking Workers exposed to other industrial chemicals (e.g., organic peroxides, solvents, and dyes) 16–74 metaphases/subject SCE did not correlate with smoking habits Statistics may have been based on cell as unit rather than individuals Significant differences in SCE at concentrations ≥ 200 mg/m^3 (46.9 ppm) with steep increases

Reference (location)	Study population (yrs employed)[a]	Number of subjects	Styrene exposure mean (range)		Results	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)	SCE/cell (mean ± SD) Exposure response	
		Plant 6 2 Control 2 Plant 7 2 Control 2 Plant 8 4 Control 2 Plant 9 7 Control 4	NR [57.5–69] NR [69–80.5] NR [80.5–92] NR (>92)	NR (615–777) NR (489–828) NR (504–909) NR (389–1,108)	19.6 ± 5.2 10.3 ± 0.1 16.1 ± 1.6* 10.4 ± 0.0 16.1 ± 0.13** 9.7 ± 1.5 15.1 ± 0.5*** 8.5 ± 1.1	occurring at about 250 mg/m^3 [57.5 ppm] Statistics: Student's *t*-test
Hansteen *et al.* 1984 (Norway)	Glass-fiber reinforced polyester plant workers (2 groups based on exposure levels) (NR)	*Exposed* total 18 group 1 11 group 2 7 *Controls* 9	13.2 (2–44) 7.5 (2–13) 22.3 (14–44)	NR (200–1,200)[e]	6.6 6.9 6.0 6.5	Controls matched on age, sex, and smoking Low exposure SCE were not significantly higher in smokers vs. non-smokers (total, exposed, or control groups) Statistics: Fisher-Irwin test, Wilcoxon two-sample ranking test
Maki-Paakkanen 1987 (Finland)	Reinforced-plastics workers (mainly laminators) (1–25 yr)	Exposed 21 Controls 21	[23 (8–60)]	(mM) 2.0 (0–7.3)	7.6 ± 0.2[f] 7.4 ± 0.2 <u>Exposure response</u> *No correlation with exposure extent or duration*	Controls matched on sex and smoking. No differences between controls and exposed in alcohol consumption, drug intake, vaccinations, recent viral infections, and previous occupational exposure to chemicals. SCE significantly higher in smoking controls than non-smoking controls Statistics: Student's *t*-test, analysis for exposure response not reported
Kelsey *et al.* 1990	Reinforced-fiberglass plastic boat building	*Smokers* exposed 7	[48] (NR)	275 (NR)	7.2 ± 1.3	All subjects were male except 1 female in controls; did not differ from exposed workers with respect to age, smoking, coffee or alcohol consumption, or

Reference (location)	Study population (yrs employed)[a]	Number of subjects	Styrene exposure mean (range)		Results	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)	SCE/cell (mean ± SD) Exposure response	
(United States)	workers (smokers 8.6 yr, nonsmokers 7.2 yr)	controls 8 *Nonsmokers* exposed 13 controls 12	[2.3] (NR) [53] (NR) [0.76] (NR)	21 (NR) 323 (NR) 13 (NR)	7.2 ± 1.3 6.5 ± 0.7 6.2 ± 0.9 Exposure response *No increase with styrene air levels or urinary metabolites*	recent viral infections or vaccinations (none reported). Some exposure to styrene in the control group Styrene exposure did not affect SCE in high SCE frequency cells (HFC); however, smokers (total population) had significantly higher SCE in HFC than non-smokers. Statistics: Student's *t*-test, ANOVA for exposure response analysis
Maki-Paakkanen *et al.* 1991 (Finland)	Reinforced plastic workers (smokers – 6.4 yr, non-smokers – 7.2 yr)	*Total* exposed 17 controls 17 *Smokers* exposed 11 controls 11 *Nonsmokers* exposed 6 controls 6	[~70] (NR)[g]	(mM/L) 9.4 (< 1–21.5) 11.0 (< 1–16.6) 6.5 (< 1–21.5)	11.4 ± 1.7 12.4 ± 1.6 12.2 ± 1.7 12.9 ± 1.4 10.2 ± 0.8 11.4 ± 1.5	Controls from a research institute Age, sex, smoking, general health, alcohol consumption, drug intake, viral infections, vaccinations, and exposure to other chemicals considered. SCE higher in smokers than non-smokers (in both the exposed and control groups). Statistics: Student's *t*-test (one-tailed)
Brenner *et al.* 1991 (United States)	Reinforced fiberglass plastic boat workers (2.7 yr)	*Exposed* total 10 high 4 low 6 *Controls* 9	11.2 (1–44) 27.2 (7–44) 6.8 (1–18)	243 (96–2,496) 523 (96–2,496) 176 (96–496)	9.7 ± 0.4[f] 10.0 ± 0.6 9.4 ± 0.5 10.1 ± 0.4	Controls were library workers at a university and differed by sex and current smoking (which were retained in the analysis), education, and medication (which could not be retained in the analysis due to small numbers of subjects). No differences with respect to age, caffeine and alcohol intake, recency of colds or X-rays, other tobacco-related exposures, and exposure to wood smoke or solvents.

Reference (location)	Study population (yrs employed)[a]	Number of subjects	Styrene exposure mean (range)		Results	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)	SCE/cell (mean ± SD) Exposure response	
						2 slide readers (25 metaphases each/subject) No effect on HFCs Statistics: Wilcoxon rank-sum test, Chi-square, ANOVA, which included gender, smoking, exposure and educational status, used to evaluate exposure response in 3 exposure groups
Sorsa *et al.* 1991 (Finland)	Reinforced-plastics industry workers from 32 workshops (NR)	*Past exp. (index pts)* *Laminators* low 12 high 13 *Other workers* low 10 high 9 *Controls* other factory 19 plastics factory 12 *All subjects* exposed 70 controls 31	43 (5–182) 11 (1–133)	(mM) 2.2 (NR)[e]	7.7 ± 1.2 7.4 ± 1.4 7.3 ± 1.0 7.6 ± 1.1 6.9 ± 0.8 7.2 ± 1.0 NR NR <u>Exposure response</u> *F = 4.66, P = 0.016 for male laminators who smoked compared to other male smoking workers. No overall association with*	Total population consisted of 248 exposed workers, including 154 laminators and 63 controls (for cytogenetic analysis). SCE results available on subset past exposure index not available on all subjects 2 control groups: 1 from the plastics industry and 1 from other industries Past exposure estimated using a grading scale based on exposure duration, urinary metabolites, and air concentrations. Exposure groups divided into two subsets based on past exposure Age and smoking significantly associated with SCE in regression analysis Statistics: Regression analysis; no details provided

Reference (location)	Study population (yrs employed)[a]	Number of subjects	Styrene exposure mean (range)		Results	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)	SCE/cell (mean ± SD) Exposure response	
					styrene exposure	
Yager *et al.* 1993 (United States)	Boat manufacturing workers (6.4 yr)	*Exposed* total 46 high 11 medium 20 low 15	[15] (NR) [40] (NR) [10] (NR) [0.8] (NR	NR	6.4 ± 0.1[f] 6.9 ± 0.3 6.5 ± 0.2 6.1 ± 0.2 <u>Exposure response</u> *Air:* *r = 0.4, P < 0.01* *Breath:* *r = 0.5, P = 0.001*	No controls; longitudinal study; subjects' exposure determined from personal air monitors and concentrations in exhaled breath on 7 days over a 1-year period Smokers equally distributed over all groups SCEs analyzed twice (replicates) for most subjects SCEs significantly increased with smoking and exposure to styrene (smoking accounted for 62% and styrene 25% of the total variability) Statistics: linear regression analysis (including smoking, alcohol intake, some dietary factors, drug intake, immunizations, infections, exposures from hobbies and home repairs) and occupational history for exposure response
Hallier *et al.* 1994 (Germany)	Reinforced-plastics workers (8 yr)	*Total* laminators 14 laminators 9[h] formers 14 controls 20 *Smokers* laminators 4 formers 13 controls 10 *Nonsmokers* laminators 10	37 (29–42) 15 (12–21) 10 (NR)	(mg/L) 652 (100–1,610) 187 (100–400) NR	10.1 ± 1.0* 8.2 ± 0.99 7.6 ± 1.5 6.6 ± 1.0 9.59 ± 0.77* 7.42 ± 1.27 7.23 ± 1.00 10.25± 1.08*	All subjects were male No occupational exposure to hazardous chemicals SCE significantly higher in smoking controls vs. nonsmoking controls SCE was lower in the 9 laminators retested ~1 year after styrene exposure was reduced by half, but controls were not retested Statistics: Mann-Whitney rank U test, Wilcoxon test for laminators after reducing exposure

Reference (location)	Study population (yrs employed)[a]	Number of subjects	Styrene exposure mean (range)		Results	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)	SCE/cell (mean ± SD) Exposure response	
		controls 10			5.98 ± 0.06	
Tates *et al.* 1994 (Germany)	Polyester resin/fiberglass plastic products production workers (4–31 yr)	*Exposed* total 46 group 1 24 group 2 22 *Controls* 23	 [16 (0–138)] [20 (0–138)] [12 (0–34)]	NR	 10.2 ± 0.9*** 10.1 ± 0.8*** 10.6 ± 0.6*** 5.6 ± 0.3 <u>Exposure response</u> *No correlation with exposure duration*	Controls matched on age, sex, and smoking Workers divided into 2 groups with similar working conditions, but blood samples were taken 1 wk apart Subjects questioned about health status, exposure to X-rays, drug use, and smoking and alcohol habits; blood samples tested for some viral infections Workers also exposed to dichloromethane [genotoxin] Significant effect (P = 0.03) of smoking in controls but not the exposed HFCs (> 9 SCEs/cell) were > 14-fold higher in exposed workers than controls; no effect of smoking on HFCs Statistics: one-tailed Mann-Whitney U test
Van Hummelen *et al.* 1994 (Belgium)	Fiberglass-reinforced-plastics workers (2.9 yr)	*Smokers* exposed 30 controls 9 *Nonsmokers* exposed 13 controls 6	 [7 (0.5–25)]	102 (11–649)	 5.47 ± 0.10[f] 5.62 ± 0.32 4.41 ± 0.20 4.94 ± 0.45 <u>Exposure response</u> *No correlation with styrene air levels or*	Study consisted of 52 exposed and 24 nonexposed workers, but cytogenetic results were not available on all subjects because of technical problems All subjects were male; control group selected from a factory that produced and repaired pallets Subjects were interviewed regarding exposure to potential carcinogens and mutagens, smoking habits, diet, viral infections, vaccinations, chemotherapy, and X-rays. Exposed were

Reference (location)	Study population (yrs employed)[a]	Number of subjects	Styrene exposure mean (range)		Results	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)	SCE/cell (mean ± SD) Exposure response	
					urinary metabolite levels	significantly older (31 vs. 27), consumed less alcohol (1 vs 2.6 drinks/d), and had more recent X-rays (0.6 vs. 3.6 yr ago) Relatively low exposure Smoking significantly increased SCE frequency; no correlation of SCE with age, medical history, or other lifestyle factors No association between styrene exposure and HFC was observed. Statistics: two-tailed Mann-Whitney U test
Artuso *et al.* 1995 (Italy)	Fiber-reinforced-plastics boat building workers (NR)	*Lab 1* low 13 high 19 controls 21 *Lab 2* low 9 high 4 controls 13	NR [0.5–28] NR [20–320] NR [0.5–28] NR [20–320]	NR	2.82 ± 0.12*[f] 3.01 ± 0.12* 2.38 ± 0.10 6.47 ± 0.50 7.32 ± 0.81 5.44 ± 0.26 <u>Exposure response</u> *Tests for linear trend significant for both labs* *Multiple regression (RR, 95% CI):* *Low: 1.22 (1.05–1.43)*	All subjects were males; controls matched for age and smoking and from the same area as exposed Subjects questioned about working activity, recent illness, exposure to X-rays, use of drugs, alcohol, coffee and smoking habits. X-rays were more frequent among controls 3 scorers from 2 labs used No significant association with smoking, alcohol consumption and diagnostic X-rays Statistics: *t*-test with Tukey adjustment, multiple linear regression, which included exposure, smoking, alcohol drinking, and exposure to diagnostic X-rays, with adjustment for age and slide reader

Reference (location)	Study population (yrs employed)[a]	Number of subjects	Styrene exposure mean (range)		Results	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)	SCE/cell (mean ± SD) Exposure response	
					High: 1.26 (1.07–1.47)	
Holz *et al.* 1995 (Germany)	Styrene production plant workers (1–34 yr)	*Total* Exposed 25 Controls 25 *Smokers* Exposed 17 controls 13 *Nonsmokers* exposed 8 controls 12	NR (0.01–> 0.9) NR [≤ 0.01]	13.3–43.9 (NR)[i] 4.3–5.5 (NR) 10–49.4 (NR) 5.6–6.3 (NR) 20.3–32.4 (NR) 2.8–4.6 (NR)	9.27 ± 1.24 9.24 ± 1.24 9.38 ± 1.37 9.67 ± 1.36 9.04 ± 0.99 8.87 ± 0.96	Controls from the same plant matched for age and sex and had similar smoking habits Controls were exposed to low levels of styrene Subjects questioned about alcohol consumption, smoking, drug use, and exposure to aromatic hydrocarbons outside the workplace Workers exposed to aromatic hydrocarbons: ethylbenzene (highest exposure), benzene, toluene, and xylene 15 metaphases/subject Statistics: Student's *t*-test
Rappaport *et al.* 1996 (United States)	Reinforced plastic boat manufacturing workers (≥ 1 yr)	*Exposed* smokers 22 nonsmokers 24	[17 (0.4–51)] [12 (0.2–54)]	NR	6.73 ± 0.22[f] 6.07 ± 0.140 <u>Exposure response</u> Styrene: $r = 0.39$, $P < 0.1$ SO– all: $r = 0.23$, NS SO– smokers: $r = 0.81$, $P = 0.015$	No controls; gender ratios not provided Correlation analysis suggested that styrene-7,8-oxide (SO) was 2,000 times more effective than styrene in producing SO biomarkers (albumin or DNA adducts) Statistics: ANOVA, Pearson's correlation, multiple linear regression
Karakaya *et al.* 1997 (Turkey)	Furniture workers (10 yr)	*Total* exposed 44 controls 41	30 (20–300)	207 (14–1,482)[e] 12 (0–38)[e]	6.2 ± 1.6** 5.23 ± 1.23	Total population consisted of 53 exposed subjects, but SCE results were not available for 9 subjects due to poor sample preparations.

Reference (location)	Study population (yrs employed)[a]	Number of subjects	Styrene exposure mean (range)		Results	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)	SCE/cell (mean ± SD) Exposure response	
		Smokers exposed 29 controls 29 *Nonsmokers* exposed 15 controls 12			6.8 ± 1.3 5.88 ± 0.76 5.2 ± 1.4** 3.66 ± 0.52 Exposure response *No significant correlation with exposure duration (although a trend was noted) for UT levels*	All subjects were male; controls were university office workers matched on age and smoking Subjects interviewed about occupational, family, and dietary history. No information on other workplace exposures SCE significantly higher ($P < 0.01$) in smokers vs. nonsmokers in controls and exposed, but not affected by age Urinary thioethers (UT) were significantly higher in exposed than controls Statistics: ANOVA, Mann-Whitney U test
Biró *et al.* 2002 (Hungary)	Oil refinery workers (NR)	exposed 10 controls 25	NR	NR	7.9 ± 0.3* [f] 6.4 ± 0.2	Subjects interviewed about age, medication, smoking and drinking habits, and medical and work histories More smokers in exposed (80%) vs. controls (20%) SCE was higher in smokers but no separate analysis was conducted for smokers and nonsmokers Statistics: Student's *t*-test
Laffon *et al.* 2002a (Spain)	Fiberglass-reinforced plastic production workers	exposed 14 controls 30	< 20 (NR)	313–353 (NR)	3.5 ± 0.06** [f] 2.6 ± 0.05 Exposure response	All subjects were male; controls were university employees, and workers were employed at least 7 yr

Reference (location)	Study population (yrs employed)[a]	Number of subjects	Styrene exposure mean (range)		Results	Comments[c]
			Air (ppm)[b]	Urinary mandelic acid (mg/g creatinine)	SCE/cell (mean ± SD) Exposure response	
	(17 yr)				*Positive correlation with exposure duration (P ≤ 0.01)*	Subjects queried about smoking and alcohol habits, medication, recent infections and vaccinations, diagnostic tests such as X-rays, and previous occupational exposure to chemicals SCE significantly increased with number of cigarettes/d in exposed but not controls More controls smoked (53%) than workers (36%), but workers had smoked longer Workers also exposed to organic peroxides, acetone, and dichloromethane SCE higher in smokers in exposed group HFC significantly increased in exposed group Statistics: Student's *t*-test, one-way ANOVA
Teixeira *et al.* 2004 (NR)	Reinforced-plastics workers from 2 small plants (1–30) yr	*Total* exposed 28 controls 28 *Men* exposed 18 controls 18 *Women* exposed 10 controls 10	27 (2–91)	401 (47–1,490)[g]	7.18 ± 0.34* [f] 6.30 ± 0.25 7.25 ± 0.51 5.99 ± 0.31 7.04 ± 0.28 6.86 ± 0.036	Controls were office workers who were similar in age, sex ratio, and smoking habits as the exposed groups Subjects queried about smoking and alcohol habits, medication, diet, X-ray exposure, and previous occupational exposure to chemicals Exposed group also exposed to low levels of toluene and acetone (< 1% styrene concentrations) ≥ 25 metaphase/individual Statistics: *t*-test

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
HFC = high SCE frequency cells, NR = not reported, SCE = sister chromatid exchange.
[a] Study population includes both sexes unless otherwise noted.

[b] [Bracketed data were converted from mg/m^3 to ppm (1 mg/m^3 styrene ≈ 0.23 ppm).]
[c] Control group included unexposed workers from the same plant as the exposed except where noted otherwise. Potential confounders (e.g., exposures to other chemicals, recent infections, vaccinations, etc.) are noted as identified by the study authors.
[d] Values reported for 5 controls but the group(s) were not identified.
[e] Sum of mandelic and phenylglyoxylic acids.
[f] Mean ± SE.
[g] Air concentration was estimated from urine mandelic acid levels.
[h] Subset of exposed workers that were sampled one year after reducing exposure.
[i] Values represent the mean concentrations measured before and after the work shift.

The general limitations [i.e., small numbers of subjects and failure to control for potential confounding factors] noted for chromosomal aberration and micronucleus studies also apply to SCE studies. Most of the studies included control groups matched on one or more of the following variables: age, gender, or smoking. Some studies that did not use matched subjects controlled for variables (such as age, gender, and smoking habits) in the analysis or reported that age, smoking, and gender distribution were similar between groups. Only two studies (Sorsa *et al.* 1991) [it was not clear whether smoking and age were controlled for in the dose-response regression analysis], and Biró *et al.* (2002) did not meet those criteria. Biro *et al.* did report that ages were similar, but smoking habits and gender differed between the exposed and referent groups. Ten studies reported that smoking was significantly correlated with SCE (Hallier *et al.* 1994, Karakaya *et al.* 1997, Kelsey *et al.* 1990, Laffon *et al.* 2002a, Maki-Paakkanen *et al.* 1991, Sorsa *et al.* 1991, Tates *et al.* 1994, Van Hummelen *et al.* 1994, Watanabe *et al.* 1983, Yager *et al.* 1993), while Meretoja *et al.* (1978a), Camurri *et al.* (1983, 1984), Hansteen *et al.* (1984), Brenner *et al.* (1991), Artuso *et al.* (1995), Holz *et al.* (1995), Rappaport *et al.* (1996), and Teixeira *et al*. (2004) did not find an association with smoking. Age was associated with SCE in the study by Sorsa *et al.* (1991) but not in another study (Van Hummelen *et al.* 1994). None of the studies reported that gender was a significant factor.

Two studies (Rappaport *et al.* 1996, Yager *et al.* 1993) had longitudinal study designs (without controls), and compared styrene exposure measured at several times during a one-year period with SCE frequency. Both studies reported positive correlations with styrene exposure (levels in exhaled air and/or other biomarkers of styrene exposure).

Nine of the remaining 20 studies reviewed reported a significant increase in SCEs in workers exposed to styrene compared with controls (Table 5-15). These nine studies included Andersson *et al.* (1980), Camurri *et al.* (1983, 1984) [considered as one study], Hallier *et al.* (1994), Tates *et al.* (1994), Artuso *et al.* (1995), Karakaya *et al.* (1997), Biro *et al.* (2002), Laffon *et al.* (2002a), and Teixeira *et al.* (2004). In addition to the exposure-response relationships observed in the longitudinal studies, Artuso *et al.* (1995) (exposure level), Laffon *et al.* (2002a) (exposure duration), and Watanabe *et al.* (1983) (total urinary metabolites) also reported significant exposure-response associations. However,

no associations were reported in other studies (Kelsey *et al.* 1990, Sorsa *et al.* 1991, Tates *et al.* 1994 [for exposure duration], Van Hummelen *et al.* 1994, and Karakaya *et al.* 1997). Workers in the study by Tate*s et al*. (1994) were exposed to dichloromethane; however, no correlation was found between dichloromethane exposure and SCE levels. Workers in other studies (Camurri *et al.* 1983, 1984, and Laffon *et al.* 2002a) were exposed to other chemicals such as organic peroxides, dyes, and acetone. Andersson *et al.* (1980) did not report results for all subjects and reported exposure as the product of styrene concentration and years exposed. Hallier *et al.* (1994) found that SCE levels in laminators decreased after technical and hygienic improvements reduced styrene levels from 37 to 15 ppm. Biró *et al.* (2002) did not provide styrene concentrations or levels of styrene metabolites in the urine.

SCE levels were not significantly higher in exposed workers than controls in the studies by Meretoja *et al.* (1978a), Watanabe *et al.* (1983, 1981), Hansteen *et al.* (1984), Mäki-Paakkanen *et al.* (1991), Mäki-Paakkanen (1987), Kelsey *et al.* (1990), Brenner *et al.* (1991), Sorsa *et al.* (1991), Van Hummelen *et al.* (1994), and Holz *et al*. (1995). There were no clear differences in the number of subjects or mean styrene concentrations between the positive and negative studies. However, most of the studies published prior to 1994 were negative while most of the studies published after 1994 were positive. The meta-analysis by Bonassi *et al.* (1996) was inconclusive regarding styrene exposure and SCE.

5.4.5 Genetic polymorphisms and susceptibility to styrene-mediated genotoxicity

Individuals may vary in their susceptibility to styrene's genotoxic effects because of differences in the ability to activate and inactivate styrene or differences in DNA-repair capacity. *CYP2E1* is one of the primary enzymes involved in the metabolism of styrene to styrene-7,8-oxide, and detoxification is mediated by mEH [also known as EPHX1] and glutathione S-transferases (GSTs); conjugation of styrene with glutathione as a minor detoxification pathway (see Section 5.1.3 for a detailed description of the metabolism of styrene). Studies have been conducted *in vitro* with human lymphocytes exposed to styrene and in styrene-exposed workers to evaluate whether polymorphisms in xenobiotic

metabolizing enzymes, DNA repair, or other critical pathways modulate genotoxic damage.

5.4.5.1 In vitro *studies*

The findings of the *in vitro* studies are summarized in Table 5-16. Human lymphocytes with polymorphisms in metabolic enzymes (coded for by *CYP1A1, CYP2E1, GSTM1, GSTT1, GSTP1*, or *mEH*) or DNA-repair enzymes (coded for by *hOGG1*, *XRCC1*, or *XRCC3*) were exposed to either styrene (usually at concentrations between 5,000 and 10,000 μM, although one study used 500 to 1,500 μM) or styrene-7,8-oxide (usually at 10 to 300 μM, although one study used 600 to 2,500 μM). Genotoxicity (single-strand breaks, *HPRT* mutations, micronuclei, or SCE) was measured and compared among the genotypes. [Most studies used only a small number of cells per genotype group. It is difficult to draw any conclusions about the effects of specific polymorphisms in modifying specific damage, because some of the polymorphisms were evaluated in only one study, or conflicting results were observed when the polymorphism was evaluated in several studies (e.g., *GSTM1* and *GSTT1*). Interpretation of the *GSTM1* and *GSTT1* studies also is complicated because the studies looked at different end points (e.g., SCE, *HPRT* mutations) or used different exposure agents (styrene or styrene-7,8-oxide), and some studies looked at combinations of *GSTM1* and *GSTT1* genotypes.] Three studies reported higher levels of genetic damage in a *GSTP1* variant; [however, the studies varied in the exposure (styrene or styrene-7,8-oxide), the end point affected (single-strand breaks or micronuclei), and the variant in which the effect was observed].

Table 5-16. Genotype analyses in *in vitro* studies with styrene and styrene-7,8-oxide

Genotypes Polymorphisms	Number of donors	Exposure (μM)		End points	Results (compared with + or wild type)	Reference
		Styrene	Styrene-7,8-oxide			
CYP1A1 Msp I (m1, m2, m4)[a]	var. 4–10 wt 20–26	5,000–10,000	–	SB	more SSB in *CYP1A1* m1 and m2 heterozygotes; less damage in m4 heterozygotes	Laffon *et al.* 2003a
CYP2E1 RsaI and DraI[b]	var. 1–6 wt 24–29	5,000–10,000	–	SB	more SSB in *CYP2E1* DraI heterozygotes	Laffon *et al.* 2003a
EPHX1 low, medium, high meH activity	6–8	–	10–100	SB	no genotype effect	Buschini *et al.* 2003
	5–16	5,000–10,000	–	SB	no genotype effect	Laffon *et al.* 2003a
	6–18	–	50–200	MN, SB	more MN and SSB in cells with lower mEH activity	Laffon *et al.* 2003b
	4–9	–	100–300	MN, SB	more MN in cells with higher mEH activity at 200 μM	Godderis *et al.* 2006
GSTM1 null	6+/6–	–	50–150	SCE	no genotype effect	Uüskula *et al.* 1995
	3+/2– cell lines[c]	–	600–2,500	HPRT mutation	more mutations in *GSTM1*-deficient cell lines	Shield and Sanderson 2004, 2001)
	5+/9–	–	10–100	SB	no genotype effect	Buschini *et al.* 2003
	15+/12–	5,000–10,000	–	SB	no genotype effect	Laffon *et al.* 2003a
	17+/13–	–	50–200	MN, SB	no genotype effect	Laffon *et al.* 2003b
	8+/12–	–	100–300	MN, SB	no genotype effect	Godderis *et al.* 2006
GSTT1 null	5+/5–	–	50–150	SCE	more SCE in *GSTT1* null	Ollikainen *et al.* 1998
	21+/6–	5,000–10,000	–	SB	no genotype effect	Laffon *et al.* 2003a
	24+/6–	–	50–200	MN, SB	no genotype effect	Laffon *et al.* 2003b
	11+/3–	–	10–100	SB	no genotype effect	Buschini *et al.* 2003
	14+/6–	–	100–300	MN, SB	more MN with *GSTT1* (considered a spurious effect)	Godderis *et al.* 2006

Genotypes Polymorphisms	Number of donors	Exposure (µM)		End points	Results (compared with + or wild type)	Reference
		Styrene	Styrene-7,8-oxide			
GSTM1 and *GSTT1* combinations	5–7	500–1,500	–	SCE	more SCE in *GSTM1* null/*GSTT1* null at high styrene level	Bernardini *et al.* 2002
GSTP1 codon 105 alone or in combination with codon 114[d]	7 wt/7 var.	–	10–100	SB	more SSB in *GSTP1* variants	Buschini *et al.* 2003
	4–18	5,000–10,000	–	SB	more SSB in *GSTP1* 105 variant	Laffon *et al.* 2003a
	4–18	–	50–200	MN, SB	more MN (nonsignificant) in *GSTP1* 105 and 114 variant (combination)	Laffon *et al.* 2003b
	3–10	–	100–300	MN, SB	no genotype effect	Godderis *et al.* 2006
hOGG1 codon 326[e]	7–13	–	100–300	MN, SB	no genotype effect	Godderis *et al.* 2006
XRCC1 codons 194[f], 280[e], 399[e]	1–18	–	100–300	MN, SB	no genotype effect	
XRCC3 codon 241[e]	3–11	–	100–300	MN, SB	no genotype effect	

+ = positive for gene; – = negative for gene; HPRT = hypoxanthine phosphoribosyltransferase; MN = micronuclei; SB = strand breaks; SCE = sister chromatid exchange; var. = variant; wt = wild type.
[a] Predicted influence on the enzyme is increased inducibility (m1), increased activity (m2), and decreased activity (m4).
[b] Predicted influence on the enzyme is unknown.
[c] These studies used established human B lymphoblastoid cell lines instead of whole-blood lymphocyte cultures from donors.
[d] Compared to the wild-type enzyme, the variant proteins show either a reduced half-life or a different catalytic efficiency toward organic substrates.
[e] Low activity.
[f] High activity.

5.4.5.2 In vivo *studies*

The findings for studies that evaluated polymorphisms in metabolizing enzymes and genotoxic effects in styrene-exposed workers are summarized in Table 5-17. Results evaluating the relationship between polymorphisms and genetic damage in both non-exposed and styrene-exposed workers are not discussed in this review. [Most of these studies determined the genotypes of fewer than 50 styrene-exposed workers for several polymorphisms, measured various genotoxic end points, and compared the amount of genetic damage among genotype groups. As with the *in vitro* studies, it is difficult to draw any conclusions regarding specific polymorphisms, because the findings were conflicting, and the studies had many limitations. The number of individuals per genotype was very small, limiting the statistical power to detect an effect. Many studies did not adjust for potential confounders and made multiple comparisons, thus increasing the possibility of obtaining false-positive results.]

One study of styrene-exposed workers evaluated polymorphisms in DNA-repair genes and genotoxicity; however, this study included both styrene-exposed workers and referents, and most of the analysis was of the whole population rather than specifically the styrene-exposed populations. Kuricova *et al*. (2005) measured DNA adducts, single-strand breaks, *HPRT* mutations, and chromosomal aberrations among 48 workers (16 males and 32 females) at a styrene-reinforced-plastics plant (the same population studied by Vodicka *et al.* (2001a); see Table 5-17). Levels of damage were compared among polymorphisms for XPD, XPG, XPC, XRCC1, XRCC3, and cyclin D1. Most of the results were presented for the entire population (which also included 24 controls), but the authors stated that styrene-exposed individuals with the *XRCC1* 399 wild-type genotype had a lower frequency of chromosomal aberrations than individuals with the variant genotype. [This was the only significant finding among the exposed population.]

[In addition to the limitations discussed (small numbers of subjects or cell lines, multiple comparisons, potential confounding, conflicting results, and very few studies evaluating similar end points and genotypes), the genetic susceptibility studies discussed evaluated only single polymorphisms. The evaluation of multiple loci or several polymorphisms in

the same pathway may be more informative for identifying populations sensitive to styrene-mediated genotoxicity.]

Table 5-17. Genotype analyses *in vivo* in workers occupationally exposed to styrene in association with biomarkers of genotoxicity

Genotypes Polymorphisms	No. of exposed workers	End points[a]	Results for exposed workers[b]	Reference
CYP1A1 MspI	44 (19 *HPRT* 29 SSB)	SSB, CA, *HPRT* mutation	no effects reported	Vodicka *et al.* 2001a
CYP2E1 RsaI and DraI	44 (19 *HPRT* 29 SSB)	SSB, CA, *HPRT* mutation	more *HPRT* mutations in DraI heterozygotes; more SSB in both RsaI and DraI heterozygotes; no effects for CA.	Vodicka *et al.* 2001a
	19	DNA adducts, *HPRT*	more adducts in heterozygotes, no effect on adducts	Vodicka *et al.* 2003
	28	SCE, MN	no effects reported	Teixeira *et al.* 2004
EPHX1 low, medium, high mEH activity	44 (19 *HPRT* 29 SSB)	SSB, CA, *HPRT* mutation	no effects reported	Vodicka *et al.* 2001a
	48	SSB	no effects reported	Buschini *et al.* 2003
	19	DNA adducts, HPRT mutation	no effects reported	Vodicka *et al.* 2003
	28	SCE, MN	decreased SCE for medium mEH activity; no effect on MN	Teixeira *et al.* 2004
GSTM1 null	44 (19 *HPRT* 29 SSB)	SSB, CA, *HPRT* mutation	no effects reported	Vodicka *et al.* 2001a
	14	SSB, SCE, MN	no effects reported, but increased PRI[c] was observed in *GTSM1* null	Laffon *et al.* 2002a
	48	SSB	fewer SSB in *GSTM1* null	Buschini *et al.* 2003
	19	DNA adducts, *HPRT* mutation	no effects reported	Vodicka *et al.* 2003
	28	SCE, MN	no effects reported	Teixeira *et al.* 2004
	95	MN, CA	no effects reported	Migliore *et al.* 2006b
GSTT1 null	44 (19 HPRT 29 SSB)	SSB, CA, *HPRT* mutation	no effects reported	Vodicka *et al.* 2001a
	14	SSB, SCE, MN	no effects reported	Laffon *et al.* 2002a
	48	SSB	more SSB in *GSTT1* null	Buschini *et al.* 2003
	19	DNA adducts, *HPRT* mutation	no effects reported	Vodicka *et al.* 2003
	28	SCE, MN	no effects reported	Teixeira *et al.* 2004

Genotypes Polymorphisms	No. of exposed workers	End points[a]	Results for exposed workers[b]	Reference
	95	MN, CA	significantly higher frequency of MN and non-significant increase in CA	Migliore *et al.* 2006b
GSTP1 codon 105 alone or in combination with codon114	44 (19 *HPRT* 29 SSB)	SSB, CA, *HPRT*	marginal effect on *HPRT* mutation[d] no effects reported for SSB and CA	Vodicka *et al.* 2001a
	19	DNA adducts, *HPRT* mutation	more *HPRT* mutations in heterozygotes, no effect on adducts	Vodicka *et al.* 2003
	28	SCE, MN	no effects reported	Teixeira *et al.* 2004
	95	MN, CA	no effects reported	Migliore *et al.* 2006b

[a]CA = chromosomal aberrations, MN = micronuclei, SSB = single-strand breaks.
[b]Results reported for exposed populations only. Some studies did report association between polymorphisms and genetic damage in the total population (controls and exposed workers) or in controls only.
[c]PRI = proliferation rate index = (MI + 2MII + 3MIII)/N, where MI, MII, and MIII = the number of metaphases in first, second, and third or subsequent divisions, and N = the total number of metaphase scored in the SCE assay.
[d]The effect was observed only when outliers were included.

5.4.6 Summary of styrene and styrene-7,8-oxide genotoxicity

Results from *in vitro* studies and *in vivo* studies in experimental animals and humans are summarized in Table 5-18. DNA adducts (primarily O^6-deoxyguanosine and N7-deoxyguanosine) have been detected in the liver and lungs of rats and mice exposed to styrene by inhalation or i.p. injection. O^6-deoxyguanosine, N^2-guanine, and N1-adenine adducts have been detected in lymphocytes of workers occupationally exposed to styrene. *In vitro* and *in vivo* studies indicated that styrene could induce DNA damage including single-strand breaks. Mutation studies in bacteria were mostly negative without metabolic activation, but some studies were positive with metabolic activation. *In vitro* mutation studies with eukaryotic cells gave mixed results. No mutation studies of styrene-exposed experimental animals were reviewed. A few studies investigated *HPRT*- and *GPA*-locus mutations in styrene-exposed workers and reported inconclusive to weak positive results. One study was positive for *HPRT* mutations but these workers also were exposed to dichloromethane. *In vitro* studies indicate that styrene can cause chromosomal aberrations, SCE, and micronuclei; whereas, *in vivo* studies in rodents were positive for SCE only. A meta-analysis of studies of occupational exposed workers reported a positive association between styrene exposure level (higher levels) and chromosomal

aberration frequency. Studies in occupationally exposed workers show conflicting responses with SCE and micronuclei formation.

Table 5-18. Genetic and related effects of styrene

Effect	In vitro	In vivo	
		Rodents	Humans
DNA adducts	NSR[a]	+	+
DNA damage[b]	+[1]	+	+
Mutations		NS	(+)
bacteria	–		NA
lower eukaryotes	±		NA
mammalian cells	±		NA
Chromosomal aberrations	+	–	(+)
Sister chromatid exchange	(+)	+	±
Micronuclei	+	–	±
Aneuploidy or polyploidy	±	+[1]	–

+ = predominantly positive results; +[1] = positive results in the only study reviewed; ± = similar number of positive and negative results or multiple studies with positive and negative results; (+) = weakly positive results; – = predominantly negative results.
NA = not applicable; NSR = no studies reviewed.
[a] Studies with styrene-7,8-oxide did cause DNA adducts *in vitro*.
[b] Includes alkali-labile sites and single-strand breaks.

5.5 Mechanistic studies and considerations

Several recent publications reviewed the possible mechanisms of styrene-induced carcinogenicity. Both genotoxic and epigenetic processes have been considered. IARC (2002) proposed two likely mechanisms for styrene carcinogenicity: (1) DNA damage in target tissues resulting from metabolic conversion of styrene to styrene-7,8-oxide and (2) the cytotoxic effects of styrene in the lungs of mice. IARC did not consider these mechanisms to be mutually exclusive and suggested that the interspecies differences in the metabolism of styrene and styrene-7,8-oxide in rats and mice were likely important. In addition, Cruzan *et al.* (2002) and The Harvard Center for Risk Analysis (Cohen *et al.* 2002) concluded that cytotoxicity and subsequent hyperplasia of lung cells must play a key role underlying development of lung tumors in mice, and they proposed several potential mechanisms that could explain how styrene exposure could cause development of hyperplasia in the mouse lung but not the rat lung. Cohen *et al.* (2002) proposed that the species differences between mice and rats (assuming that the lung tumors are caused by styrene-7,8-oxide) could be due to a combination of higher rates of styrene-7,8-oxide accumulation and greater susceptibility of the mouse lung to epoxides. IARC (2002) noted that mice are considered to be more susceptible to induction of lung tumors by epoxides and chemicals capable of being metabolized to epoxides than are rats, based on findings of lung tumors in mice but not in rats when both species were exposed to ethylene oxide, 1,3-butadiene, isoprene, or chloroprene. Cruzan *et al.* (2002) proposed that interspecies differences in styrene toxicity are most likely explained through CYP2F-generated metabolites such as 4-vinylphenol.

Some (but not all) studies in experimental animals reported increased incidences of tumors of the mammary gland or lymphatic system in rats (see Section 4), and increases in mortality or incidence of pancreatic cancer and lymphohematopoietic malignancies have been reported in some studies of styrene-exposed workers (see Section 3). However, no styrene-specific mechanistic studies or reviews of the mammary gland, pancreas, or lymphohematopoietic system as possible tumor sites were identified. (See Section 5.2 for a discussion of prolactin, styrene exposure, and breast cancer). The following sections discuss mechanistic considerations related to genotoxicity (Section 5.5.1), gene expression and apoptosis (Section 5.5.2) , oxidative stress (Section 5.5.3), cytotoxic

effects of styrene on mouse lung (Section 5.5.4), and selected styrene analogues (Section 5.5.5).

Styrene-7,8-oxide, a primary and genotoxic metabolite of styrene, is listed in the *Report on Carcinogens* as *reasonably anticipated to be a human carcinogen* based on sufficient evidence in experimental animals (NTP 2004), and IARC (1994b) concluded that there was sufficient evidence in experimental animals for its carcinogenicity. Styrene-7,8-oxide and other epoxides, or epoxide-forming chemicals, are reactive compounds. Epoxides have been associated with lung, liver, harderian gland, and circulatory system neoplasms in mice; Zymbal's gland and brain tumors in rats; and mammary gland and forestomach tumors in rats and mice (Melnick 2002). Dunnick *et al.* (1995) and Bennett and Davis (2002) reviewed findings from NTP's carcinogenesis studies and reported that epoxides or chemicals metabolized to epoxides were associated with mammary tumors in rodents. Bennett and Davis hypothesized that the mammary gland may be efficient in metabolizing chemicals to their epoxides. However, these authors also noted that not all epoxides or epoxide-forming chemicals were associated with mammary tumors. Styrene-7,8-oxide administered by oral gavage induced high incidences of both benign and malignant tumors of the forestomach in both sexes of rats (three strains tested) and in one strain of mice (IARC 1994b). One of the rat studies also included prenatal exposure followed by postnatal gastric lavage. Lijinsky (1986) also reported liver tumors in male mice in the low-dose group only. Lung and mammary tumors were not increased in these studies. No inhalation carcinogenicity studies have been conducted with styrene-7,8-oxide. However, styrene-7,8-oxide has been measured in the blood of rats and mice following oral and i.p. administration (IARC 1994b). No reports of the levels of styrene-7,8-oxide in the lungs of rats or mice exposed to styrene were identified, but a PBPK model indicated that oral administration of styrene-7,8-oxide at 275 mg/kg per day would result in higher lung levels of styrene-7,8-oxide than from metabolism of styrene administered at 40 ppm by inhalation (Sarangapani *et al.* 2002) (see Section 5.3.4 for the metabolic assumptions for this model).

An increased incidence of lung, liver, mammary gland, and lymphatic neoplasias has been reported for some studies in experimental animals (see Section 4), although the

results were not consistent across studies. Increases in mortality or incidence of lymphohematopoietic malignancies and tumors at some other sites (such as the pancreas) have also been reported in some studies of styrene-exposed workers (see Section 3). However, no styrene-specific mechanistic studies or reviews of the mammary gland, pancreas, or lymphohematopoietic system as possible tumor sites were identified. (See Section 5.2 for a discussion of prolactin, styrene exposure, and breast cancer).The following section discusses mechanistic considerations related to genotoxicity, gene expression and apoptosis, oxidative stress, cytotoxicity in mouse lung, and studies of selected styrene analogues.

5.5.1 Genotoxicity

Some DNA adducts are highly xenobiotic-specific DNA lesions that can alter DNA ultrastructure. IARC (2002) noted that a potential mechanism for the carcinogenicity of styrene is based on covalent binding of the DNA-reactive metabolite styrene-7,8-oxide. DNA adducts formed with styrene-7,8-oxide include N7-guanine, N3-adenine, O^6-guanine, N^2-guanine, N1-adenine, N^6-adenine, and N3-cytosine (see Section 5.4.1). Adducts associated with oxidative damage (e.g., 8-hydroxy-2'-deoxyguanosine) also have been reported in styrene-exposed workers (Marczynski *et al.* 1997a). N7-guanine adducts are the predominant type, but are repaired *in vivo*, whereas O^6-guanine adducts occur at a much lower frequency but are more persistent (see Section 5.4.2.1). N7-guanine and N3-adenine adducts may result in depurination or may cause single-strand breaks. Because DNA polymerase preferentially adds an adenine opposite an apurinic site, N7-guanine adducts may result in G·C→A·T transitions, and N3-adenine adducts may result in A·T→T·A transversions (Loeb and Preston 1986). The other adducts occur at base-pairing sites and may cause the following specific base-pair mutations: (1) O^6-guanine, G·C→A·T transition, (2) N2-guanine, G·C→A·T transversion (via incorporation of deoxythymidine triphosphate opposite the adduct) (Zang *et al.* 2005b), (3) N1-adenine, mutations at A·T base pairs (via blockage of a central hydrogen bonding site of the adenine residue), (4) N3-uracil, G·C→A·T transition and, to a minor extent, G·C→T·A transversion (via conversion to the N3-cytosine adduct) (Zhang *et al.* 1995), and (5) N6-guanine, A·T→G·C transition. A·T→G·C transition was the dominant type of mutation in

both styrene-7,8-oxide–exposed *HPRT* mutant clones (Bastlová and Podlutsky 1996) and in a site-specific mutation study in which a styrene-7,8-oxide adduct at the N^6-position of adenine was inserted at codon 61 in the N-*ras* gene (Latham *et al.* 1993). Weak mutagenicity was observed when *S*-styrene-7,8-oxide was bound at the α-carbon of styrene-7,8-oxide to the adenine in the second position of the codon, while the *R*-enantiomer bound at that position blocked replication of the single-stranded DNA template almost completely, and no mutagenicity was found when either the *R*- or the *S*-enantiomer was bound to the adenine in the third position of codon 61. The β-N6-dA styrene-7,8-oxide adducts have been examined as to site-specific mutagenesis in *E. coli*. These data indicate that the β-N6-dA adducts do not have significant deleterious effects on replication competence (Kanuri *et al.* 2001).

The actions of native and various site-specific mutants of HIV-1 reverse transcriptase have been examined *in vitro* on DNA templates modified with α-N6-dA adducts. For the native enzyme, activity is dependent on both the chirality of the N6-dA adducts and their sequence contexts. Replication is possible but is terminated 3 to 5 nucleotides after translesion synthesis and before reaching the end of the template (Latham and Lloyd 1994). Eight mutants of reverse transcriptase also terminate synthesis on these styrene-7,8-oxide–adducted templates. The sites of termination occur primarily 1 and 3 bases following adduct bypass, when the lesion is positioned in the major groove of the template-primer stem (Latham *et al.* 2000).

Similar replication assays have been performed using *E. coli* Klenow fragment, Sequenase 2.0, T4 polymerase holoenzyme, polymerase α, and polymerase β, *in vitro*. In all instances, lesion bypass is sensitive to both the local sequence context and the chirality of the α-N6–dA styrene-7,8-oxide adducts. For example, in the 5'-AXG-3' sequence, adducts having *R*-stereochemistry are bypassed, whereas stereochemically-identical lesions in other sequence contexts are often poor substrates. Similarly, *R*- vs. *S*-α-N6–dA adducts introduced within identical sequences are often bypassed nonequivalently. The degree of adduct-directed termination and translesion synthesis during replication is also dependent on the choice of polymerase. Templates that are poor substrates for bypass synthesis with one enzyme often read through much more

efficiently when a different polymerase is used (Latham *et al.* 1995). Similar studies have been conducted using reconstituted *E. coli* Pol III. Replication is poorly processive and strongly terminated by styrene-7,8-oxide lesions in 33-mer templates, although the same enzyme showed efficient bypass of the same adducts in M13 DNA (Latham *et al.* 1996). No data are available regarding replication by Y-family polymerases, *in vitro*.

Single-strand breaks were observed in most studies of styrene-exposed workers and occurred in a concentration-dependent manner (see Section 5.4.4.2). Most studies have reported an increase in chromosomal aberrations in styrene-exposed workers, and exposure-response relationships have been observed in several studies. A meta-analysis (Bonassi *et al.* (1996) of studies published prior to 1996 found a positive association between styrene exposure (greater than 30 ppm for an 8-hour time-weighted average) and chromosomal aberrations. The data on mutations and other types of genetic damage in humans are conflicting. Most recent studies have reported higher levels of sister chromatid exchange in styrene-exposed workers than in controls, but the study populations were small, and potential confounding was not always addressed.

As described in Section 4, styrene exposure caused lung tumors in mice but not in rats. *In vivo* experiments in rodents have shown that styrene exposure can cause DNA adducts in lung and liver in mice and rats (see Section 5.4.3.1 and Table 5-7 for a description of these studies). Comparison between animal studies is difficult because different species, organs, methods of detection, routes of administration, and exposure levels were used. Moreover, most genotoxicity studies in animals are short-term, and humans are exposed for long time periods. No correlation of adducts with tumor incidence has been observed (Nestmann *et al.* 2005), suggesting that other mechanisms of carcinogenicity may also be important. Boogaard *et al.* (2000b) reported that styrene had a low covalent binding index (CBI) relative to other known genotoxicants; the hepatic CBI (at 42 hours) was 0.19 in rats and 0.44 in mice, and the pulmonary CBI (pooled 0 and 42 hours) was 0.17 in rats and 0.24 in mice. The low levels of styrene-7,8-oxide adducts in the forestomach as the target tissue for styrene-7,8-oxide in rats and mice were judged to be insufficient to account for its carcinogenic activity by a strictly genotoxic mechanism (Phillips and Farmer 1994) (see Section 5.4.3.1). However, the reported levels of DNA binding varied

by factors of 20 to 50 among the studies. The reasons for the discrepancies were not completely understood, according to the authors; however, some of the variability could be attributed to differences in administration routes, measurement methods, and losses through depurination.

Koskinen *et al.* (2001a) compared the formation of β-N1-adenine adducts resulting from styrene exposure in mice and humans. They reported that exposure of mice to styrene at 750 mg/m^3 [173 ppm] resulted in formation of 1 β-N1-adenine adduct per 10^9 normal nucleotides, while exposure of humans at 76 mg/m^3 [17.5 ppm] resulted in 0.8 adducts per 10^9 nucleotides [the results are reported in Table 5-10 as 0.08 adducts per 10^8 nucleotides].

5.5.2 Gene expression and apoptosis

The effect of styrene-7,8-oxide on the expression of genes involved in the cell cycle and in regulation of apoptosis was studied in white blood cells exposed to styrene-7,8-oxide at a concentration of 50 or 200 μM (Laffon *et al.* 2001a). mRNA and reverse transcription polymerase chain reaction were used to analyze the expression of the genes involved in cell-cycle arrest in response to DNA damage (*p53*, *p21*) or in control of apoptosis (*bcl-2* and *bax*). Apoptotic events were detected by the DNA fragmentation assay. Data for expression were presented only in the form of graphs for individual donors (2 men and 2 women described as healthy nonsmokers aged 23 to 30). The authors reported high interindividual variation in the expression of studied genes, with no consistent pattern of increased or decreased expression. The authors did describe a difference in the cytokinesis block proliferation index (CBPI). All CBPI values for control cultures and low-exposure cultures were between 1.94 and 2.04, while the values for high-exposure cultures ranged from 1.67 to 1.78 and were significantly lower than in the controls ($P < 0.01$), indicating a delay in cell-cycle kinetics. The authors suggested that exposure to high levels of styrene-7,8-oxide might induce a delay in the cell cycle, which could allow the DNA repair system to act on the genotoxic damage produced, instead of driving the cells towards programmed cell death.

Diodovich *et al.* (2004) studied the effect of styrene on cell-cycle gene (c-*fos*, c-*jun*) expression profiles in human cord blood cells and styrene's effect on production of apoptosis-related proteins (Bax, Bcl-2, Raf-1). Exposure to styrene at 800 μM for 24 or 48 hours increased necrosis of mononuclear cord blood cells, but not apoptosis. Western blot analysis revealed induction of both c-jun and c-fos protein, but at different times, as c-jun was induced early and decreased later, while c-fos was induced only after 48 hours of exposure to styrene. Production of both Bcl-2 and Raf-1 proteins was induced by styrene exposure at all time points (6, 24, and 48 hours), whereas Bax protein was initially downregulated but recovered at the later times. The p53 protein was not produced in either unexposed or styrene-exposed cells. Macroarray analysis (see Glossary) showed that styrene-modified cord blood gene expression was associated with upregulation of monocyte chemotactic protein and downregulation of CC chemokine receptor type 1 and SLP-76 tyrosine phosphoprotein. The authors concluded that their results supported a role for styrene in promotion of cell proliferation and cell-cycle progression, which could potentially favor alterations in gene expression and genotoxic effects.

5.5.3 Oxidative stress

Marczynski *et al.* (2000) proposed a mechanism involving oxidative stress and oxidative DNA damage as the basis for the genotoxic effects of styrene resulting from an imbalance between oxidants and antioxidants in cells. Gamer *et al.* (2004) (see Section 5.2.3.2) found no evidence of oxidative stress as indicated by unchanged concentrations of 8-OH-deoxyguanosine in lung lavage fluid after 20 daily (6 hours per day during a 4-week period) exposures to styrene at 20, 40, 80, or 160 ppm. However, Roder-Stolinski *et al.* (2008) reported that exposure of human lung epithelial cells (cell line A549) to styrene *in vitro* stimulated the expression of inflammatory mediators, including chemotactic cytokine monocyte chemoattractant protein-1 (MCP-1) through activation of the NF-κB signaling pathway, and suggested that activation of the NF-κB signaling pathway was mediated via a redox-sensitive mechanism (see Section 5.2.1). Cohen *et al.* (2002) in their review suggested that the pulmonary hyperplasia that occurs in mice but not in rats likely results from oxidative damage that is caused either directly by styrene oxide or indirectly because of depletion of glutathione (GSH). As mentioned above

(Section 5.5.1) adducts associated with oxidative damage have been reported in styrene-exposed workers

5.5.4 Cytotoxic effects of styrene on mouse lung

This section discusses cytotoxicity of styrene metabolites as a possible mechanism of styrene-induced carcinogenesis. Cytotoxicity and cellular proliferation (see Section 5.2.2.2) have been observed, especially in mouse lung Clara cells, following *in vitro* and *in vivo* exposure to styrene and styrene metabolites (styrene-7,8-oxide and 4-vinylphenol). Chronic cytotoxicity can result in clonal expansion of styrene-induced, or spontaneous, mutants. Induction of lung tumors resulting from formation of cytotoxic metabolites has been proposed for other chemicals, including naphthalene. A scientific panel at the Naphthalene State-of-the-Science Symposium on the pathogenesis of respiratory tumor formation in rodents (Bogen *et al.* 2008) hypothesized that nasal tumors in rats and lung adenomas in mice occur through a cytotoxic mechanism. Metabolic activation (via Cyp2f2) was required and mouse Clara cells had the greatest capacity to metabolize naphthalene and were also highly susceptible to naphthalene-induced cytotoxicity.

Cohen *et al.* (2002), Cruzan *et al.* (2002), and IARC (2002) proposed that styrene exposure causes pulmonary hyperplasia in the mouse lung, which may play a role in the development of lung tumors. Effects of repeated styrene exposure reported in the lungs of mice, but not in rats, included focal crowding of bronchiolar cells, bronchiolar epithelial hyperplasia, and bronchiolo-alveolar hyperplasia (IARC 2002) (see Section 5.2.2.2 for a description of pneumotoxicity in rodents). Studies by Gadberry *et al*. (see Section 5.2.2.3) showed that styrene-7,8-oxide administered by i.p. injection caused pulmonary toxicity in mice, suggesting that styrene-7,8-oxide is responsible for the pneumotoxicity and that systemically available styrene-7,8-oxide can enter the lung cell. Cohen *et al.* postulated that styrene might induce cytotoxicity by directly damaging the cell or by causing glutathione depletion (see Section 5.2.2.4). Tissue damage leads to hyperplasia, which makes the tissue more sensitive to tumor development. Cohen *et al.* (2002) also stated that the role of hyperplasia does not rule out the possibility that styrene-7,8-oxide also causes genotoxic effects.

Cohen *et al.* (2002) identified three factors that they considered as possible mechanisms contributing to the development of hyperplasia in mice and subsequent development of lung tumors: (1) the presence of the cytochromes CYP2E1 and Cyp2f2, which convert styrene to styrene-7,8-oxide, in mouse lung tissues, (2) greater formation of the *R*-enantiomer of styrene-7,8-oxide, which the authors considered the more toxic and mutagenic of the two enantiomers, and (3) the susceptibility of mouse lung tissue to GSH depletion, which could reduce the detoxification of styrene-7,8-oxide. After carefully comparing these factors in rats, mice, and humans (see Section 5.3 for a review of the literature on interspecies differences, including Cohen *et al.* 2002), the authors concluded that these measures of metabolic activity and styrene-7,8-oxide accumulation in the lung do not explain why styrene caused lung tumors in mice and not rats. IARC (2002) reached a similar conclusion regarding lung-tumor susceptibility and toxicokinetic differences between mice and rats.

Cohen *et al.* (2002) proposed several reasons why styrene-7,8-oxide production in the lung did not explain the differences between mice and rats in development of pulmonary hyperplasia: (1) the Harvard PBPK model did not consistently predict a higher concentration of styrene-7,8-oxide in the lungs of mice than in rats, (2) styrene-7,8-oxide concentrations were lower in the blood of mice than in rats by an order of magnitude, and (3) the predicted concentrations of styrene-7,8-oxide in the lungs were very similar for mice exposed to styrene by inhalation at 40 to 80 ppm, which resulted in lung tumors, and rats exposed at 1,000 ppm, which did not induce lung tumors. Cohen *et al.* also stated that it was not clear that the pulmonary toxicity of the *R*-styrene-7,8-oxide was substantially greater than that of the *S*-styrene-7,8-oxide; differences in toxicity appear to be greater in the liver than in the lung (Gadberry *et al.* 1996, see Section 5.2.4). The authors also did not think that the greater sensitivity of mice to GSH depletion (see Section 5.2.2.4) could explain the differences in lung tumor susceptibility, because styrene caused hyperplasia in mice at concentrations (20 ppm) that do not cause GSH depletion. Cohen *et al.* (2002) proposed the following three possible explanations for the difference in susceptibility: (1) a greater number of Clara cells in mouse pulmonary tissue than in rat pulmonary tissue, (2) a pharmacokinetic difference at the cellular level, and (3)

a pharmacodynamic difference, such as greater susceptibility at the cellular level to injury due to less efficient DNA repair.

Similar to the first of the three factors put forward by Cohen *et al.* (2002) as possible mechanisms for the development of hyperplasia and lung tumors in mice (see above), Cruzan *et al.* (2002) proposed that interspecies differences in styrene toxicity are most likely explained through CYP2F-generated metabolites (2f2 in mice, 2F4 in rats, and 2F1 in humans). They noted that almost all of the effects of cytotoxicity and tumor formation were seen in tissues that are high in CYP2F isoforms and that CYP2F inhibitors prevented cytotoxicity (see Section 5.1.3.5). Metabolites formed from ring oxidation, including 4-vinylphenol, are about 6-fold higher in mice compared with rats, and 4-vinylphenol has been reported to be more potent than styrene-7,8-oxide as a pneumotoxicant (see Section 5.1.3.5). Also, styrene metabolism occurs primarily in Clara cells (see Sections 5.1.3.3 and 5.1.3.5), and mice produce higher levels of toxic metabolites (*R*-styrene-7,8-oxide, 4-vinylphenol, and oxidized reactive intermediates of 4-vinylphenol), and have a lower level of detoxifying epoxide hydrolase activity than rats or humans (see Sections 5.1.3.1 and 5.1.3.2). They stated that PBPK models predicted that humans do not generate sufficient levels of these metabolites in the terminal bronchioles to reach toxic levels. Cruzan *et al.* stated that the tumor profile of styrene suggests a non-genotoxic mode of action since they felt that the tumors in animals were common, reported in only one species and one site, did not occur at the 12-month sacrifice, and were associated with organ toxicity and cell turnover. Studies published after Cruzan *et al.*'s 2002 proposal that evaluated the role of Cyp2f2, ring-oxidized metabolites, and cytotoxicity in the lung are discussed in Sections 5.1.3.5 and 5.2.2.2. For example, Kaufmann *et al.* (2005) concluded that the side-chain hydroxylation pathway appeared to be of minor relevance for the pneumotoxic effects of styrene (see Section 5.2.2.2).

5.5.5 Selected styrene analogues

Studies on styrene analogues such ethylbenzene, 1-phenylethanol, 4-methylstyrene, and vinyltoluene (a mixture of 3- and 4-methylstyrene) (see Table 1-4 for structures of these analogues), provide further information on the possible relationship between formation of

ring-oxidized metabolites and the development of lung tumors in experimental animals exposed to these molecules. [However, no comprehensive reviews or evaluations of all analogues were identified in the peer-reviewed literature, and thus, only a few analogues are discussed. No long-term carcinogenicity studies (such as studies in *Cyp2f2*-knockout mice) that evaluated this proposed mechanism were identified.]

Ethylbenzene is a synthetic precursor for styrene (see Section 2.2) differing from styrene only in the absence of the double bond in the 2-carbon side chain, and 1-phenylethanol is a metabolite of both ethylbenzene and styrene (see Figure 5-1). Midorikawa *et al.* reported that ethylbenzene was metabolized to 1-phenylethanol, 2-ethylphenol, and 4-methylphenol by rat liver microsomes. The latter two metabolites were metabolically transformed to the ring-dihydroxylated metabolites ethylhydroquinone and 4-ethylcatechol (in a separate reaction), respectively, and Midorikawa *et al.* proposed further metabolism of the ethylcatechol to 4-ethyl-1,2-benzoquinone. [No *in vivo* metabolism studies were identified.] Incubation of 4-ethylcatechol with calf thymus DNA *in vitro* resulted in oxidative DNA damage, including the formation of 8-oxo-2'-deoxyguanosine (8-oxodG) in the presence of Cu(II), and the oxidative stress resulting from the formation of reactive oxygen species as a result of this proposed metabolic pathway for ethylbenzene could contribute to the carcinogenic mechanism of ethylbenzene. (Oxidative stress has been proposed to play a role in styrene-induced carcinogenicity — see above.) Ethylbenzene has been reported to induce lung tumors in male mice, liver tumors in female mice, kidney tumors in rats (both sexes), and testicular tumors in rats (Chan *et al.* 1998). Stott *et al.* (2003) reported that chronic exposure to ethylbenzene induced changes in the mouse lung, including multifocal bronchiolar/parabronchiolar hyperplasias and altered tinctorial properties. The authors proposed a nongenotoxic mode of action that was dependent upon cell proliferation and altered cell population dynamics. However, no studies were identified that evaluated the role of the ring-oxidized metabolites in lung tumor formation.

Other styrene analogues listed above, i.e., 4-methylstyrene and vinyltoluene, are not predicted to form 4-phenol metabolites because of the placement of the methyl group at the 3- or 4- position in these molecules, and no evidence for induction of mouse lung

tumors by either of these molecules was identified. However, the findings for lung cytotoxicity were mixed. 4-Methyl styrene (*para*-methylstyrene) was administered by gavage to Sprague-Dawley rats (0, 10, 50, 250, and 500 mg/kg per day) and Swiss mice (0, 10, 50, and 250 mg/kg per day) in a long-term (104 weeks) carcinogenicity study, and no increased tumor incidence was reported compared with control animals. However, Conti *et al.* (1988) reported data only for tumor incidences and did not report any endpoints for possible lung toxicity. [No inhalation studies were identified.] Vinyltoluene (a mixture of 65% to 71% 3-[*meta*-] isomer and 32% to 35% 4-[*para*-] isomer) was tested in a two-year inhalation study in F344 rats (0, 100, or 300 ppm) and B6C3F$_1$ mice (0, 10, or 25 ppm), and no increase in tumor incidences were reported (NTP 1990a). However, vinyltoluene did cause focal chronic active inflammation and diffuse hyperplasia of the respiratory epithelium, and chronic active inflammation of the bronchioles.

No ring-oxidized metabolites of 1-phenylethanol resulting from metabolism of ethylbenzene by mouse micorsomes were identified in the study by Midorikawa *et al.* (2004), and neither lung tumors nor lung cytotoxicity were observed when α-methylbenzyl alcohol (1-phenylethanol) was administered by gavage to F344 rats and B6C3F$_1$ mice at doses of 0, 375, or 750 mg/kg per day in a two-year bioassay (NTP 1990b). However, there was an increased incidence of renal tubular-cell adenoma or adenocarcinoma (combined) in male rats and transitional-cell papillomas of the urinary bladder occurred in two high-dose female rats. [No inhalation studies with 1-phenylethanol were identified.]

α-Methylstyrene, another chemical tested in a two-year inhalation study, did not significantly increase the incidence of lung tumors or cause lung cytotoxicity in mice (in the two-year study) although it did cause renal tumors and possibly leukemia in male rats and liver tumors in male (marginal) and female mice (NTP 2007). No metabolism studies evaluating whether a 4-phenol derivative of this molecule is formed during metabolism were identified, but its chemical structure does not appear to make that impossible.

5.6 Summary

5.6.1 Absorption, distribution, metabolism, and excretion

Styrene can be absorbed through inhalation, ingestion, or skin contact, but the most important route of exposure in humans in occupational settings is by inhalation, which results in rapid absorption and distribution of approximately 60% to 70% of inhaled styrene; the highest tissue concentrations are in subcutaneous fat. Food is also an important source of exposure for the general population. Metabolic activation of styrene results in formation primarily of the genotoxic metabolite styrene-7,8-oxide, which can be detoxified by glutathione conjugation or conversion to styrene glycol by microsomal epoxide hydrolase. Styrene is metabolized in both the liver and the lung, and the Clara cells in the lung are regarded as the major cell type in styrene activation following inhalation exposure. The initial step in styrene metabolism is catalyzed by cytochromes P450; CYP2E1 and Cyp2f2 are the predominant enzymes in humans and experimental animals. In animals, CYP2E1 predominates in liver, while Cyp2f2 is the primary enzyme in mouse lung. CYP2A13, CYP2F1, CYP2S1, CYP3A5, and CYP4B1 are preferentially expressed in the lung compared with liver in humans, and the human CYP2F1 has been shown to be capable of metabolizing styrene when expressed *in vitro*. Because styrene-7,8-oxide contains a chiral carbon, this and some subsequent styrene metabolites can exist as either *R*- or *S*-enantiomers. A second metabolic pathway through styrene-3,4-oxide results in formation of 4-vinylphenol, which has been detected in humans, rats, and mice *in vivo*, but the importance of 4-vinylphenol in styrene toxicity has not been well characterized. Almost all absorbed styrene is excreted as urinary metabolites, primarily mandelic acid and phenylglyoxylic acid.

5.6.2 Toxicity

Styrene exposure has been associated with numerous health effects in humans and laboratory animals. The acute toxicity of styrene is low to moderate with an oral LD_{50} of 320 mg/kg and an inhalation LC_{50} of 4,940 ppm (4-hour exposure) in mice and an oral LD_{50} of 5,000 mg/kg and an inhalation LC_{50} of 2,770 ppm (2-hour exposure) in rats. The primary effects of acute exposure to styrene in experimental animals and humans include irritation of the skin, eyes, and respiratory tract and CNS effects. Drowsiness, listlessness, muscular weakness, and unsteadiness are common signs of systemic styrene intoxication.

Several studies have reported effects on color vision, hearing threshold, reaction time, and postural stability following long-term occupational exposure to styrene at concentrations ranging from about 20 to 30 ppm. Reports of ischemic heart disease and hepatic, renal, hematological, and immunological effects have been inconsistent. Human data are insufficient to determine whether styrene is a reproductive or developmental toxicant, but effects of styrene to increase serum prolactin levels in humans have been reported.

Styrene toxicity in experimental animals is similar to that reported in humans. Exposure to styrene vapors can cause eye and respiratory tract irritation, CNS depression, and death. Clara cells are the main target of styrene pneumotoxicity, and the available data indicate increased susceptibility in the mouse. Glutathione depletion as a result of styrene exposures has been reported to be associated with damage to lung, liver, and kidney tissues. The cytotoxicity of styrene in the mouse lung, a tissue high in CYP2F isoforms, could be prevented by CYP2F inhibitors. Some studies have reported reproductive and developmental effects, but these effects were seen mostly at doses associated with maternal toxicity. Reported effects have included embryonic, fetal, and neonatal death, skeletal and kidney abnormalities, decreased birth weight, neurobehavioral abnormalities, and postnatal developmental delays. The possibility of polystyrene dimer and trimer extracts from food containers mimicking the physiological effects of estrogen have also been investigated, but with a mixture of positive and negative results.

5.6.3 Interspecies differences in metabolism, toxicity, and toxicokinetics

Species differences exist among rats, mice, and humans in the metabolism and toxicity of styrene, which may be related, at least in part, to interspecies differences in the stereochemistry of metabolism. The *R*-enantiomer, which has been suggested by some reports to be more toxic than the *S*-form, has been reported to be produced in relatively larger amounts in mouse lung than in rat lung, but the difference was less pronounced when microsomal preparations were used. In mice, the *R*-isomer of styrene-7,8-oxide was significantly more hepatotoxic than the *S*-isomer; the toxicity of the *R*-isomer also was greater in the lung, but the difference was not statistically significant.

5.6.4 Genetic and related effects

In vitro studies show that styrene-7,8-oxide forms DNA adducts and causes single-strand breaks in a dose-related manner. Several studies have shown a correlation between single-strand breaks and DNA adducts and indicate that the strand breaks, which are not generally regarded as significantly lethal or mutagenic lesions, are efficiently repaired within several hours after exposure has stopped. Adducts are formed primarily at the N7-, N^2-, and O^6-positions of guanine. N7-adducts are formed in the greatest amount but are the least persistent, while O^6-adducts are formed in the least amount but are the most persistent. Styrene-7,8-oxide was mutagenic without metabolic activation in all *in vitro* mutagenicity test systems reported and caused mutations in some studies in the presence of metabolizing enzymes. Both styrene and styrene-7,8-oxide caused cytogenetic effects (sister chromatid exchange [SCE], chromosomal aberrations, and micronuclei) in human lymphocytes or other mammalian cells *in vitro*. DNA adducts have been detected in liver and lung cells of mice and rats exposed to styrene *in vivo*, although the levels varied across studies. The majority of studies in experimental animals demonstrated an effect of both styrene-7,8-oxide and styrene exposure on single-strand breaks, while both positive and negative results for cytogenetic or clastogenic effects of styrene were reported.

DNA adducts, primarily N7- and O^6-adducts, were reported in white blood cells in all studies of styrene-exposed workers employed mainly in hand-lamination plants. In most studies in workers, single-strand breaks showed exposure-related increases; however, two studies gave negative results. The limited data on mutation frequencies in *HPRT* and *GPA* in styrene-exposed workers are inconclusive. More than half the studies measuring chromosomal aberrations have reported an increase in chromosomal aberrations in styrene-exposed workers (or subgroups of workers), and several studies have reported a positive exposure-response relationship with styrene air levels or urinary metabolites. A meta-analysis of 22 studies found a positive association between styrene exposure level and chromosomal aberration frequency when exposure levels were dichotomized as greater than or less than a threshold value of 30 ppm for an 8-hour time-weighted average. Studies of other cytogenetic markers in humans are conflicting. About half of the studies that evaluated micronucleus and SCE frequency in styrene workers were positive, and a few studies have reported significant dose-response relationships with

styrene exposure. A meta-analysis of 10 micronucleus studies was inconclusive, and a meta-analysis of 14 SCE studies indicated a slight increase in SCE frequency but, again, was too small to be conclusive. A number of studies have been published on the possible modulating role of genetic polymorphisms, mainly in xenobiotic metabolism enzymes and DNA-repair genes, at the level of various biomarkers. Some authors have suggested that genetic susceptibility (probably at many loci) may be important in styrene-mediated genotoxicity.

5.6.5 Mechanistic studies and considerations

The proposed mechanisms for the carcinogenicity of styrene include both genotoxic and epigenetic pathways. These mechanisms, which are not necessarily mutually exclusive, include: (1) metabolic conversion of styrene to styrene-7,8-oxide and subsequent induction of DNA damage in the target tissue and (2) cytotoxic effects of styrene metabolites in the mouse lung. A variety of DNA adducts (including some at base-pairing sites on nucleotides) induced by styrene and styrene-7,8-oxide has been identified in human cells, experimental animals, and occupationally exposed workers, but the covalent binding indices for both molecules are relatively low in rats and mice. The DNA damage induced by styrene exposure, including single-strand breaks, was found to correlate significantly with markers of styrene exposure in some studies of styrene workers. Styrene is mutagenic through the formation of styrene-7,8-oxide (*in vitro*). A number of studies reported a positive association between occupational exposure to styrene and the frequency of chromosomal aberrations, with some studies reporting exposure-response relationships. Some authors have suggested that polymorphisms in DNA-repair genes could put some individuals at higher risk for styrene genotoxicity or carcinogenicity.

Many researchers have tried to explain why lung tumors were observed in mice but not in rats in long-term inhalation exposure studies. Some researchers have proposed that styrene exposure causes pulmonary hyperplasia in the mouse lung, which may play a role in the development of lung tumors. Effects of repeated styrene exposure observed in the lungs of mice, but not in rats, included focal crowding of bronchiolar cells, bronchiolar epithelial hyperplasia, and bronchiolo-alveolar hyperplasia. The Harvard Center for Risk Analysis (Cohen *et al.* 2002) considered three factors as possible explanations for the

greater susceptibility of mouse lung than rat lung to development of hyperplasia leading to tumors with exposure to styrene are: (1) the presence of the styrene-metabolizing cytochromes in mouse lung tissues, (2) greater formation of the *R*-enantiomer of styrene-7,8-oxide, and (3) the susceptibility of mouse lung tissue to glutathione depletion. However, they concluded that although toxicokinetic models generally predict higher rates of metabolism by mice and rats than by humans, the models do not consistently predict a difference between the rodent species. An alternative mechanism is that interspecies differences in styrene toxicity are most likely explained through CYP2F-generated metabolites (2f2 in mice, 2F4 in rats, and 2F1 in humans) in the mouse lung. This is based on data showing that most of the effects of cytotoxicity and tumor formation were seen in mouse respiratory tissues, which are high in CYP2F isoforms, and that CYP2F inhibitors prevented cytotoxicity. Moreover, metabolites formed from ring oxidation, including 4-vinylphenol, are about 6-fold higher in mice compared with rats, and 4-vinylphenol is more potent than styrene-7,8-oxide as a pneumotoxicant.

6 References

1. ACGIH. 2007. *Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices*, Cincinnatti, OH: American Conference of Governmental Industrial Hygienists. p. 52-53, 104-105. (Support not reported. Authors affiliated with the American Conference of Governmental Industrial Hygienists.)

2. Adgate JL, Church TR, Ryan AD, Ramachandran G, Fredrickson AL, Stock TH, Morandi MT, Sexton K. 2004a. Outdoor, indoor, and personal exposure to VOCs in children. *Environ Health Perspect* 112(14): 1386-92. (Supported by the U.S. EPA and the Legislative Commission on Minnesota Resources. Authors affiliated with University of Minnesota, MN; University of Texas, TX.)

3. Adgate JL, Eberly LE, Stroebel C, Pellizzari ED, Sexton K. 2004b. Personal, indoor, and outdoor VOC exposures in a probability sample of children. *J Expo Anal Environ Epidemiol* 14 Suppl 1: S4-S13. (Supported by the Minnesota State Legislature, the U.S. EPA and the consortium of Research Triangle Institute/Environmental and Occupational Health Sciences Institute. Authors affiliated with University of Minnesota, MN; Minnesota Department of Health; Research Triangle Institute, NC.)

4. Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DE, Tice R, Waters MD, Aitio A. 2000. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. *Mutat Res* 463(2): 111-72. (Support not reported. Authors affiliated with University of Vermont, VT; TNO BIBRA International, Ltd., UK; Bureau of Chemical Hazards, Canada; Lund University, Sweden; Karolinska Institute, Sweden; National Cancer Institute, Italy; Leiden University, Netherlands; Finnish Institute of Occupational Health, Finland; University of Leicester, UK; Integrated Laboratory Systems, Inc., NC; U.S. EPA; World Health Organization.)

5. Alexander M. 1997. Environmental fate and effects of styrene. *Crit Rev Environ Scien Tech* 27(4): 383-410. (Support not reported. Authors affiliated with Cornell University, NY.)

6. 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. (Supported by Jofa AB, Malung (Sweden.) Authors affiliated with University of Uppsala, Sweden.)

7. Anttila A, Pukkala E, Riala R, Sallmén M, Hemminki K. 1998. Cancer incidence among Finnish workers exposed to aromatic hydrocarbons. *Int Arch Occup Environ Health* 71(3): 187-193. (Supported by NIEHS and the Finnish Work Environment

Fund. Authors affiliated with Finnish Cancer Registry, Finland; Finnish Institute of Occupational Health, Finland; Karolinska Institute, Sweden.)

8. Anttinen-Klemetti T, Vaaranrinta R, Mutanen P, Peltonen K. 2006. Inhalation exposure to 1,3-butadiene and styrene in styrene-butadiene copolymer production. *Int J Hyg Environ Health* 209(2): 151-8. (Supported by the Finish Work Environment Fund. Authors affiliated with Finnish Institute of Occupational Health, Finland; National Veterinary and Food Research Institute, Finland.)

9. Anwar WA, Shamy MY. 1995. Chromosomal aberrations and micronuclei in reinforced plastics workers exposed to styrene. *Mutat Res* 327(1-2): 41-47. (Support not reported. Authors affiliated with Ain Shams University, Egypt; Alexandria University, Egypt.)

10. Arand M, Müller F, Mecky A, Hinz W, Urban P, Pompon D, Kellner R, Oesch F. 1999. Catalytic triad of microsomal epoxide hydrolase: replacement of Glu^{404} with Asp leads to a strongly increased turnover rate. *Biochem J* 337 (Pt 1): 37-43. (Supported by the Deutsche Forschungsgemeinschaft and the DG XII of the European Community. Authors affiliated with University of Mainz, Germany; CNRS, France.)

11. Arfini G, Mutti A, Vescovi P, Ferroni C, Ferrari M, Giaroli C, Passeri M, Franchini I. 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(10): 826-830. (Supported by the Consiglio Nazionale delle Richerche, Progetto Finalizzato Medicina Preventiva e Riabilitative, Malattie del Sistema Nervoso and the Region Emilia-Romagna. Authors affiliated with the University of Parma, Italy; Occupational Health Service, Italy.)

12. Armstrong BG. 1998. Effect of measurement error on epidemiological studies of environmental and occupational exposures. *Occup Environ Med* 55(10): 651-656. (Support not reported. Authors affiliated with London School of Hygiene and Tropical Medicine, UK.)

13. Arrighi HM, Hertz-Picciotto I. 1994. The evolving concept of the healthy worker survivor effect. *Epidemiology* 5(2): 189-196. (Supported by the National Cancer Institute. Authors affiliated with University of North Carolina, NC; Glaxo Research Institute, NC.)

14. Artuso M, Angotzi G, Bonassi S, Bonatti S, De Ferrari M, Gargano D, Lastrucci L, Miligi L, Sbrana C, Abbondandolo A. 1995. Cytogenetic biomonitoring of styrene-exposed plastic boat builders. *Arch Environ Contam Toxicol* 29(2): 270-274. (Supported by the National Research Council, EEC STEP Project and the Italian Association for Cancer Research. Authors affiliated with Istituto Nazionale per la Ricerca sul Cancro, Italy; Health and Safety of USL, Italy; CNR Institute of Mutagenesis and Differentiation, Italy; Center for the Study and Prevention of Cancer, Italy; University of Genova, Italy.)

15. Ashley DL, Bonin MA, Cardinali FL, McCraw JM, Wooten JV. 1994. Blood concentrations of volatile organic compounds in a nonoccupationally exposed US population and in groups with suspected exposure. *Clin Chem* 40(7 Pt 2): 1401-4. (Supported by the Agency for Toxic Substances and Disease Registry. Authors affiliated with the Centers for Disease Control and Prevention, GA.)

16. ATSDR. 1992. *Toxicological Profile for Styrene*. Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdc.gov/toxprofiles/tp53.pdf.

17. Azuma Y, Nobuhara Y, Date K, Ohno K, Tanaka K, Hirrano S, Kobayashi K, Sakurai T, Chiba M, Yamada T. 2000. Biological evaluation of styrene oligomers for endocrine-disrupting effects (II). *J Food Hyg Soc Japan* 41: 109-115. (Support unknown due to foreign language. Authors affiliated with Nissin Food Products Co., Ltd., Japan.)

18. Bachmann S, Hellwig J, Jackh R, Christian MS. 1998. Uterotrophic assay of two concentrations of migrates from each of 23 polystyrenes administered orally (by gavage) to immature female Wistar rats. *Drug Chem Toxicol* 21 Suppl 1: 1-30. (Support not reported. Authors affiliated with BASF Aktiengesellschaft, Germany; Argus International, Inc., PA; Argus Research Laboratories, PA.)

19. Bakke OM, Scheline RR. 1970. Hydroxylation of aromatic hydrocarbons in the rat. *Toxicol Appl Pharmacol* 16(3): 691-700. (Supported by L. Meltzers Hoyskolefond, Norsk Medisinaldepot and Norges Almenvitenskapelige Forskningsrad. Authors affiliated with University of Bergen, Norway.)

20. Bako-Biro Z, Wargocki P, Weschler CJ, Fanger PO. 2004. Effects of pollution from personal computers on perceived air quality, SBS symptoms and productivity in offices. *Indoor Air* 14(3): 178-87. (Supported by the Danish Technical Research Council. Authors affiliated with Technical University of Denmark, Denmark; Budapest University of Technology and Economics, Hungary; UMDNJ/Robert Wood Johnson Medical School, NJ.)

21. Bakoglu M, Karademir A, Ayberk S. 2004. An evaluation of the occupational health risks to workers in a hazardous waste incinerator. *J Occup Health* 46(2): 156-64. (Support not reported. Authors affiliated with University of Kocaeli, Turkey.)

22. Baldwin RM, Shultz MA, Buckpitt AR. 2005. Bioactivation of the pulmonary toxicants naphthalene and 1-nitronaphthalene by rat CYP2F4. *J Pharmacol Exp Ther* 312(2): 857-65. (Supported by NIEHS and the National Center for Research Resources. Authors affiliated with University of California - Davis, CA.)

23. Barale R. 1991. The genetic toxicology of styrene and styrene oxide. *Mutat Res* 257(2): 107-126. (Support not reported. Authors affiliated with Universita di Ferrara, Italy.)

24. Barlow T, Dipple A. 1998. Aralkylation of guanosine with para-substituted styrene oxides. *Chem Res Toxicol* 11(1): 44-53. (Supported by the National Cancer Institute.

Authors affiliated with NCI-Frederick Cancer Research and Development Center, USA.)

25. Barlow T, Takeshita J, Dipple A. 1998. Deamination and Dimroth rearrangement of deoxyadenosine-styrene oxide adducts in DNA. *Chem Res Toxicol* 11(7): 838-845. (Supported by NCI. Authors affiliated with NCI-Frederick Cancer Research and Development Center, USA.)

26. Barlow T, Dipple A. 1999. Formation of deaminated products in styrene oxide reactions with deoxycytidine. *Chem Res Toxicol* 12(10): 883-6. (Supported by the National Cancer Institute. Authors affiliated with NCI-Frederick Cancer Research and Development Center, USA.)

27. Bastlová T, Vodièka P, Peterková K, Hemminki K, Lambert B. 1995. Styrene oxide-induced HPRT mutations, DNA adducts and DNA strand breaks in cultured human lymphocytes. *Carcinogenesis* 16(10): 2357-2362. (Supported by the Swedish Cancer Society, the Swedish Environmental Protection Board and the EU Environmental Program. Authors affiliated with Karolinska Institute, Sweden; Czech Academy of Sciences, Czech Republic; Regional Institute of Hygiene, Czech Republic.)

28. Bastlová T, Podlutsky A. 1996. Molecular analysis of styrene oxide-induced hprt mutation in human T-lymphocytes. *Mutagenesis* 11(6): 581-591. (Supported by the Swedish Cancer Society, the Swedish Environmental Protection Board and the Swedish Tobacco Co. Research Fund. Authors affiliated with the Karolinska Institute, Sweden; Institute of Theoretical and Experimental Biophysics, Russia.)

29. Batterman S, Chung-Yu P, Braun J. 2002. Levels and composition of volatile organic compounds on commuting routes in Detroit, Michigan. *Atmos Environ* 36: 6015-6030. (Support not reported. Authors affiliated with University of Michigan, MI; Chung Hwa College of Medical Technology, Taiwan; Intel Corp., OR.)

30. Batterman S, Hatzivasilis G, Jia C. 2006. Concentrations and emissions of gasoline and other vapors from residential vehicle garages. *Atmos Environ* 40(10): 1828-1844. (Supported by the American Chemistry Council, Michigan Education and Research Center, and NIOSH. Authors affiliated with University of Michigan, MI.)

31. Batterman S, Jia C, Hatzivasilis G. 2007. Migration of volatile organic compounds from attached garages to residences: a major exposure source. *Environ Res* 104(2): 224-40. (Supported by the American Chemistry Council. Authors affiliated with University of Michigan, MI.)

32. 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): 855-868. (Supported by the Chemical Manufacturers Association Styrene Program Panel. Authors affiliated with Litton Bionetics, Inc., USA; Gulf Oil Corporation, USA; Chemical Manufacturers Association, USA; George Washington University, USA.)

33. 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. (Supported by the U.S. EPA. Authors affiliated with U.S. EPA; University of North Carolina at Chapel Hill, NC.)

34. Bennett LM, Davis BJ. 2002. Identification of mammary carcinogens in rodent bioassays. *Environ Mol Mutagen* 39(2-3): 150-7. (Supported by the U.S. Department of Energy, University of California and Lawrence Livermore National Laboratory. Authors affiliated with Lawrence Livermore National Laboratory, CA; NIEHS, NC.)

35. 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): 753-759. (Supported by the European Commission and ISPESL. Authors affiliated with University of Parma Medical School, Italy.)

36. Bergamaschi E, Smargiassi A, Mutti A, Cavazzini S, Vettori MV, Alinovi R, Franchini I, Mergler D. 1997. Peripheral markers of catecholaminergic dysfunction and symptoms of neurotoxicity among styrene-exposed workers. *Int Arch Occup Environ Health* 69(3): 209-214. (Supported by the Commission of the European Communities and the Quebec FCAR. Authors affiliated with University of Parma, Italy; Universite du Quebec a Montreal, Canada.)

37. Bernardini S, Hirvonen A, Järventaus H, Norppa H. 2002. Influence of *GSTM1* and *GSTT1* genotypes on sister chromatid exchange induction by styrene in cultured human lymphocytes. *Carcinogenesis* 23(5): 893-897. (Supported by the Ministry of Foreign Affairs and by the EU Programme Quality of Life, Management of Living Resources. Authors affiliated with Finnish Institute of Occupational Health, Finland.)

38. 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. (Supported by the Fonds National Suisse de la Recherche Scientifique. Authors affiliated with University of Lausanne, Switzerland.)

39. Berthiaume S, Ring K-L. 2006. *Chemical Economics Handbook Marketing Research Report: Styrene*. 694.3000 A. SRI Consulting. (Support not reported. Authors affiliated with SRI Consulting.)

40. Bi X, Sheng G, Feng Y, Fu J, Xie J. 2005. Gas- and particulate-phase specific tracer and toxic organic compounds in environmental tobacco smoke. *Chemosphere* 61(10): 1512-22. (Supported by the National Scientific Foundation of China and Guangzhou Institite of Geochemistry, Chinese Academy of Sciences. Authors affiliated with Chinese Academy of Science, China; Shanghai University, China.)

41. Bieche I, Narjoz C, Asselah T, Vacher S, Marcellin P, Lidereau R, Beaune P, de Waziers I. 2007. Reverse transcriptase-PCR quantification of mRNA levels from cytochrome (CYP)1, CYP2 and CYP3 families in 22 different human tissues.

Pharmacogenet Genomics 17(9): 731-42. (Supported by the Ligue Nationale contre le Cancer and its Comite Regional de Hauts-de-Seine and the Canceropole of ile-de-France. Authors affiliated with INSERM; Universite Paris Descartes, France; Hopital Europeen Georges Pompidou, France; Hopital Beaujon, France; Centre Rene Huguenin.)

42. Bigbee WL, Grant SG, Langlois RG, Jensen RH, Anttila A, Pfäffli P, Pekari K, Norppa H. 1996. Glycophorin A somatic cell mutation frequencies in Finnish reinforced plastics workers exposed to styrene. *Cancer Epidemiol Biomarkers Prev* 5(10): 801-810. (Support not reported. Authors affiliated with University of pittsburgh Cancer Institute, PA; Lawrence Livermore National Laboratory, CA; University of California, CA; Institute of Occupational Health, Finland.)

43. Biró A, Pállinger 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): 133-140. (Supported by the Hungarian Scientific Research Fund and the Fodor Jozsef National Center for Public Health. Authors affiliated with National Institute of Chemical Safety, Hungary; Molecular Immunological Research Group of Hungarian Academy of Sciences, Hungary; Semmelweis University, Hungary.)

44. 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. (Supported by the Research Council of Norway and NIH. Authors affiliated with National Institute of Public Health, Norway; The National Hospital, Norway.)

45. Block JB (1976). A Kentucky study: 1950-1975, Proceedings of the NIOSH Styrene-Butadiene Briefing, Covington, Kentucky, April 30, 1976. (As cited by IARC.)

46. Bloomfield CD, Lindquist LL, Brunning RD, Yunis JJ, Coccia PF. 1978. The Philadelphia chromosome in acute leukemia. *Virchows Arch B Cell Pathol* 29(1-2): 81-91. (Supported by the National Cancer Institute, the Masonic Hospital Fund, Inc., and the Minnesota Medical Foundation. Authors affiliated with University of Minnesota, MN.)

47. BLS. 2007. *Occupational Employment and Wages, May 2006. Fiberglass Laminators and Fabricators*. U.S. Department of Labor, Bureau of Labor Statistics. Last updated 10/24/07. http://www.bls.gov/oes/current/oes512091.htm. Last accessed 11/29/07.

48. Boers JE, Ambergen AW, Thunnissen FB. 1999. Number and proliferation of clara cells in normal human airway epithelium. *Am J Respir Crit Care Med* 159(5 Pt 1): 1585-91. (Support not reported. Authors affiliated with Maastricht University, Netherlands.)

49. Bogen KT, Benson JM, Yost GS, Morris JB, Dahl AR, Clewell HJ, Krishnan K, Omiecinski CJ. 2008. Naphthalene metabolism in relation to target tissue anatomy, physiology, cytotoxicity, and tumorigenic mechanism of action. *Regul Toxicol Pharmacol* 51: S27-S36. (Supported by the U.S. EPA, the Electric Power Research Institute, the American Petroleum Institute, the Naphthalene Council, Inc., the Association of Railroads, the American Coke and Coal Chemicals Institute, the National Petrochemical Refiners Association and Regulatory Checkbook. Authors affiliated with Exponent Health and Environmental, CA; Lovelace Respiratory Research Institutem, USA; University of Utah, UT; University of Connecticut School of Pharmacy, CT; Ohio State University Comprehensive Cancer Center, OH; CIIT Centers for Health Research, USA; University of Montreal, Canada; Pennsylvania State Unviversity, PA.)

50. 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: 217-225. (Supported by the Italian Association for Cancer Research and the EEC. Authors affiliated with Istituto Nazionale per la Ricerca sul Cancro and CSTA, Italy.)

51. 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(3): 145-154. (Support not reported. Authors affiliated with Dow Chemical Company, MI.)

52. Bond JA. 1989. Review of the toxicology of styrene. *CRC Crit Rev Toxicol* 19(3): 227-249. (Supported by the U.S. Department of Energy and NIEHS. Authors affiliated with Lovelace Biomedical and Environmental Research Institute, NM; Institute of Occupational Health, Germany.)

53. Boogaard PJ, de Kloe KP, Sumner SC, 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(1): 161-172. (Supported by the Styrene Information and Research Center. Authors affiliated with Shell International Chemicals, Netherlands; Chemical Industry Institute of Toxicology, NC.)

54. Boogaard PJ, de Kloe KP, Wong BA, Sumner SC, Watson WP, van Sittert NJ. 2000b. Quantification 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(2): 203-16. (Supported by the Styrene Information and Research Center. Authors affiliated with Shell International Chemicals, Netherlands; Chemical Industry Institute of Toxicology, NC.)

55. Brenner DD, Jeffrey AM, Latriano L, Wazneh L, Warburton D, Toor M, Pero RW, Andrews LR, Walles S, Perera FP. 1991. Biomarkers in styrene-exposed boatbuilders. *Mutat Res* 261(3): 225-236. (Supported by NIH and NIEHS. Authors affiliated with Columbia University School of Public Health, NY; Conte Institute for Environmental Health, MA; Columbia Presbyterian Medical Center, NY;

National Institute of Occupational Health, Sweden; New York University Medical Center, NY.)

56. Brooks SM, Anderson L, Emmett E, Carson A, Tsay JY, Elia V, Buncher R, Karbowsky R. 1980. The effects of protective equipment on styrene exposure in workers in the reinforced plastics industry. *Arch Environ Health* 35(5): 287-94. (Supported by NIEHS, the Center for the Study of the Human Environment, and the Society of Plastics Industry. Authors affiliated with University of Cincinnati, OH.)

57. Brown HS. 1985. Estimation of human daily dose of volatile organic contaminants in drinking water. In *Chemical Safety and Regulations Compliance, Proceedings Course. Meeting Date, 1983.* Homburger F, Marquis JK, eds. Switzerland: Karger. p. 42-49. (Support and author affiliations not reported.)

58. Brown NA, Lamb JC, Brown SM, Neal BH. 2000. A review of the developmental and reproductive toxicity of styrene. *Regul Toxicol Pharmacol* 32(3): 228-247. (Support not reported. Authors affiliated with University of London, UK; Bouk & Lee, Inc., VA; Jellinek, Schwartz, and Connolly, Inc., VA.)

59. 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): 107-113. (Supported by the U.S. National Cancer Institute and the American Cancer Society. Authors affiliated with the American Health Foundation, NY.)

60. Buschini A, De Palma G, Poli P, Martino A, Rossi C, Mozzoni P, Scotti E, Buzio L, Bergamaschi E, Mutti A. 2003. Genetic polymorphism of drug-metabolizing enzymes and styrene-induced DNA damage. *Environ Mol Mutagen* 41(4): 243-252. (Supported by the Italian Ministry of Health, ISPESL. Authors affiliated with University of Parma, Italy.)

61. Byfält Nordqvist M, Löf 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. (Supported by the Swedish Work Environment Fund and the Swedish Board of Natural Protection. Authors affiliated with National Board of Occupational Safety and Health, Sweden; Wallenberg Laboratory, Sweden.)

62. 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. (Support not reported. Authors affiliated with USL, Italy.)

63. 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. (Support not reported. Authors affiliated with USL, Italy.)

64. 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): 336-348. (Support not reported. Authors affiliated with National Cancer Institute, MD.)

65. Cantoreggi S, Lutz WK. 1992. Investigation of the covalent binding of styrene-7,8-oxide to DNA in rat and mouse. *Carcinogenesis* 13(2): 193-197. (Supported by the European Chemical Industry Ecology and Toxicology Center. Authors affiliated with Swiss Federal Institute of Technology, Switzerland; University of Zurich, Switzerland.)

66. Cantoreggi S, Lutz WK. 1993. Covalent binding of styrene to DNA in rat and mouse. *Carcinogenesis* 14(3): 355-360. (Supported by the European Chemical Industry Ecology and Toxicology Center. Authors affiliated with Swiss Federal Institute of Technology, Switzerland; University of Zurich, Switzerland.)

67. Carlson G. 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. (Supported by the Styrene Information and Research Center. Authors affiliated with Purdue University, IN.)

68. Carlson G. 2004b. Influence of selected inhibitors on the metabolism of the styrene metabolite 4-vinylphenol in wild-type and CYP2E1 knockout mice. *J Toxicol Environ Health A* 67(12): 905-9. (Supported by the Styrene Research and Information Center. Authors affiliated with Purdue University, IN.)

69. Carlson GP. 1997b. Effects of inducers and inhibitors on the microsomal metabolism of styrene to styrene oxide in mice. *J Toxicol Environ Health* 51(5): 477-488. (Supported by NIH and the Styrene Information and Research Center. Authors affiliated with Purdue University, IN.)

70. 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. (Supported by NIH and the Styrene Information and Research Center. Authors affiliated with Purdue University, IN.)

71. Carlson GP, Mantick NA, Powley MW. 2000. Metabolism of styrene by human liver and lung. *J Toxicol Environ Health A* 59(8): 591-5. (Supported by NIOSH, the Styrene Information and Research Center and the U.S. EPA. Authors affiliated with Purdue University, IN.)

72. Carlson GP, Perez Rivera AA, Mantick NA. 2001. Metabolism of the styrene metabolite 4-vinylphenol by rat and mouse liver and lung. *J Toxicol Environ Health A* 63(7): 541-551. (Supported by the Styrene Information and Research Center. Authors affiliated with Purdue University, IN.)

73. Carlson GP. 2002. Effect of the inhibition of the metabolism of 4-vinylphenol on its hepatotoxicity and pneumotoxicity in rats and mice. *Toxicology* 179(1-2): 129-136. (Supported by the Styrene Information and Research Center. Authors affiliated with Purdue University, IN.)

74. Carlson GP. 2004a. Comparison of the susceptibility of wild-type and CYP2E1 knockout mice to the hepatotoxic and pneumotoxic effects of styrene and styrene oxide. *Toxicol Lett* 150(3): 335-339. (Supported by the Styrene Information and Research Center. Authors affiliated with Purdue University, IN.)

75. CDC. 2007. *In-Depth Survey Report: Styrene Exposures During Fiber Reinforced Plastic Boat Manufacturing*. EPHB 306-17a. Cincinnati, OH: U.S. Department of Health and Human Services. 32 pp.

76. Chakrabarti S, Duhr MA, Senécal-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. (Supported by the Institut de recherche en sante et securite du travail du Quebec. Authors affiliated with Universite de Montreal, Canada.)

77. Chakrabarti S, Zhang XX, Richer CL. 1997. Influence of duration of exposure to styrene oxide on sister chromatid exchanges and cell-cycle kinetics in cultured human blood lymphocytes in vitro. *Mutat Res* 395(1): 37-45. (Supported by the Institut de recherche en sante et securite du travail du Quebec. Authors affiliated with Universite de Montreal, Canada.)

78. Chan CC, Shie RH, Chang TY, Tsai DH. 2006. Workers' exposures and potential health risks to air toxics in a petrochemical complex assessed by improved methodology. *Int Arch Occup Environ Health* 79(2): 135-42. (Supported by the Taiwan Environmental Agency. Authors affiliated with National Taiwan University, Taiwan; Industrial Technology Research Institute, Taiwan.)

79. Chan PC, Hasemani JK, Mahleri J, Aranyi C. 1998. Tumor induction in F344/N rats and B6C3F1 mice following inhalation exposure to ethylbenzene. *Toxicol Lett* 99(1): 23-32. (Support not reported. Authors affiliated with NIEHS, NC; IIT Research Institute, IL.)

80. Charles SM, Batterman S, Jia C. 2007. Composition and emissions of VOCs in main- and side-stream smoke of research cigarettes. *Atmos Environ* 41(26): 5371-5384. (Supported by the University of Michigan Tobacco Research Center. Authors affiliated with University of Michigan, MI.)

81. ChemIDPlus. 2008a. *Styrene*. National Library of Medicine. http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp and (search "styrene"). Last accessed: 7/13/04.

82. ChemIDPlus. 2008b. *Styrene-7,8-oxide*. National Library of Medicine. http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp and (search "styrene oxide"). Last accessed: 7/13/04.

83. ChemIDPlus. 2008c. *Ethyl Benzene*. National Library of Medicine. http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp and (search "ethyl benzene"). Accessed on 9/18/08.

84. ChemIDPlus. 2008d. *1-Phenylethanol*. National Library of Medicine. http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp and (search "1-phenylethanol"). Accessed on 9/18/08.

85. ChemIDPlus. 2008e. *3-Methylstyrene*. National Library of Medicine. http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp and (search "3-methylstyrene"). Accessed on 9/18/08.

86. ChemIDPlus. 2008f. *4-Methylstyrene*. National Library of Medicine. http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp and (search "4-methylstyrene"). Accessed on 9/18/08.

87. Cheresources. 2008a. *Basics of Polystyrene Production*. Chemical Engineer's Resource Page. http://www.cheresources.com/polystyzz.shtml. Accessed on 5/13/08.

88. Cheresources. 2008b. *Styrene Monomer Production (Dow Process)*. Chemical Engineer's Resource Page. http://www.cheresources.com/polystymonzz.shtml. Accessed on 9/18/08.

89. Chmielewski J. 1976. Clinical and experimental research into the pathogenesis of toxic effect of styrene. V. Impact of styrene on carbohydrate balance in people in the course of their work. *Bull Inst Marit Trop Med Gdynia* 27(2): 177-184. (Support not reported. Authors affiliated with the Institute of Maritime and Tropical Medicine in Gydnia.)

90. Choi JO, Jitsunari F, Asakawa F, Sun Lee D. 2005. Migration of styrene monomer, dimers and trimers from polystyrene to food simulants. *Food Addit Contam* 22(7): 693-9. (Supported by the Korea Food and Drug Administration and the Ministry of Education, Korea. Authors affiliated with Kyungnam University, Korea; Kagawa University.)

91. Christakopoulos A, Bergmark E, Zorcec V, Norppa H, Mäki-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): 255-263. (Supported by the Swedish Work Environment Fund, Swedish Cancer Society, Ecetoc Task Force on Styrene and Shell International Research. Authors affiliated with Stockholm University, Sweden; Institute of Occupational Health, Finland; National Public Health Institute, Finland; Reserca, Sweden.)

92. Chung JK, Yuan W, Liu G, Zheng J. 2006. Investigation of bioactivation and toxicity of styrene in CYP2E1 transgenic cells. *Toxicology* 226(2-3): 99-106. (Supported by NIH. Authors affiliated with Northeastern University, MA; University of Washington, WA.)

93. Clay P. 2004. Styrene monomer does not induce unscheduled DNA synthesis in the mouse liver following inhalation exposure. *Mutagenesis* 19(6): 489-92. (Supported

by CEFIC Styrenics Steering Committee. Authors affiliated with Syngenta CTL, UK.)

94. 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. (Supported by the European Union, National Research Council and the Ministry of University and Scientific Research, Italy. Authors affiliated with University of Pavia, Italy; IRCCS Salvatore Maugeri Foundation, Italy.)

95. 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(2): 94-99. (Support not reported. Authors affiliated with University of Southhampton, UK.)

96. Cohen JT, Carlson G, Charnley G, Coggon D, Delzell E, Graham JD, Greim H, Krewski D, Medinsky M, Monson R, Paustenbach D, Petersen B, Rappaport S, Rhomberg L, Ryan PB, Thompson K. 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): 1-263. (Supported by the Styrene Information and Research Center. Authors affiliated with Harvard School of Public Health, MA; Purdue University, IN; Health Risk Strategies, Washington, D.C.; University of Southampton, UK; University of Alabama at Birmingham, AL; GSF-Institute of Toxicology, Germany; University of Ottawa, Canada; Exponent, CA; Novigen Sciences, Inc., Washington, D.C.; University of North Carolina, NC; Gradent Corporation, MA; Rollins School of Public Health of Emory University, GA.)

97. Collins AR, Dobson VL, Dušinská M, Kennedy G, Štetina R. 1997. The comet assay: what can it really tell us? *Mutat Res* 375(2): 183-193. (Supported by the EC Concerted Action on DNA Repair and Cancer, the Royal Society Collaborative, and the Scottish Office Agriculture and Fisheries Department. Authors affiliated with Rowett Research Institute, UK; Institute of Preventative and Clinical Medicine, Slovak Republic; University of Ulster at Coleraine, UK; Academy of Sciences of the Czech Republic, Czech Republic.)

98. Collins AR. 1998. Molecular epidemiology in cancer research. *Mol Aspects Med* 19(6): 359-432. (Support not reported. Authors affiliated with Rowett Research Institute, UK.)

99. 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. In *Living in a Chemical World*, Annals of the New York Academy of Sciences, vol. 534. Maltoni C, Selikoff IJ, eds. New York, NY: New York Academy of Sciences.

pp. 203-234. (Support not reported. Authors affiliated with Institute of Oncology "F. Addarii", Italy.)

100. Coyle YM, Hynan LS, Euhus DM, Minhajuddin AT. 2005. An ecological study of the association of environmental chemicals on breast cancer incidence in Texas. *Breast Cancer Res Treat* 92(2): 107-14. (Supported by the Natalie Ornish Fund, the Clay Weed Memorial Trust Fund, and CREW, Dallas. Authors affiliated with University of Texas Southwestern Medical Center, TX.)

101. Crandall MS. 1981. Worker exposure to styrene monomer in the reinforced plastic boat-making industry. *Am Ind Hyg Assoc J* 42(7): 499-502. (Support not reported. Authors affiliated with NIOSH.)

102. Cruzan G, Cushman JR, Andrews LS, Granville GC, Miller RR, Hardy CJ, Coombs DW, Mullins PA. 1997. Subchronic inhalation studies of styrene in CD rats and CD-1 mice. *Fundam Appl Toxicol* 35(2): 152-165. (Supported by the Styrene Information and Research Center. Authors affiliated with ToxWorks, NJ; Chevron Research and Technology, CA; ARCO Chemical Company, PA; Shell Canada Ltd., Canada; Dow Chemical Company, MI; Huntingdon Research Center, UK.)

103. 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): 266-281. (Supported by Styrene Information and Research Center. Authors affiliated with ToxWorks, NJ; Chevron Research and Technology, CA; ARCO Chemical Company, PA; Shell Canada Ltd., Canada; Dow Chemical Company, MI; Huntingdon Research Center, UK; Research Pathology Services, PA.)

104. Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Bevan C, Hardy CJ, Coombs DW, Mullins PA, Brown WR. 2001. Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. *J Appl Toxicol* 21(3): 185-198. (Supported by the Styrene Information and Research Center. Authors affiliated with ToxWorks, NJ; Chevron Research and Technology, CA; Rohm & Hass Company, PA; Shell Canada Ltd., Canada; Dow Chemical Company, MI; Huntingdon Research Center, UK; BP Amoco Chemical Co., IL; Research Pathology Services, PA.)

105. 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): 308-319. (Supported by the Styrene Information and Research Center and SIRC. Authors affiliated with ToxWorks, NJ; Purdue University, IL; Dow Chemical Company, MI; Rohm & Haas Chemical Company, PA; Lyondell Chemical Company, TX; BP Amoco Chemical Company, IL; Chevron Research and Technology Company, CA.)

106. Cruzan G, Carlson GP, Turner M, Mellert W. 2005a. Ring-oxidized metabolites of styrene contribute to styrene-induced Clara-cell toxicity in mice. *J Toxicol Environ*

Health A 68(3): 229-237. (Supported by the Styrene Information and Research Center. Authors affiliated with ToxWorks, NJ; Purdue University, IN; BASFAktiengesellschaft, Germany.)

107. Csanady GA, Mendrala AL, Nolan RJ, Filser JG. 1994. A physiological pharmacokinetic model for styrene and styrene-7,8-oxide in mouse, rat and man. *Arch Toxicol* 68(3): 143-157. (Supported by the European Center for Ecotoxicology and Toxicology of Chemicals. Authors affiliated with GSF-Institute for Toxicology, Germany; Dow Chemical Company, MI.)

108. Csanády 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. (Supported by the Styrene Steering Committee of the European Chemical Industry Council. Authors affiliated with GSF-Institute of Toxicology, Germany; Technische Universitat Munchen, Germany; BASF AG, Germany.)

109. Dalton P, Cowart B, Dilks D, Gould M, Lees PS, Stefaniak A, Emmett E. 2003. Olfactory function in workers exposed to styrene in the reinforced-plastics industry. *Am J Ind Med* 44(1): 1-11. (Supported by the Styrene Information and Research Center and NIH. Authors affiliated with Monell Chemical Senses Center, PA; Johns Hopkins University, MD; University of Pennsylvnia, PA.)

110. Date K, Ohno K, Azuma Y, Hirano S, Kobayashi K, Sakurai T, Nobuhara Y, Yamada T. 2002. Endocrine-disrupting effects of styrene oligomers that migrated from polystyrene containers into food. *Food Chem Toxicol* 40(1): 65-75. (Support not reported. Authors affiliated with Nissin Food Products Co., Ltd., Japan.)

111. De Palma G, Manini P, Mozzoni P, Andreoli R, Bergamaschi E, Cavazzini S, Franchini I, Mutti A. 2001. Polymorphism of xenobiotic-metabolizing enzymes and excretion of styrene-specific mercapturic acids. *Chem Res Toxicol* 14(10): 1393-1400. (Supported by the European Commission. Authors affiliated with University of Parma, Italy.)

112. De Piceis Polver P, Fenoglio C, Nano R, Coccini T, Bertone V, Vaccarone R, Gerzeli G. 2003. Styrene hepatotoxicity in rats treated by inhalation or intraperitoneally: a structural investigation. *Histol Histopathol* 18(1): 49-54. (Supported by the Ministry of the University and Scientific Research, Italy. Authors affiliated with University of Pavia, Italy; IRCCS S. Maugeri Foundation, Italy.)

113. 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): 163-170. (Support not reported. Authors affiliated with Centraal Laboratorium, Netherlands.)

114. Delbressine LP, Van Bladeren PJ, Smeets FL, Seutter-Berlage F. 1981. Stereoselective oxidation of styrene to styrene oxide in rats as measured by mercapturic acid excretion. *Xenobiotica* 11(9): 589-594. (Support not reported.

Authors affiliated with University of Nijmegen, Netherlands; University of Leiden, Netherlands.)

115. 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): 182-189. (Supported by the International Institute of Synthetic Rubber Producers. Authors affiliated with University of Alabama at Birmingham, AL; McMaster University, Canada; Texas A&M University, TX.)

116. 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: 515-534. (Supported by the University of Alabama at Birmingham and the International Institute of Synthetic Rubber Producers. Authors affiliated with University of Alabama at Birmingham, AL.)

117. Delzell E, Sathiakumar N, Graff J, Matthews R. 2005. Styrene and ischemic heart disease mortality among synthetic rubber industry workers. *J Occup Environ Med* 47(12): 1235-43. (Supported by the Styrene Information Research Center. Authors affiliated with University of Alabama at Birmingham, AL; Wayne State University, MI.)

118. 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. *Res Rep Health Eff Inst*(132): 1-63; discussion 65-74. (Supported by U.S. EPA. Authors affiliated with University of Alabama at Birmingham, AL.)

119. Ding X, Kaminsky LS. 2003. Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu Rev Pharmacol Toxicol* 43: 149-73. (Supported by NIH, U.S. EPA and NIEHS. Authors affiliated with State University of New York, NY.)

120. Diodovich C, Bianchi MG, Bowe G, Acquati F, Taramelli R, Parent-Massin D, Gribaldo L. 2004. Response of human cord blood cells to styrene exposure: evaluation of its effects on apoptosis and gene expression by genomic technology. *Toxicology* 200(2-3): 145-157. (Support not reported. Authors affiliated with ECVAM Institute for Health and Consumer Protection, Italy; Universita del Insubria, Italy; Universite de Bretagne Occidentale, France.)

121. Dolmierski R, Szczepanik M, Danielewicz-Garbalinska G, Kunikowska D, Mickiewicz W, Chomicz M, Glosnicka R. 1983. Mutagenic action of styrene and its metabolites. 1. Chromosome aberration in persons exposed to the action of styrene. Introductory investigations. *Bull Inst Marit Trop Med Gdynia* 34(1-2): 89-93. (Support not reported. Authors affiliated with the Institute of Maritime and Tropical Medicine in Gdynia.)

122. Drummond L, Caldwell J, Wilson HK. 1989. The metabolism of ethylbenzene and styrene to mandelic acid: stereochemical considerations. *Xenobiotica* 19(2): 199-207. (Support not reported. Authors affiliated with Occupational Medicine and Hygiene Laboratories, UK; St. Mary's Hospital Medical School, UK.)

123. Dumas S, Parent ME, Siemiatycki J, Brisson J. 2000. Rectal cancer and occupational risk factors: A hypothesis-generating, exposure-based case-control study. *Int J Cancer* 87(6): 874-879. (Support not reported. Authors affiliated with INRS-Institut Armand-Frappier, Canada; Universite Laval, Canada; McGill University, Canada.)

124. Dunnick JK, Elwell MR, Huff J, Barrett JC. 1995. Chemically induced mammary gland cancer in the National Toxicology Program's carcinogenesis bioassay. *Carcinogenesis* 16(2): 173-9. (Support not reported. Authors affiliated with NIEHS, NC.)

125. Dutkiewicz T, Tyras H. 1968. Skin absorption of toluene, styrene, and xylene by man. *Br J Ind Med* 25(3): 243. (Support not reported. Authors affiliated with Medical Academy, Poland.)

126. Dypbukt JM, Costa LG, Manzo L, Orrenius S, Nicotera P. 1992. Cytotoxic and genotoxic effects of styrene-7,8-oxide in neuroadrenergic Pc 12 cells. *Carcinogenesis* 13(3): 417-424. (Supported by CFN and Fondazione Clinica del Lavero, IRCCS, Italy. Authors affiliated with Karolinska Institute, Sweden; University of Washington, WA; Department of Internal Medicine, Italy; IRCCS, Italy.)

127. Eitaki Y, Kawai T, Kishi R, Sakurai H, Ikeda M. 2008. Stability in Urine of Authentic Phenylglyoxylic and Mandelic Acids as Urinary Markers of Occupational Exposure to Styrene. *J Occup Health* Pre-publication. (Support not reported. Authors affiliated with Japan Industrial Safety and Health Association, Japan; Hokkaido University Graduate School of Medicine, Japan; Kyoto Industrial Health Associatio, Japan.)

128. Elia VJ, Anderson LA, Macdonald TJ, Carson A, Buncher CR, Brooks SM. 1980. Determination of urinary mandelic and phenylglyoxylic acids in styrene exposed workers and a control population. *Am Ind Hyg Assoc J* 41(12): 922-6. (Supported by the Society of Plastics Industries and NIEHS. Authors affiliated with University of Cincinnati College of Medicine, OH.)

129. Engelhardt G, Gamer A, Vodicka P, Bárta 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. (Support not reported. Authors affilitated with BASF Aktiengesellschaft, Germany; Academy of Sciences of the Czech Republic, Czech Republic; Charles University, Czech Republic.)

130. Engström J. 1978. Styrene in subcutaneous adipose tissue after experimental and industrial exposure. *Scand J Work Environ Health* 4(Suppl 2): 119-120. (Support

not reported. Authors affiliated with National Board of Occupational Safety and Health, Sweden.)

131. EPA. 1997a. *Summary of Findings from the Boat Manufacturing Presumptive MACT Process: Styrene Emission Control Options*. 8198-30-09. U.S. Environmental Protection Agency. 53 pp.

132. EPA. 1997b. *Air Emissions from Scrap Tire Combustion*. Research Triangle Park, NC: U.S. Environmental Protection Agency. 117 pp. http://www.epa.gov/ttn/catc/dir1/tire_eng.pdf.

133. EPA. 2003. National emissions standards for hazardous air pollutants: reinforced plastic composite production. *Fed Register* 68(76): 19375-19443.

134. EPA. 2007. *Regulatory Impact Analysis. Control of Hazardous Air Pollutants from Mobile Sources. Chapter 2, Emission Inventories*. EPA420-R-07-002. U.S. Environmental Protection Agency. 115 pp.

135. EPA. 2008a. *Monitor Values Report - Hazardous Air Pollutants: Styrene*. U.S. Environmental Protection Agency. http://www.epa.gov/air/data. Accessed on 2/15/08.

136. EPA. 2008b. *Indoor Environment Management. Pollution Prevention*. U.S. Environmental Protection Agency. http://www.epa.gov/appcdwww/iemb/pollprev.htm. Accessed on 5/14/08.

137. Eriksson K, Wiklund L. 2004. Dermal exposure to styrene in the fibreglass reinforced plastics industry. *Ann Occup Hyg* 48(3): 203-208. (Supported by the EU, the National Institute for Working Life, Sweden, and the University Hospital of Northern Sweden. Authors affiliated with University Hospital of Northern Sweden, Sweden; National Institute for Working Life, Sweden; Alcontrol Laboratories, Sweden.)

138. EU. 2002. *European Union Risk Assessment Report. Styrene. Part I - Environment*. EUR 20541 EN. Luxembourg: European Communites.

139. Everhart J, Wright D. 1995. Diabetes mellitus as a risk factor for pancreatic cancer. A meta-analysis. *Jama* 273(20): 1605-9. (Support not reported. Authors affiliated with National Institute of Diabetes and Digestive and Kidney Diseases, MD.)

140. Fabry L, Léonard A, Roberfroid M. 1978. Mutagenicity tests with styrene oxide in mammals. *Mutat Res* 51(3): 377-381. (Supported by the "Fonds de la Recherche Fondamentale Collective" and the Belgian Nuclear Energy Study Center. Authors affiliated with CEN-SFK, Belgium; University of Louvain, Belgium.)

141. Fail PA, Hines JW, Zacharewski T, Wu ZF, Borodinsky L. 1998. Assessment of polystyrene extract for estrogenic activity in the rat uterotrophic model and an in vitro recombinant receptor reporter gene assay. *Drug Chem Toxicol* 21 Suppl 1:

101-21. (Supported by the Polystyrene Work Group of the Society of the Plastics Industry, Inc.'s Food, Drug and Cosmetic Materials Packaging Committee. Authors affiliated with Research Triangle Institute, NC; Michigan State University, MI; Keller and Heckman, LLP, Washington, D.C.)

142. Fairfax RE, Swearngin S. 2005. Styrene overexposure in a bathtub manufacturing facility. *J Occup Environ Health* 2(11): D82-D86. (Support not reported. Authors affiliated with U.S. Department of Labor.)

143. FDA. 2006. *U.S. Food and Drug Administration - Total Diet Study. Market Baskets 1991-3 and 2003-4*. College Park, MD: U.S. Food and Drug Administraton. 127 pp. http://www.cfsan.fda.gov/~comm/tds-res.html.

144. Feng B, Zhou L, Passarelli M, Harris CM, Harris TM, Stone MP. 1995. Major groove (R)-alpha-(N6-adenyl)styrene oxide adducts in an oligodeoxynucleotide containing the human N-ras codon 61 sequence: conformations of the R(61,2) and R(61,3) sequence isomers from 1H NMR. *Biochemistry* 34(43): 14021-36. (Supported by NIH and the Vanderbilt Center in Molecular Toxicology. Authors affiliated with Vanderbilt University, TN; Memorial Sloan-Kettring Cancer Center, NY; Georgetown University, MD; University of Maine at Farmington, ME.)

145. Feng B, Voehler M, Zhou L, Passarelli M, Harris CM, Harris TM, Stone MP. 1996. Major groove (S)-alpha-(N6-adenyl)styrene oxide adducts in an oligodeoxynucleotide containing the human N-ras codon 61 sequence: conformations of the S(61,2) and S(61,3) sequence isomers from 1H NMR. *Biochemistry* 35(23): 7316-29. (Supported by NIH, Vanderbilt Center for Molecular Toxicology, University of Wisconsin and USDA. Authors affiliated with Vanderbilt University, TN; Memorial Sloan-Kettering Cancer Center, NY; Georgetown University, MD; University of Maine at Farmington, ME.)

146. Filser JG, Kessler W, Csanády GA. 2002. Estimation of a possible tumorigenic risk of styrene from daily intake via food and ambient air. *Toxicol Lett* 126(1): 1-18. (Supported by the Styrene Steering Committee of the European Center for Ecotoxicology and Toxicology of Chemicals. Authors affiliated with GSF Institute of Toxicology, Germany.)

147. Fishbein L. 1992. Exposure from occupational versus other sources. *Scand J Work Environ Health* 18(Suppl 1): 5-16. (Support not reported. Authors affiliated with Risk Science Institute, Washington, D.C; U.S. FDA.)

148. 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. (Support not reported. Authors affiliated with BASF Aktiengesellschaft, Germany.)

149. Flodin U, Fredriksson M, Persson B, Hardell L, Axelson O. 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. (Supported by

the Swedish Work Environment Fund. Authors affiliated with University Hospital, Sweden; Region Hospital, Sweden.)

150. Forni A, Goggi E, Ortisi E, Cacchetti R, Cortona G, Sesana G, Alessio L. 1988. Cytogenic findings in styrene workers in relation to exposure. In *Enviromental Hygiene*. Seemayer NH, Hadnagy W, eds. Berlin, Germany: Springer. p. 159-162. (Supported by the Ministry of Education. Authors affiliated with University of Milan, Italy; Unit of Occupational Health, Italy; Desio Hospital Italy; University of Brescia, Italy.)

151. 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): 231-239. (Support not reported. Authors affiliated with Deutsches Krebsforschungszentrum, Germany; BASF Aktiengesellschaft, Germany.)

152. Fuente A, McPherson B. 2006. Organic solvents and hearing loss: The challenge for audiology. *Int J Audiol* 45(7): 367-81. (Support not reported. Authors affiliated with University of Hong Kong, China.)

153. Fukami T, Katoh M, Yamazaki H, Yokoi T, Nakajima M. 2008. Human cytochrome P450 2A13 efficiently metabolizes chemicals in air pollutants: naphthalene, styrene, and toluene. *Chem Res Toxicol* 21(3): 720-5. (Supported by the Smoking Research Foundation and the Japan Society for the Promotion of Science. Authors affiliated with Kanazawa University, Japan; Shawa Pharmaceutical University, Japan.)

154. Fustinoni S, Colosio C, Colombi A, Lastrucci L, Yeowell-O'Connell K, Rappaport SM. 1998. Albumin and hemoglobin adducts as biomarkers of exposure to styrene in fiberglass-reinforced-plastics workers. *Int Arch Occup Environ Health* 71(1): 35-41. (Support not reported. Authors affiliated with Istituti Clinici di Perfezionamento, Italy; Universita degli Studi di Milano, Italy; Medicina del Lavoro di Pietrasanta, Italy; University of North Carolina, NC.)

155. Gadberry MG, DeNicola DB, Carlson GP. 1996. Pneumotoxicity and hepatotoxicity of styrene and styrene oxide. *J Toxicol Environ Health* 48(3): 273-294. (Supported by Purdue University, the AFPE, NIH and the Styrene Information and Research Center. Authors affiliated with Purdue University, IN.)

156. 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. (Support not reported. Authors affiliated with Institut National de Recherche et de Sécurité, France.)

157. Galassi C, Kogevinas M, Ferro G, Biocca M. 1993. Biological monitoring of styrene in the reinforced plastics industry in Emilia Romagna, Italy. *Int Arch Occup Environ Health* 65(2): 89-95. (Supported by the European Science Foundation. Authors affiliated with Local Health Unit of Scandiano, Italy; IARC, France; SEDI, Italy.)

158. 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): 1655-1667. (Support not reported. Authors affiliated with BASF Aktiengesellschaft, Germany; Experimental Toxicology Services, Netherlands.)

159. Gapstur SM, Gann PH, Lowe W, Liu K, Colangelo L, Dyer A. 2000. Abnormal glucose metabolism and pancreatic cancer mortality. *Jama* 283(19): 2552-8. (Supported by NCI and the National Heart, Lung and Blood Institute. Authors affiliated with Northwestern University Medical School, IL; Robert H. Lurie Comprehensive Cancer Center; Veterans Affairs Chicago Health Care System, IL.)

160. Gérin M, Siemiatycki J, Désy 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(2): 144-156. (Supported by the Institut de recherche en sante et en securite du travail du Quebec, the Fonds de la recherche en sante du Quebec, the National Health Research And Development Program of Canada and the National Cancer Institute of Canada. Authors affiliated with Universite de Montreal, Canada; Universite du Quebec, Canada; McGill University, Canada; Health Protection Branch, Health Canada; University of Ottawa, Canada.)

161. 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): 636-644. (Support not reported. Authors affiliated with Fondazione Salvatore Maugeri, Italy; Il Sezione di Medicina del Lavoro, Italy; Sezione di Medicina Preventiva, Italy.)

162. Gobba F, Ghittori S, Imbriani M, Cavalleri A. 2000. Evaluation of half-mask respirator protection in styrene-exposed workers. *Int Arch Occup Environ Health* 73(1): 56-60. (Support not reported. Authors affiliated with Microbiologiche e Biostatistiche Universita di Modena e Reggio Emilia, Italy; Fondazione Salvatore Maugeri, Italy; Universita di Pavia, Italy.)

163. 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. (Supported by the Belgian Offices for Scientific, Technical, and Cultural Affairs, the EU Cancer Risk Biomarkers Project and ECETOC. Authors affiliated with Katholieke Universiteit Leuven, Belgium; Vrije Universiteit Brussel, Belgium; Universite Catholique de Louvain, Belgium.)

164. Godderis L, Aka P, Mateuca R, Kirsch-Volders M, Lison D, Veulemans H. 2006. Dose-dependent influence of genetic polymorphisms on DNA damage induced by styrene oxide, ethylene oxide and gamma-radiation. *Toxicology* 219(1-3): 220-9. (Support not reported. Authors affiliated with Katholieke Universiteit Leuven,

Belgium; Vrije Universiteit Brussel, Belgium; Catholic University of Louvain, Belgium.)

165. 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): 916-932. (Supported by the Health Effects Institute, Massachusetts. Authors affiliated with Wayne State University School of Medicine, MI; University of Alabama at Birmingham, AL; University of Minnesota, MN.)

166. Green T, Toghill A, Foster JR. 2001a. The role of cytochromes P-450 in styrene induced pulmonary toxicity and carcinogenicity. *Toxicology* 169(2): 107-17. (Supported by the Styrene Steering Committee and the Styrene Information and Research Center. Authors affiliated with Syngenta Central Toxicology Laboratory, UK.)

167. Green T, Lee R, Toghill A, Meadowcroft S, Lund V, Foster J. 2001b. 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. (Supported by the Styrene Information and Research Center. Authors affiliated with Syngenta Central Toxicology Laboratory, UK; University College London, UK.)

168. Guenel P, Imbernon E, Chevalier A, Crinquand-Calastreng A, Goldberg M. 2002. Leukemia in relation to occupational exposures to benzene and other agents: a case-control study nested in a cohort of gas and electric utility workers. *Am J Ind Med* 42(2): 87-97. (Supported by Électricité de France-Gaz de France. Authors affiliated with Hôpital National de Saint-Maurice, France; EDF-GDF, France.)

169. Guest MJ. 1997. Styrene Copolymers. In *Handbook of Thermoplastics*. Olabisi O, ed. New York, NY: Marcel Dekker, Inc. p. 161-175. (Support not reported. Authors affiliated with Dow Chemical Company, TX.)

170. Guillemin MP, Berode M. 1988. Biological monitoring of styrene: a review. *Am Ind Hyg Assoc J* 49(10): 497-505. (Support not reported. Authors affiliated with Institute of Occupational Health and Hygiene, Switzerland.)

171. Guo H, Lee SC, Chan LY, Li WM. 2004a. Risk assessment of exposure to volatile organic compounds in different indoor environments. *Environ Res* 94(1): 57-66. (Supported by Hong Kong Polytechnic University and the Research Grant Council of the Hong Kong Government. Authors affiliated with Hong Kong Polytechnic University, China.)

172. 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. (Supported by the Swedish Work Environment Fund, the Swedish Cancer Society, and the Ellen, Walter and Lennart Hesselman's Foundation for Scientific Research. Authors affiliated with University Hospital, Sweden; Central Hospital, Sweden; Halsoklippan, Sweden.)

173. 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. (Support not reported. Authors affiliated with Institut fur Arbeitsphysiologie an der Universitat Dortmund, Germany; Arbeitsmedizinische Zentren des TUV Rheinland, Germany.)

174. Hallier E, Goergens HW, Karels H, Golka K. 1995. A note on individual differences in the urinary excretion of optical enantiomers of styrene metabolites and of styrene-derived mercapturic acids in humans. *Arch Toxicol* 69(5): 300-5. (Support not reported. Authors affiliated with Institut fur Arbeitsphysiologie, Universitat Dortmund, Germany.)

175. 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. (Support not reported. Authors affiliated with Telemark Sentralsjukehus, Norway.)

176. Härkönen H, Tola S, Korkala ML, Hernberg S. 1984. Congenital malformations, mortality and styrene exposure. *Ann Acad Med Singapore* 13(Suppl 2): 404-407. (Support not reported. Authors affiliated with Institute of Occupational Health, Finland.)

177. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA, Bloomfield CD. 2000. The World Health Organization classification of hematological malignancies report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. *Mod Pathol* 13(2): 193-207. (Supported by Becton-Dickinson, Berlex Laboratories/Schering Berlin, Bristol-Meyers Squibb, the Cure for Lymphoma Foundation, Coulter Corporation, Dako A/S, F. Hoffmann-La Roche Ltd., the Leukemia Clinical Research Foundation, the Swiss Federal Office of Public Health, the National Cancer Institute, the University of Chicago Cancer Research Center and the World Health Organization. Authors affiliated with Massachusetts General Hopital, MA; Harvard Medical School, MA; National Cancer Institute, MD; Hotel Dieu, France; Hopital Necker, France; University of Wurzburg, Germany; University of Chicago, IL; St. Bartholomew's Hospital, UK; Ohio State University, OH.)

178. Harvey PW. 2005. Human relevance of rodent prolactin-induced non-genotoxic mammary carcinogenesis: prolactin involvement in human breast cancer and significance for toxicology risk assessments. *J Appl Toxicol* 25(3): 179-83. (Support not reported. Authors affiliated with Covance Laboratories, UK.)

179. Harvilchuck JA, Carlson GP. 2006. Comparison of styrene and its metabolites styrene oxide and 4-vinylphenol on cytotoxicity and glutathione depletion in Clara cells of mice and rats. *Toxicology* 227(1-2): 165-72. (Supported by the Styrene Information and Research Center. Authors affiliated with Purdue University, IN.)

180. Haufroid V, Jakubowski M, Janasik B, Ligocka D, Buchet JP, Bergamaschi E, Manini P, Mutti A, Ghittori S, Arand M, Hangen N, Oesch F, Hirvonen A, Lison D. 2002b. Interest of genotyping and phenotyping of drug-metabolizing enzymes for the interpretation of biological monitoring of exposure to styrene. *Pharmacogenetics* 12(9): 691-702. (Supported by the National Fund for Scientific Research, Belgium and the European Commission. Authors affiliated with Catholic University of Louvain, Belgium; Nofer Institute of Occupational Medicine, Poland; University of Parma, Italy; Fondazione Salvatore Maugeri, Italy; University of Mainz, Germany; Finnish Institute of Occupational Health, Finland.)

181. Haufroid V, Lison D. 2005. Mercapturic acids revisited as biomarkers of exposure to reactive chemicals in occupational toxicology: a minireview. *Int Arch Occup Environ Health* 78(5): 343-54. (Support not reported. Authors affiliated with Université catholique de Louvain, Belgium.)

182. HazDat. 2008. *Internet HazDat - Site Contaminant Query*. Agency for Toxic Substances and Disease Registry. http://www2.atsdr.cdc.gov/gsql/sitecontam.script and type "styrene" into the Contaminant Name field. Accessed on 5/13/08.

183. HealthCanada. 1993. *Priority Substances List Assessment Report: Styrene*. Ottawa, Canada: Government of Canada, Environment Canada, Health Canada. 48 pp.

184. Hemminki K, Hesso A. 1984. Reaction products of styrene oxide with guanosine in aqueous media. *Carcinogenesis* 5(5): 601-607. (Support not reported. Authors affiliated with Institute of Occupational Health, Finland.)

185. Hemminki K, Koskinen M, Rajaniemi H, Zhao CY. 2000. DNA adducts, mutations, and cancer 2000. *Regul Toxicol Pharmacol* 32(3): 264-275. (Support not reported. Authors affiliated with Karolinska Institute, Sweden.)

186. Henderson LM, Speit G. 2005. Review of the genotoxicity of styrene in humans. *Mutat Res* 589(3): 158-191. (Supported by the Styrene Steering Committee. Authors affilitated with Henderson Scientific Consultancy, UK; Universitatsklinikum, Germany.)

187. Hennard C, Finneman J, Harris CM, Harris TM, Stone MP. 2001. The nonmutagenic (*R*)- and (*S*)-beta-(N^6-adenyl)styrene oxide adducts are oriented in the major groove and show little perturbation to DNA structure. *Biochemistry* 40(33): 9780-9791.(Supported by the NIH, the Vanderbilt Center in Molecular Toxicology, University of Wisconsin, NSF, and USDA. Authors affiliated with Vanderbilt University, TN).

188. Herrero ME, Arand M, Hengstler JG, Oesch F. 1997. Recombinant expression of human microsomal epoxide hydrolase protects V79 Chinese hamster cells from styrene oxide- but not from ethylene oxide-induced DNA strand breaks. *Environ Mol Mutagen* 30(4): 429-39. (Support not reported. Authors affiliated with University of Mainz, Germany.)

189. Higuchi Y. 2004. Glutathione depletion-induced chromosomal DNA fragmentation associated with apoptosis and necrosis. *J Cell Mol Med* 8(4): 455-464. (Support not reported. Authors affiliated with Kanazawa University Graduate School of Medical Science, Japan.)

190. Hillis DR, Davis AD. 1995. *Waste Reduction: Strategies for Fiberglass Fabricators*. North Carolina Division of Pollution Prevention and Environmental Assistance. http://www.p2pays.org/ref/01/00368.pdf. Last accessed: 3/14/05. (Supported by the Office of Waste Reduction, North Carolina Department of Environment, Health and Natural Resources. Authors affiliated with ECU.)

191. Hillis DR. 1997. *Establishing Waste Reduction Benchmarks and Good Manufacturing Practice for Open Mold Laminating*. North Carolina Division of Pollution Prevention and Environmental Assistance. http://www.p2pays.org/ref/01/00372.pdf. Last accessed: 3/14/05. (Supported by the North Carolina Division of Pollution Prevention and Environmental Assistance. Authors affiliated with East Carolina University, NC.)

192. 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(5): 347-352. (Support not reported. Authors affiliated with Epidemiology and Medical Statistics Unit, UK.)

193. Hoet P, Lison D. 2008. Ototoxicity of toluene and styrene: state of current knowledge. *Crit Rev Toxicol* 38(2): 127-70. (Supported by CONCAWE. Authors affiliated with Universite Catholique de Louvain, Belgium.)

194. Hofmann 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. (Supported by the Styrene Steering Committee of the European Chemical Industry Council. Authors affiliated with GSF-National Research Center for Environment and Health, Germany; Technische Universität München, Germany.)

195. 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. (Supported by the Swedish Cancer Society. Authors affiliated with University Hospital, Sweden.)

196. Hogstedt B, Akesson B, Axell K, Gullberg B, Mitelman F, Pero RW, Skerfving S, Welinder H. 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. (Supported by the Swedish Work Environment Fund and the Swedish Cancer Society. Authors affiliated with Central Hospital, Sweden; University Hospital, Sweden; University of Lund, Sweden; Occupational Health Center, Sweden; Wallenberg Laboratory, Sweden.)

197. Holmes MJ, Hart A, Northing P, Oldring PK, Castle L, Stott D, Smith G, Wardman O. 2005. Dietary exposure to chemical migrants from food contact materials: a probabilistic approach. *Food Addit Contam* 22(10): 907-19. (Supported by the Association of European Producers of Steel for Packaging, Association of Plastcs Manufacturers in Europe, Plastics Europe, European Council of Paint, Printing Ink and Artists Colours Industry, Confederation of European Paper Industries, European Aluminum Association, European Phenolic Resins Association, Ecological and Toxicological Association of Dyes and Organic Pigments, European Wax Federation, Food Contact Additives Panel, Flexible Packaging Europe, and the European Secretariat of Manufacturers of Light Metal Packaging. Authors affiliated with Department for Environment, Food and Rural Affairs, UK; Valspar Corporation, UK.)

198. Holz O, Scherer G, Brodtmeier S, Koops F, Warncke K, Krause T, Austen A, Angerer J, Tricker AR, Adlkofer F, Rüdiger HW. 1995. Determination of low level exposure to volatile aromatic hydrocarbons and genotoxic effects in workers at a styrene plant. *Occup Environ Med* 52(6): 420-428. (Supported by the German Ministry of Research and Technology. Authors affiliated with University of Vienna; University of Hamburg; Analtyischbiologisches Forschungslabor.)

199. Horvath E, Pongracz K, Rappaport S, Bodell WJ. 1994. ^{32}P-post-labeling detection of DNA adducts in mononuclear cells of workers occupationally exposed to styrene. *Carcinogenesis* 15(7): 1309-1315. (Supported by NIOSH, NIEHS and the University of California. Authors affiliated with University of California, CA; University of North Carolina, NC.)

200. Howard PH. 1989. Styrene. In *Handbook of Environmental Fate and Exposure Data for Organic Chemicals*, vol 1. Large Production and Priority Pollutants. Lewis Publishers. p. 490-498. (Support not reported. Author affiliations not indicated.)

201. HSDB. 2008a. *Styrene*. National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB and search "styrene". Last Accessed: 5/08/08.

202. HSDB. 2008b. *Styrene-7,8-oxide*. National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB and search "styrene oxide". Accessed on 9/18/08.

203. Hsieh L-L, Chang C-C, Sree U, Lo J-G. 2006. Determination of volatile organic compounds in indoor air of buildings in nuclear power plants, Taiwan. *Water Air Soil Poll* 170(1-4): 107-121. (Supported by the National Science Council and the Taiwan Power Company. Authors affiliated with National Hsing-Hua University, Taiwan; Academia Sinica, Taiwan; Yuan-Pei Institute of Science and Technology, Taiwan.)

204. Huff JE. 1984. Styrene, Styrene Oxide, Polystyrene, and ß-Nitrostyrene/Styrene Carcinogenicity in Rodents. In *Industrial Hazards of Plastics and Synthetic*

Elastomers. Järvisalo J, Pfäffli P, Vainio H, eds. New York, NY: Alan R. Liss, Inc. p. 227-238. (Support not reported. Authors affiliated with NIEHS.)

205. Hukkanen J, Pelkonen O, Hakkola J, Raunio H. 2002. Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. *Crit Rev Toxicol* 32(5): 391-411. (Supported by the Academy of Finland, the Biomed2 program (EUROCYP Project) and TEKES (Technology Development Center, Finland.) Authors affiliated with University of Oulu, Finland; Lapland Central Hospital, Finland; University of Kuopio, Finland.)

206. Hynes DE, DeNicola DB, Carlson GP. 1999. Metabolism of styrene by mouse and rat isolated lung cells. *Toxicol Sci* 51(2): 195-201. (Supported by NIH and the Styrene Information and Research Center. Authors affiliated with Purdue University, IN.)

207. IARC. 1979. *Styrene, Polystyrene and Styrene-Butadiene Copolymers*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 19. Lyon, France: International Agency for Research on Cancer. p. 231-74.

208. IARC. 1994a. *Styrene*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 60. Lyon, France: International Agency for Research on Cancer.

209. IARC. 1994b. *Styrene Oxide*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 60. Lyon, France: International Agency for Research on Cancer.

210. IARC. 1999. *1,3-Butadiene*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 71. Lyon, France: International Agency for Research on Cancer. p. 109-225.

211. IARC. 2002. *Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 82. Lyon, France: International Agency for Research on Cancer.

212. ICIS. 2008. *Styrene-Butadiene Rubber (SBR) Uses and Outlook*. Reed Business Information Limited. http://www.icis.com/v2/chemicals/9076467/styrene-butadiene-rubber/uses.html. Accessed on 9/15/08.

213. IISRP. 1973. *Synthetic Rubber: The Story of an Industry*, New York: International Institute of Synthetic Rubber Producers. p. 28-87. (Support not reported. Authors affiliated with International Institute of Synthetic Rubber Producers.)

214. Ikeda M, Koizumi A, Miyasaka M, Watanabe T. 1982. Styrene exposure and biologic monitoring in FRP boat production plants. *Int Arch Occup Environ Health* 49(3-4): 325-39. (Supported by the Ministry of Education, Science and Culture of

the Government of Japan. Authors affiliated with Tohoku University School of Medicine, Japan.)

215. Inaoka T, Nagano M, Kitano T, Ushijima K, Minamoto K, R. T, Koyanagi A. 2002. Biological monitoring of styrene in FRP-making small industries in Kumamoto, Japan. Winter-summer difference and effect of protective masks in practical working conditions. *J Occup Health* 44: 83-88. (Support not reported. Authors affiliated with Kumamoto University School of Medicine, Japan; the Chemo-sero-therapeutic Research Institute; Japan Red-Cross Hospital.)

216. Iregren A, Johnson A-C, Nylen P. 2005a. Low-level styrene exposure and color vision in Swedish styrene workers. *Environ Toxicol Pharm* 19(3): 511-516. (Support not reported. Authors affiliated with National Institute for Working Life, Sweden; Karolinska Institute, Sweden.)

217. Ishidate M, Jr., Yoshikawa K. 1980. Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation--a comparative study on mutagens and carcinogens. *Arch Toxicol Suppl* 4: 41-44. (Support not reported. Authors affiliated with National Institute of Hygiene Sciences, Japan.)

218. 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. (Support not reported. Authors affiliated with Research Institute of Preventive Medicine, Czechoslovakia.)

219. Jacobs A. 1989. Benzene and leukaemia. *Br J Haematol* 72(2): 119-21. (Support not reported. Authors affiliated with University of Wales College of Medicine.)

220. Jantunen K, Mäki-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. (Supported by the swedish work Environment Fund. Authors affiliated with the Institute of Occupational Health, Finland.)

221. Jensen AA, Breum NO, Bacher J, Lynge E. 1990. Occupational exposures to styrene in Denmark 1955-88. *Am J Ind Med* 17(5): 593-606. (Support not reported. Authors affiliated with the Danish National Institute of Occupational Health, Denmark; Danish Cancer Society, Denmark; Danish Institute of Technology, Denmark.)

222. Jersey GC, Balmer MF, Quast JF, Park CN, Schuetz DJ, Beyer JE, Olson KJ, McCollister SB, Rampy LW. 1978. *Two-Year Chronic Inhalation Toxicity and Carcinogenicity Study on Monomeric Styrene in Rats. Final Report*. Michigan: Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A. (Unpublished study.)

223. Johanson G, Ernstgard L, Gullstrand E, Lof A, Osterman-Golkar S, Williams CC, Sumner SC. 2000. Styrene oxide in blood, hemoglobin adducts, and urinary

metabolites in human volunteers exposed to (13)C(8)-styrene vapors. *Toxicol Appl Pharmacol* 168(1): 36-49. (Supported by the Styrene Information and Research Center. Authors affiliated with National Institute for Working Life, Sweden; University Hospital, Sweden; Arrhenius Laboratories for Natural Sciences, Sweden; CIIT, NC.)

224. Johnson AC, Morata TC, Lindblad AC, Nylen PR, Svensson EB, Krieg E, Aksentijevic A, Prasher D. 2006. Audiological findings in workers exposed to styrene alone or in concert with noise. *Noise Health* 8(30): 45-57. (Supported by NoiseChem and the Swedish Council for Working Life and Social Research. Authors affiliated with Karolinska Institutet, Sweden; National Institute for Working Life, Sweden; Department of Work and Physical Environment, Sweden; NIOSH; Roehampton University, UK; University College London, UK.)

225. Johnson AC. 2007. Relationship between styrene exposure and hearing loss: review of human studies. *Int J Occup Med Environ Health* 20(4): 315-25. (Support not reported. Authors affiliated with Karolinska Institute, Sweden.)

226. Kanuri M, Finneman J, Harris CM, Harris TM, Lloyd RS. 2001. Efficient nonmutagenic replication bypass of DNAs containing beta-adducts of styrene oxide at adenine N^6. *Environ. Mol. Mutagen.* 38(4): 357-360.

227. Karakaya AE, Karahalil B, Yilmazer M, Aygün N, Sardas S, Burgaz S. 1997. Evaluation of genotoxic potential of styrene in furniture workers using unsaturated polyester resins. *Mutat Res* 392(3): 261-268. (Supported by the Research Fund of the Turkish Scientific and Research Council. Authors affiliated with Gazi University, Turkey.)

228. Karlgren M, Miura S, Ingelman-Sundberg M. 2005. Novel extrahepatic cytochrome P450s. *Toxicol Appl Pharmacol* 207(2 Suppl): 57-61. (Supported by the Swedish Research Council and NIH. Authors affiliated with Karolinska Institutet, Sweden.)

229. 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. (Supported by the Styrene Steering Committee. Authors affiliated with BASF Aktiengesellschaft, Germany; Dow Chemical Company, Switzerland.)

230. 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. (Support not reported. Authors affiliated with Harvard School of Public Health, MA; University of Massachuesetts Medical Center, MA; Mt. Sinai School of Medicine, NY.)

231. Khanna S, Rao GS, Dogra RK, Shukla LJ, Srivastava SN, Dhruv SP, Shanker R. 1994. Styrene induced pancreatic changes in rodents. *Indian J Exp Biol* 32(1): 68-71. (Support not reported. Authors affiliated with Industrial Toxicology Research Center, India.)

232. Kim H, Wang RS, Elovaara E, Raunio H, Pelkonen O, Aoyama T, Vainio H, Nakajima T. 1997. Cytochrome P450 isozymes responsible for the metabolism of toluene and styrene in human liver microsomes. *Xenobiotica* 27(7): 657-665. (Support not reported. Authors affiliated with Chungbuk National University, Korea; Finnish Institute of Occupational Health, Finland; University of Oulu, Finland.)

233. Kim JK, Shin HS, Lee JH, Lee JJ, Lee JH. 2003. Genotoxic effects of volatile organic compounds in a chemical factory as evaluated by the Tradescantia micronucleus assay and by chemical analysis. *Mutat Res* 541(1-2): 55-61. (Support not reported. Authors affiliated with Korea Atomic Energy Research Institute, South Korea; Chungnam National University, South Korea; Yonsei University, South Korea; Yong-In University, South Korea.)

234. Kitamura S, Ohmegi M, Sanoh S, Sugihara K, Yoshihara S, Fujimoto N, Ohta S. 2003. Estrogenic activity of styrene oligomers after metabolic activation by rat liver microsomes. *Environ Health Perspect* 111(3): 329-34. (Supported by the Japanese Ministry of Education, Science, Sports and Culture and the Japan Society for the Promotion of Science. Authors affiliated with Hiroshima University, Japan.)

235. Kligerman AD, Allen JW, Erexson GL, Morgan DL. 1993. Cytogenetic studies of rodents exposed to styrene by inhalation. In *Butadiene and Styrene: Assessment of Health Hazards*, IARC Scientific Publications No. 127. Sorsa M, Peltonen K, Vainio H, Hemminki K, eds. Lyon, France: International Agency for Research on Cancer. p. 217-24. (Support not reported. Authors affiliated with U.S. EPA; Environmental Health Research and Testing, Inc,, NC; NIEHS.)

236. Kogevinas M, Ferro G, Saracci R, Andersen A, Biocca M, Coggon D, Gennaro V, Hutchings S, Kolstad H, Lundberg I, Lynge E, Partanen T. 1993. Cancer mortality in an international cohort of workers exposed to styrene. In *Butadiene and Styrene: Assessment of Health Hazards*, IARC Scientific Publications No. 127. Sorsa M, Peltonen K, Vainio H, Hemminki K, eds. Lyon, France: International Agency for Cancer Research. p. 289-300. (Supported by the CEC. Authors affiliated with IARC; the Cancer Registry of Norway; Presidio Multizonale di Prevenzione, Italy; University of Southampton, UK; Istituto Nazionale per la ricerca sul cancro, Italy; Health and Safety Execute, UK; University of Aarhus, Denmark; Karolinska Hospital, Sweden; Dansih Cancer Registry; Institute of Occupational Health, Finland.)

237. Kogevinas M, Ferro G, Andersen A, Bellander T, Biocca M, Coggon D, Gennaro V, Hutchings S, Kolstad H, Lundberg I, Lynge E, Partanen T, Saracci R. 1994a. Cancer mortality in a historical cohort study of workers exposed to styrene. *Scand J Work Environ Health* 20(4): 251-261. (Supported by the Commission of the European Communities. Authors affiliated with IARC; Cancer Registry of Norway; Karolinska Hospital, Sweden; Presidio Multizonale di Prevenzione, Italy; University of Southampton, UK; Istituto Nazionale per la ricerca sul cancro, Italy; Health and

Safety Executive, UK; University of Aarhus, Denmark; Danish Cancer Registry; Institute of Occupational Health, Finland.)

238. Kogevinas M, Ferro G, Saracci R, Andersen A, Bellander T, Biocca M, Breum N, Coggon D, Gennaro V, Hutchings S, Jensen A, Kolstad H, Lundberg I, Lynge E, Pannett B, Pfaeffli P. 1994b. *IARC Historical multicentric cohort study of workers exposed to styrene*. IARC Technical Report 94/002. Lyon: International Agency for Research on Cancer. (as cited by IARC 2002.)

239. Kolstad H, Olsen J. 1999. Why do short term workers have high mortality? *Am J Epidemiol* 149(4): 347-352. (Support not reported. Authors affiliated with University of Aarhus, Denmark.)

240. Kolstad HA, Lynge E, Olsen J. 1993. Cancer incidence in the Danish reinforced plastics industry. In *Butadiene and Styrene: Assessment of Health Hazards*, IARC Scientific Publications vol. 127. Sorsa M, Peltonen K, Vainio H, Hemminki K, eds. Lyon, France: International Agency for Cancer Research. p. 301-308. (Supported by the Health Fund, Aarhus University Research Foundation, the Danish Working Environment Fund, the Danish Research Academy, and the Danish Cancer Society. Authors affiliated with University of Aarhus, Denmark; Danish Cancer Society, Denmark.)

241. 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(4): 272-278. (Supported by the Health Fund, Aarhus University Research Foundation, the Danish Working Environment Fund, the Danish Research Academy, and the Danish Cancer Society. Authors affiliated with University of Aarhus, Denmark; Danish Cancer Society, Denmark; National Institute of Occupational Health, Denmark.)

242. 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(5): 320-327. (Supported by the Health Fund, Aarhus University Research Foundation, the Danish Working Environment Fund, the Danish Research Academy, and the Danish Cancer Society. Authors affiliated with University of Aarhus, Denmark; Danish Cancer Society, Denmark; Danish Institute for Clinical Epidemiology, Denmark; Danish Epidemiology Science Center, Denmark.)

243. Kolstad HA, Pedersen B, Olsen J, Lynge E, Jensen G, Lisse I, Philip P, Pedersen NT. 1996. Clonal chromosome aberrations in myeloid leukemia after styrene exposure. *Scand J Work Environ Health* 22(1): 58-61. (Supported by the Health Fund, Aarhus University Research Foundation, the Danish Working Environment Fund, the Danish Research Academy, and the Danish Cancer Society. Authors affiliated with University of Aarhus, Denmark; Danish Cancer Society, Denmark; Danish Epidemiology Center, Denmark; Fredericksberg Hospital, Denmark;

Hvidore Hospital, Denmark; Finsen Laboratory, Denmark; University Hospital of Odense, Denmark.)

244. Kolstad HA, Sønderskov J, Burstyn I. 2005. Company-level, semi-quantitative assessment of occupational styrene exposure when individual data are not available. *Ann Occup Hyg*(Pre-publication): 1-11. (Supported by the Danish Working Environment Fund. Authors affiliated with Aarhus University Hospital, Denmark; Utrecht University, Netherlands; University of Alberta, Canada.)

245. Korn M, Wodarz R, Drysch K, Schoknecht W, Schmahl FW. 1985. Stereometabolism of styrene in man: gas chromatographic determination of phenylethyleneglycol enantiomers and phenylethanol isomers in the urine of occupationally-exposed persons. *Arch Toxicol* 58(2): 110-114. (Support not reported. Authors affiliated with Institut fur Arbeits-und Sozialmedizin der Universitat Tubigen, Germany.)

246. Korn M, Gfrörer W, Filser JG, Kessler W. 1994. Styrene-7,8-oxide in blood of workers exposed to styrene. *Arch Toxicol* 68(8): 524-527. (Support not reported. Authors affiliated with Institut fur Arbeits-und Sozialmedizin der Universitat Tubigen, Germany; GSF-Institut fur Toxikologie, Germany.)

247. Koskinen M, Schweda EKH, Hemminki K. 1999. Alkylation of anionic DNA bases by styrene-7,8-oxide. *J Chem Soc Perkin Trans* 2: 2441-2445. (Supported by the Swedish Council for Work Life Research. Authors affiliated with Karolinska Institute, Sweden; Center for Nutrition and Toxicology, Sweden; Clinical Research Center, Sweden.)

248. Koskinen M, Vodicka P, Hemminki K. 2000a. Adenine N3 is a main alkylation site of styrene oxide in double-stranded DNA. *Chem Biol Interact* 124(1): 13-27. (Supported by the Swedish Work Environment Fund and GACR. Authors affiliated with Karolinska Institute, Sweden; Czech Academy of Science, Czech Republic.)

249. Koskinen M, Calebiro D, Hemminki K. 2000b. Styrene oxide-induced 2'-deoxycytidine adducts: implications for the mutagenicity of styrene oxide. *Chem Biol Interact* 126(3): 201-213. (Supported by Swedish Council for Work Life Research. Authors affiliated with Karolinska Institute, Sweden.)

250. Koskinen M, Vodicka P, Hemminki K. 2001a. Identification of 1-adenine DNA adducts in workers occupationally exposed to styrene. *J Occup Environ Med* 43(8): 694-700. (Supported by the European Communities and Swedish Council for Work Life Research. Authors affiliated with Karolinska Institute, Sweden; Academy of Sciences of the Czech Republic, Czech Republic.)

251. Koskinen M, Vodickova L, Vodicka P, Warner SC, Hemminki K. 2001b. Kinetics of formation of specific styrene oxide adducts in double-stranded DNA. *Chem Biol Interact* 138(2): 111-24. (Supported by the European Communities and the Swedish Council for Work Life Research. Authors affiliated with Karolinska Institute,

Sweden; National Institute of Public Health, Czech Republic; Academy of Sciences of the Czech Republic, Czech Republic.)

252. Kraeling MEK, Bronaugh RL. 2005. In vitro percutaneous absorption of acrylamide and styrene from cosmetic vehicles through fuzzy rat and human skin. *Cutan Ocular Toxicol* 24: 65-79. (Support not reported. Authors affiliated with U.S. FDA.)

253. Kuricova M, Naccarati A, Kumar R, Koskinen M, Dusinska M, Tulinska J, Vodickova L, Liskova A, Jahnova E, Fuortes L, Haufroid V, Hemminki K, Vodicka P. 2005. DNA repair and cyclin D1 polymorphisms and styrene-induced genotoxicity and immunotoxicity. *Toxicol Appl Pharmacol*(207): S302-S309. (Supported by GACR, NIEHS and EU Diephy. Authors affiliated with Academy of Sciences of the Czech Republic, Czech Republic; German Cancer Research Center, Germany; Orion Pharma, Finsland; Karolinska Institute, Sweden; RB SMU-Institute Prevent. Clin. Med., Slovak republic; University of Iowa, IA; Catholic University Louvain, Belgium.)

254. Laffon B, Pásaro E, Méndez J. 2001a. Effects of styrene-7,8-oxide over *p53*, *p21*, *bcl-2* and *bax* expression in human lymphocyte cultures. *Mutagenesis* 16(2): 127-132. (Supported by the Spanish Ministry of Education and Xunta de Galicia. Authors affiliated with Universidade da Coruna, Spain.)

255. Laffon B, Pásaro E, Méndez J. 2001b. 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(1-2): 163-172. (Supported by the Spanish Ministry of Education and Xunta de Galicia. Authors affiliated with Universidade da Coruna, Spain.)

256. Laffon B, Pásaro E, Méndez J. 2002a. Evaluation of genotoxic effects in a group of workers exposed to low levels of styrene. *Toxicology* 171(2-3): 175-186. (Supported by the Universidade da Coruna and Xunta de Galicia. Authors affiliated with Universidade da Coruna, Spain.)

257. Laffon B, Pásaro E, Méndez J. 2002b. DNA damage and repair in human leukocytes exposed to styrene-7,8-oxide measured by the comet assay. *Toxicol Lett* 126(1): 61-8. (Supported by the Universidade da Coruna and Xunta de Galicia. Authors affiliated with Universidade da Coruna, Spain.)

258. Laffon B, Perez-Cadahia B, Pasaro E, Mendez J. 2003a. Individual sensitivity to DNA damage induced by styrene *in vitro*: influence of cytochrome p450, epoxide hydrolase and glutathione S-transferase genotypes. *Toxicology* 186(1-2): 131-41. (Supported by the Xunta de Galicia. Authors affiliated with Universidade da Coruna, Spain.)

259. Laffon B, Pérez-Cadahia B, Pásaro E, Méndez J. 2003b. Effect of epoxide hydrolase and glutathione *S*-tranferase genotypes on the induction of micronuclei and DNA damage by styrene-7,8-oxide in vitro. *Mutat Res* 536(1-2): 49-59. (Supported by the Xunta de Galicia. Authors affiliated with Universidade da Coruna, Spain.)

260. Latham GJ, Zhou L, Harris CM, Harris TM, Lloyd RS. 1993. The replication fate of *R*- and *S*-styrene oxide adducts on adenine N^6 is dependent on both the chirality of the lesion and the local sequence context. *J Biol Chem* 268(31): 23427-23434. (Support not reported. Authors affiliated with Vanderbilt University School of Medicine, TN; University of Texas, TX.)

261. Latham GJ, Lloyd RS. 1994. Deoxynucleotide polymerization by HIV-1 reverse transcriptase is terminated by site-specific styrene oxide after translesion synthesis. *J. Biol. Chem.* 269: 28527-28530. (Supported by the U.S. Public Health Service and the American Cancer Society. Authors affiliated with Vanderbilt University, TN; University of Texas, TX.)

262. Latham GJ, Harris CM, Harris TM, Lloyd RS. 1995. The efficiency of translesion synthesis past single styrene oxide DNA adducts *in vitro* is polymerase-specific. *Chem. Res. Toxicol.* 8: 422-430. (Supported by USPHS and ACS. Authors affiliated with Vanderbilt University School of Medicine, TN; University of Texas Medical Branch, TX.)

263. Latham GJ, McNees AG, DeCorte B, Harris CM, Harris TM, O'Donnell M, Lloyd RS. 1996. Comparison of the efficiency of synthesis past single bulky DNA adducts *in vivo* and *in vitro* by the polymerase III holenzyme. *Chem. Res. Toxicol.* 9: 1167-1175. (Supported by the U.S. Public Health Service. Authors affiliated with Vanderbilt University, TN; Cornell University Medical College, NY; University of Texas, TX; University of Oregon, OR.)

264. Latham GJ, Forgacs E, Beard WA, Prasad R, Bebenek K, Kunkel TA, Wilson SH, Lloyd RS. 2000. Vertical-scanning mutagenesis of a critical tryptophan in the 'minor groove binding track' of HIV-1 reverse transcriptase. Major groove DNA adducts identify specific protein interactions in the minor groove. *J. Biol. Chem.* 275(20): 15025-15033. (Supported by NIH . Authors affiliated with University of Texas, TX; NIEHS; Ambion, Inc., TX.)

265. Latif F, Moschel RC, Hemminki K, Dipple A. 1988. Styrene oxide as a stereochemical probe for the mechanism of aralkylation at different sites on guanosine. *Chem Res Toxicol* 1(6): 364-369. (Supported by NCI, DHHS, and the International Union Against Cancer. Authors affiliated with NCI- Frederick Cancer Research Facility, MD.)

266. Lattime RE. 2000. Styrene-Butadiene Rubber. In *Kirk-Othmer Encyclopedia of Chemical Technology Online Edition*. Jon Wiley & Sons, Inc. 17 pp. http://mrw.interscience.wiley.com/emrw/9780471238966/kirk/article/styrlatt.a01/current/abstract?hd=All,lattime. (Support not reported. Authors affiliated with the Goodyear Tire and Rubber Company.)

267. Lazutka JR, Lekevicius R, Dedonytė V, Maciuleviciutė-Gervers L, Mierauskienė J, Rudaitienė S, Slapšytė G. 1999. Chromosomal aberrations and sister-chromatid exchanges in Lithuanian populations: effects of occupational and environmental

exposures. *Mutat Res* 445(2): 225-239. (Supported by the Lithuanian Minsitry of Health Care, the Municipality of the Kedainiai district, the Open Society Fund - Lithuania and the National Sciences Program 'Atomic Energetics and Environment' Authors affiliated with Vilnius University, Lithuania.)

268. Le PT, Harris CM, Harris TM, Stone MP. 2000. Altered electrophoretic migration of polycyclic aromatic hydrocarbon and styrene oxide adducts at adenine N^6 correlates with adduct-induced structural disorder. *Chem. Res. Toxicol.* 13(2): 63-71. (Supported by NIH. Authors affiliated with Vanderbilt University, TN.)

269. Leavens TL, Farris GM, James RA, Shah R, Wong VA, Marshall MW, Bond JA. 1997. Genotoxicity and cytotoxicity in male B6C3F1 mice following exposure to mixtures of 1,3-butadiene and styrene. *Environ Mol Mutagen* 29(4): 335-345. (Support not reported. Authors affiliated with University of North Carolina, NC; Chemical Industry Institute of Toxicology, NC.)

270. Lee CW, Dai YT, Chien CH, Hsu DJ. 2006. Characteristics and health impacts of volatile organic compounds in photocopy centers. *Environ Res* 100(2): 139-49. (Supported by the National Science Council of the Republic of China. Authors affiliated with National Kaohsiung First University of Science and Technology, Taiwan; Chung Hwa College of Medical Technology, Taiwan; Kaoshan Industrial Safety and Health, Inc., Taiwan; Chang Jung Christian University, Taiwan.)

271. 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(4): 175-81. (Support not reported. Authors affiliated with Catholic University Medical College, South Korea; Finnish Institute of Occupational Health, Finland.)

272. Lees PS, Stefaniak A, Emmett EA, Dalton P. 2003. Exposure assessment for study of olfactory function in workers exposed to styrene in the reinforced-plastics industry. *Am J Ind Med* 44(1): 12-23. (Supported by the Styrene Information and Research Center. Authors affiliated with Johns Hopkins University, MD; Hospital of the University of Pennsylvania, PA; Monell Chemical Senses Center, PA.)

273. Lemasters GK, Carson A, Samuels SJ. 1985. Occupational styrene exposure for 12 product categories in the reinforced-plastics industry. *Am Ind Hyg Assoc J* 46(8): 434-441. (Supported by the EPA. Authors affiliated with University of Cincinnati College of Medicine, OH; University of California, CA.)

274. Lemen RA, Young R (1976). Investigations of health hazards in SBR facilities, Proceedings of the NIOSH Styrene-Butadiene Briefing, Covington, Kentucky, April 30, 1976. (As cited by IARC.)

275. Lemen RA, Meinhardt TJ, Crandall MS, Fajen JM, Brown DP. 1990. Environmental epidemiologic investigations in the styrene-butadiene rubber production industry. *Environ Health Perspect* 86: 103-6. (Support not reported. Authors affiliated with NIOSH.)

276. Leung MK, Liu CH, Chan AH. 2005. Occupational exposure to volatile organic compounds and mitigation by push-pull local exhaust ventilation in printing plants. *J Occup Health* 47(6): 540-7. (Supported by the Occupational Safety and Health Council of Hong Kong. Authors affiliated with University of Hong Kong, China.)

277. Lickly TD, Lehr KM, Welsh GC. 1995a. Migration of styrene from polystyrene foam food-contact articles. *Food Chem Toxicol* 33(6): 475-481. (Support not reported. Authors affiliated with Dow Chemical Company, MI.)

278. Lickly TD, Breder CV, Rainey ML. 1995b. A model for estimating the daily dietary intake of a substance from food-contact articles: styrene from polystyrene food-contact polymers. *Regul Toxicol Pharmacol* 21(3): 406-17. (Support not reported. Authors affiliated with Dow Chemical Company, MI; Keller and Heckman, Washington, D.C.; Society of the Plastics Industry, Inc., MI; Dow Plastics, MI.)

279. Lijinsky W. 1986. Rat and mouse forestomach tumors induced by chronic oral administration of styrene oxide. *J Natl Cancer Inst* 77(2): 471-476. (Supported by the Public Health Service. Authors affiliated with Litton Bionetics, Inc., NCI-Frederick Cancer Research Facility, MD.)

280. Liljelind I, Rappaport S, Eriksson K, Andersson J, Bergdahl IA, Sunesson AL, Jarvholm B. 2003. Exposure assessment of monoterpenes and styrene: a comparison of air sampling and biomonitoring. *Occup Environ Med* 60(8): 599-603. (Supported by the Swedish Council for Work Life Research, Center for Environmental Research and NIEHS. Authors affiliated with Umeå University, Sweden; University of North Carolina, NC; Norrland's University Hospital, Sweden; University Hospital, Sweden; National Institute for Working Life, Sweden.)

281. Limasset JC, Simon P, Poirot P, Subra I, Grzebyk M. 1999. Estimation of the percutaneous absorption of styrene in an industrial situation. *Int Arch Occup Environ Health* 72(1): 46-51. (Support not reported. Authors affiliated with INRS, France.)

282. Linhart I, Gut I, Smejkal J, Novak J. 2000. Biotransformation of styrene in mice. Stereochemical aspects. *Chem Res Toxicol* 13(1): 36-44. (Supported by the Internal Grant Agency of the Ministry of Health, Czech Republic and the Institute of Chemical Technology, Prague. Authors affiliated with Institute of Chemical Technology, Czech Republic; National Institute of Public Health, Czech Republic.)

283. Linhart I. 2001. Stereochemistry of styrene biotransformation. *Drug Metab Rev* 33(3-4): 353-367. (Supported by the Ministry of Education of the Czech Republic. Authors affiliated with the Institute of Chemical Technology, Czech Republic.)

284. Linnainmaa K, Meretoja T, Sorsa M, Vainio H. 1978a. Cytogenetic effects of styrene and styrene oxide on human lymphocytes and Allium cepa. *Scand J Work Environ Health* 4 Suppl 2: 156-62. (Supported by the Finnish Cultural Foundation. Authors affiliated with the Institute of Occupational Health, Finland.)

285. Linnainmaa K, Meretoja T, Sorsa M, Vainio H. 1978b. Cytogenetic effects of styrene and styrene oxide. *Mutat Res* 58(2-3): 277-86. (Supported by the National Research Council for Sciences (Academy of Finland.) Authors affiliated with Institute of Occupational Health, Finland; University of Helsinki, Finland.)

286. Liu SF, Rappaport SM, Rasmussen J, Bodell WJ. 1988a. Detection of styrene oxide-DNA adducts by ^{32}P-postlabeling. *Carcinogenesis* 9(8): 1401-4. (Supported by the University of California, NIOSH, CDC, and NIEHS. Authors affiliated with University of California, CA.)

287. Liu SF, Rappaport SM, Pongracz K, Bodell W. 1988b. Detection of Styrene Oxide-DNA Adducts in Lymphocytes of a Worker Exposed to Styrene. In *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention*, IARC Scientific Publications No. 89. Bartsch H, Hemminki K, O'Neill IK, eds. Lyon, France: International Agency for Research on Cancer. p. 217-222. (Supported by NIOSH, CDC, and the University of California. Authors affiliated with University of California Berkeley, CA; University of California, San Francisco, CA.)

288. Liu SF, Fang QM, Jin ZL, Rappaport MS. 2001. Investigation of protein-styrene oxide adducts as a molecular biomarker of human exposed to styrene. *J Environ Sci (China)* 13(4): 391-7. (Supported by the National Natural Science Foundation of China. Authors affiliated with Chinese Academy of Sciences, China; University of North Carolina, NC.)

289. Loeb LA, Preston BD. 1986. Mutagenesis by apurinic/apyrimidinic sites. *Annu Rev Genet* 20: 201-230. (Supported by NIH, EPA, and NCI. Authors affiliated with University of Washington, WA.)

290. Löf A, Gullstrand E, Byfält Nordqvist M. 1983. Tissue distribution of styrene, styrene glycol and more polar styrene metabolites in the mouse. *Scand J Work Environ Health* 9(5): 419-430. (Support not reported. Authors affiliated with National Board of Occupational Safety and Health, Sweden.)

291. Loh MM, Houseman EA, Gray GM, Levy JI, Spengler JD, Bennett DH. 2006. Measured concentrations of VOCs in several non-residential microenvironments in the United States. *Environ Sci Technol* 40(22): 6903-11. (Supported by the American Chemical Council and the International Society for Exposure Analysis. Authors affiliated with Harvard School of Public Health, MA; University of California, CA.)

292. 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(5): 283-287. (Supported by BASF Corporation, Chevron Chemical Company, Conoco, Inc., Dow Chemical company, Exxon Company, Goodyear Tire and Rubber Company, Mitsubishi, Canadian OXY Offshore Production Company, PetroTex, Phillips Petroleum company, Quantum

Chemical Corporation, Solutia, Inc., Texaco, Inc., Texas Petrochemicals Corporation, Union Carbide Corporation, Uniroyal Goodrich Tire Company. Authors affiliated with Epidemiology Resources, Inc., MA.)

293. Lozano PR, Miracle ER, Krause AJ, Drake M, Cadwallader KR. 2007. Effect of cold storage and packaging material on the major aroma components of sweet cream butter. *J Agric Food Chem* 55(19): 7840-6. (Supported by the California Dairy Research Foundation. Authors affiliated with University of Illinois, IL; North Carolina State University, NC.)

294. 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. (Supported by NIDR, NCI, NIEHS, University of California. Authors affiiated with University of California, CA; EPA; University of North Carolina, NC; Battelle Center for Public Health Research and Evaluation, WA.)

295. Luderer U, Collins TF, Daston GP, Fischer LJ, Gray RH, Mirer FE, Olshan AF, Setzer RW, Treinen KA, Vermeulen R. 2005. NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Styrene. *Birth Defects Res B Dev Reprod Toxicol* 77(2): 110-93. (Support not reported. Authors affiliated with University of California, CA; U.S. FDA; Proctor & Gamble Co., OH; Michigan State University, MI; Johns Hopkins University, MD; United Auto Workers, MI; University of North Carolina, NC; Schering-Plough Research Institute, NJ; NCI.)

296. Lutz WK, Cantoreggi S, Velic I. 1993. DNA Binding and Stimulation of Cell Division in the Carcinogenicity of Styrene 7,8-oxide. In *Butadiene and Styrene: Assessment of Health Hazards*, IARC Scientific Publications No. 127. Lyon, France: International Agency for Research on Cancer. p. 245-252. (Support not reported. Authors affiliated with Swiss Federal Institute of Technology, Switzerland; University of Zurich, Switzerland.)

297. Ma M, Umemura T, Mori Y, Gong Y, Saijo Y, Sata F, Kawai T, Kishi R. 2005. Influence of genetic polymorphisms of styrene-metabolizing enzymes and smoking habits on levels of urinary metabolites after occupational exposure to styrene. *Toxicol Lett* 160(1): 84-91. (Supported by the Japanese Minsitry of Education, Science, Sports, Culture and Technology and the Japanese Industrial Safety and Health Association. Authors affiliated with Hokkaido University Graduate School of Medicine, Japan; Osaka Occupational Health Service Center, Japan.)

298. Macaluso M, Larson R, Delzell E, Sathiakumar N, Hovinga M, Julian J, Muir D, Cole P. 1996. Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. *Toxicology* 113(1-3): 190-202. (Supported by the International Institute of Synthetic Rubber Producers. Authors affiliated with University of Alabama, AL; Texas A&M University, TX; McMaster University, Canada.)

299. 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. (Supported by the International Institute of Synthetic Rubber Producers and the Olefins Panel of the Chemical Manufacturers Association. Authors affiliated with University of Alabama, AL; CDC.)

300. 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. (Support not reported. Authors affiliated with Fondazione Salvatore Maugeri, Italy; Universita di Pavita, Italy; Universita di Modena, Italy.)

301. Magnavita N, Placentino RA, Chiusolo P, Fiorini A, Laurenti L, Sica S. 2002. Work-related acute leukemia and mucor mycosis in a boat-builder. *Haematologica* 87(12): ECR42. (Support not reported. Authors affiliated with Catholic University School of Medicine, Italy.)

302. 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. (Supported by the Swedish Environment Fund. Authors affiliated with Institute of Occupational Health, Finland.)

303. 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. (Supported by the Swedish Work Environment Fund. Authors affiliated with Institute of Occupational Health, Finland; National Institute of Occupational Health, Sweden; University of Stockholm.)

304. Maltoni C, Failla G, Kassapidis G. 1979. First experimental demonstration of the carcinogenic effects of styrene oxide. Long-term bioassays on Sprague-Dawley rats by oral administration. *Med Lav* 5: 358-362. (Support not reported. Authors affiliated with Institute of Oncology and Tumour Center, Italy.)

305. Maltoni C, Ciliberti A, Carretti D. 1982. Experimental contributions in identifying brain potential carcinogens in the petrochemical industry. In *Brain Tumors in the Chemical Industry*, Annals of the New York Academy of Sciences vol. 381. Selikoff IJ, Hammond EC, eds. New York, NY: New York Academy of Sciences. p. 216-249. (Support not reported. Authors affiliated with the Institute of Oncology, Italy.)

306. Manini P, De Palma G, Mozzoni P, Andreoli R, Poli D, Bergamaschi E, Franchini I, Mutti A. 2002a. GSTM1 polymorphism and styrene metabolism: insights from an acute accidental exposure. *Toxicol Lett* 134(1-3): 201-208. (Supported by the

European Community. Authors affiliated with University of Parma Medical School, Italy.)

307. Manini P, Andreoli R, Poli D, De Palma G, Mutti A, Niessen WM. 2002b. Liquid chromatography/electrospray tandem mass spectrometry characterization of styrene metabolism in man and in rat. *Rapid Commun Mass Spectrom* 16(24): 2239-2248. (Supported by the European Community and Azienda Ospedaliera di Parma. Authors affiliated with Universita delgi Studi di Parma, Italy; Hyphen MassSpec Consultancy, Netherlands.)

308. 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): 160-169. (Supported by the European Community, Italian Ministry of Health and the Instituto Superiore per la Prevenzione e la Sicurezza del Lavoro. Authors affiliated with University of Parma, Italy; Nofer Institute of Occupational Medicine, Poland; Czech Academy of Sciences, Czech Republic; Finnish Institute of Occupational Health.)

309. Marczynski B, Rozynek P, Elliehausen HJ, Korn M, Baur X. 1997a. 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. (Support not reported. Authors affiliated with University of Bochum, Germany; Bau-BG Hannover, Germany.)

310. Marczynski B, Peel M, Baur X. 1997b. Changes in high molecular weight DNA fragmentation following human blood exposure to styrene-7,8-oxide. *Toxicology* 120(2): 111-7. (Supported by Deutscher Akademischer Austauschidienst. Authors affiliated with University of Bochum, Germany; Carleton University, Canada.)

311. Matanoski G, Francis M, Correa-Villaseñor A, Elliott E, Santos-Burgoa C, Schwartz L. 1993. Cancer epidemiology among styrene-butadiene rubber workers. In *Butadiene and Styrene: Assessment of Health Hazards*, IARC Scientific Publications No. 127. Sorsa M, Peltonen K, Vainio H, Hemminki K, eds. Lyon, France: International Agency for Research on Cancer. p. 363-374. (Supported by NIOSH. Authors affiliated with Johns Hopkins University, MD.)

312. 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. In *Preventative Strategies for Living in a Chemical World: a Symposium in Honor of Irving J. Selikoff*, Annals of the New York Academy of Sciences vol. 837. Bingham E, Rall DP, eds. New York: New York Academy of Sciences. p. 157-169. (Supported by NIH and EPA. Authors affiliated with Johns Hopkins University, MD; Battelle, VA; Escuela de Salud Publica de Mexico, Mexico.)

313. Matanoski GM, Schwartz L. 1987. Mortality of workers in styrene-butadiene polymer production. *J Occup Med* 29(8): 675-680. (Supported by the International Institute of Synthetic Rubber Producers. Authors affiliated with Johns Hopkins University, MD.)

314. 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: 107-117. (Support not reported. Authors affiliated with Johns Hopkins School of Hygiene and Public Health, MD; Instituto Nacional de Salud Publica, Mexico.)

315. Matanoski GM, Tao XG. 2003. Styrene exposure and ischemic heart disease: A case-cohort study. *Am J Epidemiol* 158(10): 988-995. (Supported by the Health Effects Institute. Authors affiliated with Johns Hopkins Bloomberg School of Public Health, MD.)

316. Matsuoka A, Hayashi M, Ishidate M, Jr. 1979. Chromosomal aberration tests on 29 chemicals combined with S9 mix *in vitro*. *Mutat Res* 66: 277-290. (Supported by the Ministry of Health and Welfare, Japan. Authors affiliated with National Institute of Hygienic Sciences, Japan.)

317. McConnell EE, Swenberg JA. 1993. Styrene and styrene oxide: results of studies on carcinogenicity in experimental animals. In *Butadiene and Styrene: Assessment of Health Hazards*, IARC Scientific Publications No. 127. Sorsa M, Peltonen K, Vainio H, Hemminki K, eds. Lyon, France: International Agency for Research on Cancer. p. 323-333. (Support not reported. Authors affiliated with University of North Carolina, NC.)

318. McConnell EE, Swenberg JA. 1994. Review of styrene and styrene oxide long-term animal studies. *Crit Rev Toxicol* 24(55): S49-55. (Support not reported. Authors affiliated with University of North Carolina, NC.)

319. McDougal JN, Jepson GW, Clewell HJ, 3rd, Gargas ML, Andersen ME. 1990. Dermal absorption of organic chemical vapors in rats and humans. *Fundam Appl Toxicol* 14(2): 299-308. (Support not reported. Authors affiliated with Harry G. Armstrong Aerospace Medical Research Laboratory, OH.)

320. McMichael AJ, Spirtas R, Gamble JF, Tousey PM. 1976a. Mortality among rubber workers: Relationship to specific jobs. *J Occup Med* 18(3): 178-185. (Support not reported. Authors affiliated with University of North Carolina, NC.)

321. McMichael AJ, Andjelkovic DA, Tyroler HA. 1976b. Cancer mortality among rubber workers: an epidemiologic study. In *Occupational Carcinogenesis*, Annals of the New York Academy of Sciences vol. 271. Saffiotti U, Wagoner JK, eds. New York, NY: New York Academy of Sciences. p. 125-37. (Support not reported. Authors affiliated with University of North Carolina, NC.)

322. Meinhardt TJ, Young RJ, Hartle RW. 1978. Epidemiologic investigations of styrene-butadiene rubber production and reinforced plastics production. *Scand J Work Environ Health* 4(Suppl 2): 240-246. (Support not reported. Authors affiliated with NIOSH.)

323. 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(4): 250-259. (Support not reported. Authors affiliated with NIOSH.)

324. Melnick RL. 2002. Carcinogenicity and mechanistic insights on the behavior of epoxides and epoxide-forming chemicals. In *Carcinogenesis Bioassays and Protecting Public Health*, Annals of the New York Academy of Sciences vol. 982. Mehlman M, Bingham E, Landrigan PJ*et al, eds.*, eds. New York, NY: New York Academy of Sciences. pp. 177-189. (Support not reported. Authors affiliated with NIEHS.)

325. Meretoja T, Vainio H, Sorsa M, Harkonen H. 1977. Occupational styrene exposure and chromosomal aberrations. *Mutat Res* 56(2): 193-197. (Support not reported. Authors affiliated with University of Helsinki, Finland.)

326. 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. (Support not reported. Authors affiliated with Institute of Occupational Health, Finland.)

327. Michaud DS. 2004. Epidemiology of pancreatic cancer. *Minerva Chir* 59(2): 99-111. (Support not reported. Authors affiliated with Harvard School of Public Health, MA.)

328. Midorikawa K, Uchida T, Okamoto Y, Toda C, Sakai Y, Ueda K, Hiraku Y, Murata M, Kawanishi S, Kojima N. 2004. Metabolic activation of carcinogenic ethylbenzene leads to oxidative DNA damage. *Chem Biol Interact* 150(3): 271-81. (Supported by the Ministry of Education, Science, Sports and Culture of Japan. Authors affiliated with Mie University School of Medicine, Japan; Meijo University, Japan.)

329. Migliore L, Naccarati A, Zanello A, Scarpato R, Bramanti L, Mariani M. 2002. Assessment of sperm DNA integrity in workers exposed to styrene. *Hum Reprod* 17(11): 2912-2918. (Supported by the EC and the Italian Ministero del Lavoro e della Previdenza Sociale. Authors affiliated with University of Pisa, Italy; Dipartimento di Prevenzione U.O. Igiene y Medicina del Lavoro, Italy.)

330. Migliore L, Naccarati A, Coppede F, Bergamaschi E, De Palma G, Voho A, Manini P, Jarventaus H, Mutti A, Norppa H, Hirvonen A. 2006a. Cytogenetic biomarkers, urinary metabolites and metabolic gene polymorphisms in workers exposed to styrene. *Pharmacogenet Genomics* 16(2): 87-99. (Supported by EC, the Italian

Ministero del Lavoro e della Previdenza Sociale and the Academy of Finland. Authors affiliated with University of Pisa, Italy; University of Parma, Italy; Finnish Institute of Occupational Health, Finland.)

331. Migliore L, Colognato R, Naccarati A, Bergamaschi E. 2006b. Relationship between genotoxicity biomarkers in somatic and germ cells: findings from a biomonitoring study. *Mutagenesis* 21(2): 149-52. (Supported by EC and the Italian Ministero del Lavoro e della Previdenza Sociale. Authors affiliated with University of Pisa, Italy; Academy of Sciences of the Czech Republic, Czech Republic; University of Parma, Italy.)

332. Miller RR, Newhook R, Poole A. 1994. Styrene production, use, and human exposure. *Crit Rev Toxicol* 24: S1-S10. (Support not reported. Authors affiliated with Dow Chemical Company, MI; Health Canada; Dow Europe, Switzerland.)

333. Miller SL, Branoff S, Nazaroff WW. 1998. Exposure to toxic air contaminants in environmental tobacco smoke: An assessment for California based on personal monitoring data. *J Expo Anal Environ Epidemiol* 8(3): 287-311. (Supported by the ARB. Authors affiliated with University of Colorado, CO; EPA; University of California, CA.)

334. Minamoto K, Nagano M, Inaoka T, Futatsuka M. 2002. Occupational dermatoses among fibreglass-reinforced plastics factory workers. *Contact Dermatitis* 46(6): 339-47. (Support not reported. Authors affiliated with Kumamoto University School of Medicine, Japan.)

335. MnTAP. 2007. *Reducing Volatile Emissions in teh Fiber Reinforced Plastics Industry*. Minneapolis, MN: Minnesota Technical Assistance Program. 4 pp. http://www.mntap.umn.edu. (Supported by the State of Minnesota. Authors affiliated with the University of Minnesota, MN.)

336. Morgan DL, Cooper SW, Carlock DL, Sykora JJ, Sutton B, Mattie DR, McDougal JN. 1991. Dermal absorption of neat and aqueous volatile organic chemicals in the Fischer 344 rat. *Environ Res* 55(1): 51-63. (Supported by the U.S. Air Force. Authors affiliated with NSI, Inc., NC; H.G. Armstrong Aerospace Medical Research Laboratory, OH.)

337. Morgan DL, Mahler JF, O'Connor RW, Price HC, Jr., Adkins B, Jr. 1993b. Styrene inhalation toxicity studies in mice. I. Hepatotoxicity in B6C3F1 mice. *Fundam Appl Toxicol* 20(3): 325-335. (Suport not reported. Authors affiliated with NIEHS; ManTech Environmental Technology, Inc., NC.)

338. Morgan DL, Mahler JF, Dill JA, Price HC, Jr., O'Connor RW, Adkins B, Jr. 1993c. Styrene inhalation toxicity studies in mice. II. Sex differences in susceptibility of B6C3F1 mice. *Fundam Appl Toxicol* 21(3): 317-325. (Support not reported. Authors affiliated with NIEHS; Battelle Pacific Northwest Laboratories, WA; ManTech Environmental Technology, Inc., NC.)

339. Morris JB. 2000. Uptake of styrene in the upper respiratory tract of the CD mouse and Sprague-Dawley rat. *Toxicol Sci* 54(1): 222-228. (Supported by the Styrene Information and Research Center. Authors affiliated with the School of Pharmacy, CT.)

340. Moschel RC, Hemminki K, Dipple A. 1986. Hydrolysis and rearrangement of O^6-substituted guanosine products resulting from reaction of guanosine with styrene oxide. *J Org Chem* 51: 2952-2955. (Supported by NCI and DHHS. Authors affiliated with NCI-Frederick Cancer Research Facility, MD.)

341. Mutti A, Vescovi PP, Falzoi M, Arfini G, Valenti G, Franchini I. 1984. Neuroendocrine effects of styrene on occupationally exposed workers. *Scand J Work Environ Health* 10(4): 225-228. (Supported by the Consiglio Nazionale delle Ricerche, Progetto Finalizzato Medicina Preventiva e Riabilitativa, Malattie del Sistema Nervosa, and by the Department of Social Security, Region Emilia-Romagna. Authors affiliated with University of Parma, Italy.)

342. Mutti A, Smargiassi A. 1998. Selective vulnerability of dopaminergic systems to industrial chemicals: risk assessment of related neuroendocrine changes. *Toxicol Ind Health* 14(1-2): 311-323. (Supported by the European Union. Authors affiliated with University of Parma Medical School, Italy.)

343. Nakajima T, Elovaara E, Gonzalez FJ, Gelboin HV, Raunio H, Pelkonen O, Vainio H, Aoyama T. 1994a. Styrene metabolism by cDNA-expressed human hepatic and pulmonary cytochromes P450. *Chem Res Toxicol* 7(6): 891-896. (Supported by the Ministry of Education, Science and Culture, Japan. Authors affiliated with Shinshu University School of Medicine, Japan; Institute of Occupational Health, Finland; NCI, MD; University of Oulu, Finland; IARC, France.)

344. Nakajima T, Wang RS, Elovaara E, Gonzalez FJ, Gelboin HV, Vainio H, Aoyama T. 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): 637-642. (Supported by the Japan Ministry of Education, Science and Culture. Authors affiliated with Shinshu University School of Medicine, Japan; Institute of Occupational Health, Finland; NCI, MD; IARC, France.)

345. Nakayama S, Nishide T, Horike T, Kishimoto T, Kira S. 2004. Evaluation of the efficiency of respiratory protective equipment based on the biological monitoring of styrene in fibreglass reinforced plastics industries. *J Occup Health* 46(2): 132-40. (Supported by the Okayama Occupational Health Promotion Center. Authors affiliated with Okayama University Graduate School of Medicine and Dentistry, Japan; Okayama Occupational Health Promotion Center, Japan; Okayama Industrial Injury Hospital, Japan.)

346. Nano R, Rossi A, Fenoglio C, Polver PD. 2000. Evaluation of a possible styrene-induced damage to the haematopoietic tissues in the rat. *Anticancer Res* 20(3A):

1615-1619. (Supported by the Commission of the European Communities, CNR and MURST Projects, Italy. Authors affiliated with University of Pavia, Italy; CNR, Italy.)

347. Nazaroff WW, Singer BC. 2004. Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. *J Expo Anal Environ Epidemiol* 14 Suppl 1: S71-7. (Supported by the Cigarette and Tobacco Surtax Fund of the State of California through the Tobacco-Related Research Program of the University of California and the U.S. Department of Energy. Authors affiliated with University of California, CA; Ernest Orlando Lawrence Berkeley National Laboratory, CA.)

348. NCHS. 2000. *Third National Health and Nutrition Examination Survey, 1988-1994, NHANES III Priority Toxicants Reference Range Study Data File (Series 11, No. 4A)*. Hyattsville, MD: U.S. Department of Health and Human Services, National Center for Health Statistics, Centers for Disease Control and Prevention. 58 pp.

349. NCI. 1979a. *Bioassay of Styrene for Possible Carcinogenicity*. Technical Report Series No. 185. Bethesda, MD: National Cancer Institute.

350. NCI. 1979b. *Bioassay of a Solution of beta-Nitrostyrene and Styrene for Possible Carcinogenicity*. Technical Report Series No. 170. Bethesda, MD: National Cancer Institute. 96 pp.

351. Negri S, Maestri L, Andreoli R, Manini P, Mutti A, Imbriani M. 2006. Mercapturic acids of styrene in man: comparability of the results obtained by LC/MS/MS and by HPLC-fluorimeter, and stability of samples under different storage conditions. *Toxicol Lett* 162(2-3): 225-33. (Supported by the European Community. Authors affiliated with Salvatore Maugeri Foundation, Italy; University of Parma, Italy; University of Pavia, Italy.)

352. Nerin C, Acosta D. 2002. Behavior of some solid food simulants in contact with several plastics used in microwave ovens. *J Agric Food Chem* 50(25): 7488-92. (Supported by the Spanish Ministry of Science and Technology and the AECI. Authors affiliated with University of Zaragoza, Spain.)

353. 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. (Support not reported. Authors affiliated with CANTOX Health Sciences International, Canada; NOVA Chemicals, Inc., VA.)

354. Nhamburo PT, Kimura S, McBride OW, Kozak CA, Gelboin HV, Gonzalez FJ. 1990. The human CYP2F gene subfamily: identification of a cDNA encoding a new cytochrome P450, cDNA-directed expression, and chromosome mapping. *Biochemistry* 29(23): 5491-9. (Support not reported. Authors affiliated with National Institutes of Health, MD.)

355. Nichols WK, Mehta R, Skordos K, Mace K, Pfeifer AM, Carr BA, Minko T, Burchiel SW, Yost GS. 2003. 3-methylindole-induced toxicity to human bronchial

epithelial cell lines. *Toxicol Sci* 71(2): 229-36. (Supported by the National Heart, Lung and Blood Institute of the NIH. Authors affiliated with University of Utah, UT; Nestle Research Center, Switzerland; University of New Mexico, NM; GlaxoSmithKline, NC; State University of New Jersey, NJ.)

356. Nicholson WJ, Selikoff IJ, Seidman H. 1978. Mortality experience of styrene-polystyrene polymerization workers. Initial findings. *Scand J Work Environ Health* 4(Suppl 2): 247-252. (Support not reported. Authors affiliated with Mount Sinai School of Medicine, NY; American Cancer Society, NY.)

357. NIOSH. 2008. *International Chemical Safety Cards: Polystyrene*. National Institute for Occupational Safety and Health. Updated on 11/25/03. http://www.cdc.gov/niosh/ipcsneng/neng1043.html. Accessed on 5/1/08.

358. Nishi Y, Hasegawa MM, Taketomi M, Ohkawa Y, Inui N. 1984. Comparison of 6-thioguanine-resistant mutation and sister chromatid exchanges in Chinese hamster V79 cells with forty chemical and physical agents. *Cancer Res* 44(8): 3270-9. (Support not reported. Authors affiliated with the Japan Tobacco and Salt Public Corporation, Japan.)

359. Nishimura M, Yaguti H, Yoshitsugu H, Naito S, Satoh T. 2003. Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sensitivity real-time reverse transcription PCR. *Yakugaku Zasshi* 123(5): 369-75. (Support not reported. Authors affiliated with Otsuka Pharmaceutical Factory, Inc., Japan; Non-Profit Organization Human and Anima Bridging Research Organization, Japan.)

360. NLM. 2008. *Household Products Database*. National Institutes of Health, National Library of Medicine. http://hpd.nlm.nih.gov/. Last accessed: 4/8/08.

361. Nordenson I, Beckman L. 1984. Chromosomal aberrations in lymphocytes of workers exposed to low levels of styrene. *Hum Hered* 34(3): 178-82. (Supported by the Swedish Work Environment Fund and the Nordic Council. Authors affiliated with University of Umea, Sweden.)

362. Norppa H, Sorsa M, Pfaeffli P, Vainio H. 1980a. Styrene and styrene oxide Induce SCEs and are metabolized in human lymphocyte cultures. *Carcinogenesis* 1(4): 357-361. (Supported by the National Research Council for Sciences, Academy of Finland. Authors affiliated with Institute of Occupational Health, Finland.)

363. Norppa H, Hemminki K, Sorsa M, Vainio H. 1981b. Effect of monosubstituted epoxides on chromosome aberrations and SCE in cultured human lymphocytes. *Mutat Res* 91(3): 243-250. (Supported by the National Research Council for Sciences, Academy of Finland. Authors affiliated with Institute of Occupational Health, Finland.)

364. Norppa H, Vainio H. 1983. Induction of sister-chromatid exchanges by styrene analogues in cultured human lymphocytes. *Mutat Res* 116(3-4): 379-87. (Supported

by the Emil Aaltonen Foundation. Authors affiliated with the Institute of Occupational Health, Finland.)

365. Norppa H, Vainio H, Sorsa M. 1983a. Metabolic activation of styrene by erythrocytes detected as increased sister chromatid exchanges in cultured human lymphocytes. *Cancer Res* 43(8): 3579-82. (Supported by the Swedish Work Environment Fund. Authors affiliated with Institute of Occupational Health, Finland.)

366. Norppa H, Tursi F, Einistö P. 1983b. Erythrocytes as a Metabolic Activation System in Mutagenicity Studies. In *Mutagenesis and Genetic Toxicology*. Janiaud P, Averbeck D, Moustacchi E, eds. Paris, France: INSERM. p. 35-50. (Support not reported. Authors affiliated with Institute of Occupational Health, Finland.)

367. Norppa H, Tursi F. 1984. Erythrocyte-Mediated Metabolic Activation Detected by SCE. In *Sister Scromatid Exchange*, vol. 29B. Tice RR, Hollander A, eds. New York: Plenum Press. p. 547-559. (Supported by the Swedish Work Environment Fund and the European Science Foundation. Authors affiliated with Institute of Occupational Health, Finland; Istituto di Ricerche Farmacologiche "Mario Negri," Italy.)

368. Norppa H. 2003. Genetic susceptibility, biomarker responses, and cancer. *Mutat Res* 544(2-3): 339-348. (Supported by the European Union and the Finnish Work Environment Fund. Authors affiliated with the Finnish Institute of Occupational Health, Finland.)

369. NRC. 2008. *National Response Center*. National Response Center. http://www.nrc.uscg.mil/nrchp.html. Accessed on 5/13/08.

370. NSC. 2004. *Styrene Chemical Backgrounder*. National Safety Council. http://www.nsc.org/library/chemical/styrene.htm. Last accessed: 12/29/04.

371. NTP. 1976a. *Report on the Carcinogenesis Bioassay of Chloroform*. Technical Report Series. Bethesda, MA: National Institutes of Health. 70 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

372. NTP. 1976b. *Carcinogenesis Bioassay of Trichloroethylene*. Technical Report Series No. 2. NCI-CG-TR-2. Bethesda, MD: National Institutes of Health. 225 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

373. NTP. 1977. *Bioassay of Tetrachloroethylene for Possible Carcinogenicity*. Technical Report Series No. 13. NCI-CG-TR-13. Bethesda, MD: National Institutes of Health. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

374. NTP. 1978a. *Bioassay of 1,1,2,2-Tetrachloroethane for Possible Carcinogenicity*. Technical Report Series No. 27. NCI-CG-TR-27. Bethesda, MD: National Institutes

of Health. 96 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

375. NTP. 1978b. *Bioassay of Chloropicrin for Possible Carcinogenicity*. Technical Report Series No. 65. NCI-CG-TR-65. Bethesda, MD: National Institutes of Health. 90 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

376. NTP. 1978c. *Bioassay of 1,1-Dichloroethane for Possible Carcinogenicity*. Technical Report Series No. 66. NCI-CG-TR-66. Bethesda, MD: National Institutes of Health. 102 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

377. NTP. 1978d. *Bioassay of Hexachloroethane for Possible Carcinogenicity*. Technical Report Series No. 68. NCI-CG-TR-68. Bethesda, MD: National Institutes of Health. 106 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

378. NTP. 1978e. *Bioassay of Allyl Chloride for Possible Carcinogenicity*. Technical Report Series No. 73. NCI-CG-TR-73. Bethesda, MD: National Institutes of Health. 110 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

379. NTP. 1978f. *Bioassay of 1,1,2-Trichloroethane for Possible Carcinogenicity*. Technical Report Series No. 74. NCI-CG-TR-74. Bethesda, MD: National Institutes of Health. 104 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

380. NTP. 1978g. *Bioassay of 3-Sulfolene for Possible Carcinogenicity*. Technical Report Series No. 102. NCI-CG-TR-102. Bethesda, MD: National Institutes of Health. 102 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

381. NTP. 1978h. *Bioassay of Trichlorofluoromethane for Possible Carcinogenicity*. Technical Report Series No. 106. NCI-CG-TR-106. Bethesda, MD: National Institutes of Health. 99 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

382. NTP. 1978i. *Bioassay of Iodoform for Possible Carcinogenicity*. Technical Report No. 110. NC-CG-TR-110. Bethesda, MD: National Institutes of Health. 105 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

383. NTP. 1979a. *Bioassay of a Solution of Beta-Nitrostyrene and Styrene for Possible Carcinogenicity*. Technical Report Series No. 170. NCI-CG-TR-170. Bethesda, MD: National Institutes of Health. 98 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

384. NTP. 1979b. *Bioassay of Styrene for Possible Carcinogenicity.* Technical Report Series No. 185. NCI-CG-TR-185. Bethesda, MD: National Institutes of Health. 108 pp. http://ntp.niehs.nih.gov/?objectid=D16D6C59-F1F6-975E-7D23D1519B8CD7A5.

385. NTP. 1990a. *Toxicology and Carcinogenesis Studies of Vinyl Toluene (Mixed Isomers) (65%-71% meta-Isomer and 32%-35% para-Isomer) in F344/N Rats and B6C3F$_1$ mice (Inhalation Studies)*. Technical Report Series No. 375. NIH Publication No. 90-2830. Research Triangle Park, NC: National Toxicology Program. 193 pp.

386. NTP. 1990b. *Toxicology and Carcinogenesis Studies of alpha-Methylbenzyl Alcohol in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)*. Technical Report Series No. 369. NIH Pub. No. 89-2824. Research Triangle Park, NC: National Toxicology Program. 177 pp.

387. NTP. 2000. *NTP Technical Report on the Toxicology and Carcinogenesis Studies of Naphthalene in F344/N Rats (Inhalation Studies)*. NTP TR 500. NIH Publication No. 01-4434. Research Triangle Park, NC: National Toxicology Program. 176 pp.

388. NTP. 2004. *Report on Carcinogens* 11th ed., Research Triangle Park, NC: National Toxicology Program. p. III-278.

389. NTP. 2006. *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Styrene*. NIH Publication No. 06-4475. Research Triangle Park, NC: National Toxicology Program. 190 pp.

390. NTP. 2007. *NTP Technical Report on the Toxicology and Carcinogenesis Studies of alpha-Methylstyrene in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)*. NTP TR 543. NIH Publication No. 08-4474. Research Triangle Park, NC: National Toxicology Program. 216 pp.

391. Nylander-French LA, Kupper LL, Rappaport SM. 1999. An investigation of factors contributing to styrene and styrene-7,8-oxide exposures in the reinforced-plastics industry. *Ann Occup Hyg* 43(2): 99-105. (Supported by the Finnish Work Environment Fund, NIEHS and NCI. Authors affiliated with University of North Carolina, NC.)

392. Oberheitmann B, Frentzel-Beyme R, Hoffmann W. 2001. An application of 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(1): 23-29. (Support not reported. Authors affiliated with University of Bremen, Germany; Bremen Institute for Prevention Research, Germany.)

393. Oesch F, Herrero ME, Hengstler JG, Lohmann M, Arand M. 2000. Metabolic detoxification: implications for thresholds. *Toxicol Pathol* 28(3): 382-387. (Supported by the Deutsche Forschungsgemeinschaft and the European Community. Authors affiliated with University of Mainz, Germany.)

394. Ohashi Y, Nakai Y, Ikeoka H, Koshimo H, Nakata J, Esaki Y, Horiguchi S, Teramoto K. 1986. Degeneration and regeneration of respiratory mucosa of rats after exposure to styrene. *J Appl Toxicol* 6(6): 405-12. (Support not reported. Authors affiliated with Osaka City University Medical School, Japan.)

395. 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. (Support not reported. Authors affiliated with Nissin Food Products Co., Ltd., Japan.)

396. Ohno K, Azuma Y, Date K, Nakano S, Kobayashi T, Nagao Y, Yamada T. 2002. Estrogenicity of styrene oligomers and assessment of estrogen receptor binding assays. *Environ Health Perspect* 110(7): A384-5; author reply A385-6. (Support not reported. Authors affiliated with Nissin Food Products Co., Ltd., Japan.)

397. Ohno K, Azuma Y, Date K, Nakano S, Kobayashi T, Nagao Y, Yamada T. 2003. Evaluation of styrene oligomers eluted from polystyrene for estrogenicity in estrogen receptor binding assay, reporter gene assay, and uterotrophic assay. *Food Chem Toxicol* 41(1): 131-41. (Support not reported. Authors affiliated with Nissin Food Products Co., Ltd., Japan.)

398. Ohyama KI, Nagai F, Tsuchiya Y. 2001. Certain styrene oligomers have proliferative activity on MCF-7 human breast tumor cells and binding affinity for human estrogen receptor. *Environ Health Perspect* 109(7): 699-703. (Support not reported. Authors affiliated with Tokyo Metropolitan Research Laboratory of Public Health, Japan; Kogakuin University, Japan.)

399. Okun AH, Beaumont JJ, Meinhardt TJ, Crandall MS. 1985. Mortality patterns among styrene-exposed boatbuilders. *Am J Ind Med* 8(3): 193-205. (Support not reported. Authors affiliated with NIOSH.)

400. 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 Mol Mutagen* 31(4): 311-315. (Support not reported. Authors affiliated with Finnish Institute of Occupational Health, Finland.)

401. Ong CN, Shi CY, Chia SE, Chua SC, Ong HY, Lee BL, Ng TP, Teramoto K. 1994. Biological monitoring of exposure to low concentrations of styrene. *Am J Ind Med* 25(5): 719-30. (Supported by the Singapore Turf Club. Authors affiliated with National University of Singapore, Singapore; Osaka City University Medical School, Japan.)

402. 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): 445-460. (Support not reported. Authors affiliated with the Finnish Institute of Occupational Health, Finland.)

403. 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(1-2): 111-116. (Supported by the Styrene Steering Committee of the European Chemical Industry Council. Authors affiliated with University of Wurzburg, Germany.)

404. 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): 575-584. (Support not reported. Authors affiliated with NIEHS; Hebrew University, Israel.)

405. Painter SL, Zegar IS, Tamura PJ, Bluhm S, Harris CM, Harris TM, Stone MP. 1999. Influence of the *R*(61,2)- and *S*(61,2)-alpha-(N^6-adenyl)styrene oxide adducts on the A•C mismatched base pair in an oligodeoxynucleotide containing the human *N-ras* codon 61. *Biochemistry* 38(27): 8635-8646. (Supported by NIH, University of Wisconsin, USDA and Vanderbilt Center in Molecular Toxicology. Authors affiliated with Vanderbilt University, TN; Volunteer State Community College, TN; State University, KS.)

406. 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. (Supported by the Commission of European Communities. Authors affiliated with Istituto oli Ricerche Farmacologiche "Mario Negri" Italy; Academy of Medicine, Poland.)

407. Parent ME, Hua Y, Siemiatycki J. 2000. Occupational risk factors for renal cell carcinoma in Montreal. *Am J Ind Med* 38(6): 609-618. (Supported by Health Canada, the National Cancer Institute of Canada, the Institut de recherche en sante du Quebec, the Fonds de la recherche en sante du Quebec, and the Medical Research Council of Canada. Authors affiliated with INRS-Institut Armand-Frappier, Canada; McGill University, Canada.)

408. Pauwels W, Vodicèka P, Severi M, Plná K, Veulemans H, Hemminki K. 1996. Adduct formation on DNA and haemoglobin in mice intraperitoneally administered with styrene. *Carcinogenesis* 17(12): 2673-2680. (Supported by the EU Environment and PECO Program, the Swedish Medical Council, the National Environmental Protection Board, the Swedish Cancer Fund, the Belgian Incentive Program for Health Hazards, and the Services of the Prime Minister, Czech Ministry of Health. Authors affiliated with Katholieke Universiteit Leuven, Belgium; Czech Academy of Sciences, Czech Republic; Karolinska Institute, Sweden.)

409. Pauwels W, Veulemans H. 1998. Comparison of ethylene, propylene and styrene 7,8-oxide in vitro adduct formation on N-terminal valine in human haemoglobin and on N-7-guanine in human DNA. *Mutat Res* 418(1): 21-33. (Supported by the Belgian Program for Health Hazards and the Services of the Prime Minister, Scientific, Technical and Cultural Affairs. Authors affiliated with Katholieke Universiteit Lewen, Belgium.)

410. Payne-Sturges DC, Burke TA, Breysse P, Diener-West M, Buckley TJ. 2004a. Personal exposure meets risk assessment: a comparison of measured and modeled exposures and risks in an urban community. *Environ Health Perspect* 112(5): 589-98. (Supported by U.S. EPA, Mickey Leland National Center for Urban Air Toxics Research, Johns Hopkins Risk Science and Public Policy Institute, Johns Hopkins Center for Urban Environmental Health, and the Maryland Cigarette Restitution Fund. Authors affiliated with Johns Hopkins University, MD.)

411. Pekari K, Nylander-French L, Pfaffli P, Sorsa M, Aitio A. 1993. Biological monitoring of exposure to styrene - assessment of different approaches. *J Occup Med Toxicol* 2(2): 115-126. (Supported by the Finnish Work Environment Fund. Authors affiliated with Institute of Occupational Health, Finland.)

412. Pelkonen O, Raunio H. 1997. Metabolic activation of toxins: tissue-specific expression and metabolism in target organs. *Environ Health Perspect* 105 Suppl 4: 767-74. (Supported by the Academy of Finland. Authors affiliated with University of Oulu, Finland.)

413. 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(2): 119-23. (Supported by the National Research Council for Sciences (Academy of Finland.) Authors affiliated with Institute of Occupational Health, Finland.)

414. Perera FP, Weinstein IB. 2000. Molecular epidemiology: recent advances and future directions. *Carcinogenesis* 21(3): 517-524. (Supported by NIH, Cancer Center Core, NIEHS, U.S. Army, Department of Energy, Gladys and Roland Harriman Foundation, the Bauman Family Foundation, the Robert Wood Johnson Foundation, W. Alton Jones Foundation, New York Community Trust, Irving A. Hansen memorial Foundation, the National Foundation for Cancer Research, T.J. Martell Foundation, and the Alma Toorock Memorial for Cancer Research. Authors affiliated with Columbia University, NY.)

415. Pero RW, Bryngelsson T, Hoegstedt B, Akesson B. 1982. Occupational and in vitro exposure to styrene assessed by unscheduled DNA synthesis in resting human lymphocytes. *Carcinogenesis* 3(6): 681-685. (Supported by the Swedish Council for Planning and Coordination of Research in "Chemical Health Risks in our Environment" by the Swedish Workers Protection Fund and the National Board of Health and Social Welfare in Sweden. Authors affiliated with University of Lund, Sweden; Lund University Hospital, Sweden.)

416. Pezzagno G, Ghittori S, Imbriani M, Capodaglio E. 1985. Urinary elimination of styrene in experimental and occupational exposure. *Scand J Work Environ Health* 11(5): 371-380. (Support not reported. Authors affiliated with University of Pavia, Italy; Medical Centre of Pavia, Italy.)

417. Pfaffli P, Saamanen A. 1993. The Occupational Scene of Styrene. In *Butadiene and Styrene: Assessment of Health Hazards*, IARC Scientific Publications No. 127.

Sorsa M, Peltonen K, Vainio H, Hemminki K, eds. Lyon, France: International Agency for Research on Cancer. pp. 15-26. (Support not reported. Authors affiliated with Institute of Occupational Health, Finland; Technical Research Center of Finland.)

418. Pfäffli P, Hesso A, Vainio H, Hyvönen M. 1981. 4-Vinylphenol excretion suggestive of arene oxide formation in workers occupationally exposed to styrene. *Toxicol Appl Pharmacol* 60(1): 85-90. (Support not reported. Authors affiliated with Institute of Occupational Health, Finland; Muncipal Health Center, Finland.)

419. Phillips DH, Farmer PB. 1994. Evidence for DNA and protein binding by styrene and styrene oxide. *Crit Rev Toxicol* 24(46): S35-46. (Supported by the Medical Research Council and the Cancer Research Campaign. Authors affiliated with Haddow Laboratories, UK; University of Leicester, UK.)

420. Pinches SE, Apps P. 2007. Production in food of 1,3-pentadiene and styrene by Trichoderma species. *Int J Food Microbiol* 116(1): 182-5. (Support not reported. Authors affiliated with Council for Scientific and Industrial Research, South Africa.)

421. Pinkerton KE, Avadhanam KP, Peake JL, Plopper CG. 1997. Tracheobronchial Airways. In *Comprehensive Toxicology*, vol. 8. Sipes IG, McQueen CA, Gandolfi AJ, eds. Oxford, UK: Elseviers Science, Ltd. p. 23-44. (Support not reported. Authors affiliated with University of California - Davis, CA.)

422. Plopper CG, Hill LH, Mariassy AT. 1980a. Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung. III. A study of man with comparison of 15 mammalian species. *Exp Lung Res* 1(2): 171-180. (Support not reported. Authors affiliated with NIEHS; University of California Davis, CA.)

423. Plopper CG, Mariassy AT, Hill LH. 1980b. Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung: I. A comparison of rabbit, guinea pig, rat, hamster, and mouse. *Exp Lung Res* 1(2): 139-154. (Support not reported. Authors affiliated with NIEHS; University of California Davis, CA.)

424. Plotnick HB, Weigel WW. 1979. Tissue distribution and excretion of ^{14}C-styrene in male and female rats. *Res Commun Chem Pathol Pharmacol* 24(3): 515-524. (Support not reported. Authors affiliated with NIOSH.)

425. 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. (Support not reported. Authors affiliated with Charles University, Czechoslovakia; Psychiatric Research Institute, Czechoslovakia.)

426. Ponomarkov V, Tomatis L. 1978. Effects of long-term oral administration of styrene to mice and rats. *Scand J Work Environ Health* 4(Suppl 2): 127-135. (Support not reported. Authors affiliated with IARC, France.)

427. Ponomarkov V, Cabral JR, Wahrendorf J, Galendo D. 1984. A carcinogenicity study of styrene-7,8-oxide in rats. *Cancer Lett* 24(1): 95-101. (Support not reported. Authors affiliated with IARC, France; Cancer Research Center, Russia.)

428. Qian C, Dipple A. 1995. Different mechanisms of aralkylation of adenosine at the 1- and N^6-positions. *Chem Res Toxicol* 8(3): 389-395. (Supported by NCI and DHHS. Authors affiiated with NCI-Frederick Cancer Research and Development Center, MD; Reproductive Technology Laboratories, CA.)

429. Rahman Q, Abidi P, Afaq F, Schiffmann D, Mossman BT, Kamp DW, Athar M. 1999. Glutathione redox system in oxidative lung injury. *Crit Rev Toxicol* 29(6): 543-568. (Support not reported. Authors affiliated with Industrial Toxicology Research Center, India; Hamdard University, India; Northwestern Unversity Medical School, IL; University of Vermont College of Medicine, VT; University of Rostock, Germany.)

430. Ramsey JC, Young JD, Karbowski RJ, Chenoweth MB, McCarty LP, Braun WH. 1980. Pharmacokinetics of inhaled styrene in human volunteers. *Toxicol Appl Pharmacol* 53(1): 54-63. (Support not reported. Authors affiliated with Dow Chemical Company, MI.)

431. Ramsey JC, Andersen ME. 1984. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol Appl Pharmacol* 73(1): 159-75. (Support not reported. Authors affiliated with Dow Chemical, USA, MI; Air Force Aerospace Medical Research Laboratory, OH.)

432. 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): 5410-5416. (Supported by NIOSH. Authors affiliated with University of North Carolina, NC; University of Californa, CA; Electric Power Research Institute, CA.)

433. Raunio H, Rautio A, Pelkonen O. 1999. The CYP2A subfamily: function, expression and genetic polymorphism. *IARC Sci Publ*(148): 197-207. (Supported by the Finnish Academy of Sciences and the European Commission Biomed1 and Biomed2 Programmes. Authors affiliated with University of Oulu, Finland.)

434. Rennix CP, Quinn MM, Amoroso PJ, Eisen EA, Wegman DH. 2005. Risk of breast cancer among enlisted Army women occupationally exposed to volatile organic compounds. *Am J Ind Med* 48(3): 157-67. (Supported by Breast Cancer Research Grants Program and the Massachusetts Department of Public Health. Authors affiliated with Navy Environmental Health Center, VA; University of Massachusetts, MA; U.S. Army Research Institute for Environmental Medicine, MA.)

435. Riihimäki V, Pfäffli P. 1978. Percutaneous absorption of solvent vapors in man. *Scand J Work Environ Health* 4(1): 73-85. (Support not reported. Authors affiliated with Institute of Occupational Health, Finland.)

436. Roberts ES, Alworth WL, Hollenberg PF. 1998. Mechanism-based inactivation of cytochromes P450 2E1 and 2B1 by 5-phenyl-1-pentyne. *Arch Biochem Biophys* 354(2): 295-302. (Supported by NIH. Authors affiliated with University of Michigan, MI; Tulane University, LA.)

437. Roder-Stolinski C, Fischader G, Oostingh GJ, Feltens R, Kohse F, von Bergen M, Morbt 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*. (Support not reported. Authors affiliated with UFZ - Helmholtz Centre for Environmental Research, Germany; University of Salzburg, Austria; Martin-Luther-University Halle-Wittenberg, Germany.)

438. Rodriguez-Arnaiz R. 1998. Biotransformation of several structurally related 2B compounds to reactive metabolites in the somatic *w/w+* assay of *Drosophila melanogaster*. *Environ Mol Mutagen* 31(4): 390-401. (Support not reported. Authors affiliated with Facultad de Ciencias, Mexico.)

439. 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): 165-176. (Support not reported. Authors affiliated with NIOSH.)

440. Säämänen A. 1998. *Methods to Control Styrene Exposure in the Reinforced Plastics Industry*. VTT Publications 354. Technical Research Centre of Finland. (Support not reported. Author affiliated with VTT Manufacturing Technology.)

441. Sandell J, Marniemi J, Parkki MG, Aitio A. 1978. Effects of inhalation and cutaneous exposure to styrene on drug metabolism enzymes in the rat. *Res Commun Chem Pathol Pharmacol* 19(1): 109-118. (Supported by NIH and the Juho Vainio Foundation, Finland. Authors affiliated with University of Turku, Finland; Social Insurance Institution, Finland.)

442. Santos-Burgoa C, Matanoski GM, Zeger S, Schwartz L. 1992. Lymphohematopoietic cancer in styrene-butadiene polymerization workers. *Am J Epidemiol* 136(7): 843-854. (Supported by the International Institute of Synthetic Rubber Producers. Authors affiliated with Escuela de Salud Publica de Mexico; Johns Hopkins University School of Hygiene and Public Health, MD.)

443. Sapkota A, Williams D, Buckley TJ. 2005. Tollbooth workers and mobile source-related hazardous air pollutants: how protective is the indoor environment? *Environ Sci Technol* 39(9): 2936-43. (Supported by the Johns Hopkins Education and Research Center Pilot Project Research Training Fund, NIEHS and EPA. Authors affiliated with Johns Hopkins Bloomberg School of Public Health, MD.)

444. 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. (Supported by the Styrene Information and Research Center. Authors affiliated with

The K.S. Crump Group, Inc., NC; ToxWorks, NJ; ENVIRON International Corporation, LA; Colorado State University, CO.)

445. Sasaki YF, Izumiyama F, Nishidate E, Matsusaka N, Tsuda S. 1997. Detection of rodent liver carcinogen genotoxicity by the alkaline single-cell gel electrophoresis (Comet) assay in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow). *Mutat Res* 391(3): 201-14. (Supported by the tutikawa Memorial Fund for Study in Mammalian Mutagenicity and Shirakaba Farm Co. Ltd. Authors affiliated with Hachinohe National College of technology, Japan; Iwate University, Japan.)

446. Sathiakumar N, Delzell E, Hovinga M, Macaluso M, Julian JA, Larson R, Cole P, Muir DCF. 1998. Mortality from cancer and other causes of death among synthetic rubber workers. *Occup Environ Med* 55(4): 230-235. (Supported by the International Institute of Synthetic Rubber Producers. Authors affiliated with University of Alabama, AL; McMaster University; Texas A & M University, TX.)

447. Sathiakumar N, Graff J, Macaluso M, Maldonado G, Matthews R, Delzell E. 2005. An updated study of mortality among North American synthetic rubber industry workers. *Occup Environ Med* 62(12): 822-829. (Support not reported. Authors affiliated with University of Alabama at Birmingham, AL; Wayne State University School of Medicine, MI; University of Minnesota, MN.)

448. Savela K, Hesso A, Hemminki K. 1986. Characterization of reaction products between styrene oxide and deoxynucleosides and DNA. *Chem Biol Interact* 60(3): 235-246. (Supported by the Swedish Work Environment Fund. Authors affiliated with Institute of Occupational Health, Finland.)

449. Scélo G, Constantinescu V, Csiki I, Zaridze D, Szeszenia-Dabrowska N, Rudnai P, Lissowska J, Fabiánová E, Cassidy A, Slamova A, Foretova L, Janout V, Fevotte J, Fletcher T, Mannetje A, Brennan P, Boffetta P. 2004. Occupational exposure to vinyl chloride, acrylonitrile and styrene and lung cancer risk (Europe). *Cancer Causes Control* 15(5): 445-452. (Supported by the European Commisson's INCO-COPERNICUS Programme, the Polish State Committee for Scientific Research and IARC. Authors affiliated with IARC, France; Institute of Hygiene, Romania; Cancer Research Center,Russia; Institute of Occupational Medicine, Poland; National Institute of Environmental Health, Hungary; Cancer Center and M. Sklodowska-Curie Institute of Oncology, Poland; Specialized State Health Institute, Slovakia; University of Liverpool, UK; Charles University, Czech Republic; Masaryk Cancer Institute, Czech Republic; Palacky University of Medicine, Czech Republic; Universite Claude Bernard, France; London School of Hygiene and Tropical Medicine, UK.)

450. Scott D, Preston RJ. 1994a. A re-evaluation of the cytogenetic effects of styrene. *Mutat Res* 318(3): 175-203. (Supported by the Styrene Steering Committee and the Cancer Research Campaign. Authors affiliated with Paterson Institute for Cancer Research, UK; Chemical Industry Institute of Toxicology, NC.)

451. Scott D, Preston RJ. 1994b. A critical review of the cytogenetic effects of styrene with an emphasis on human population monitoring: a synopsis. *Crit Rev Toxicol* 24(Suppl 1): S47-8. (Support not reported. Authors affiliated with Paterson Institute for Cancer Research, UK; Chemical Industry Institute of Toxicology, NC.)

452. Seidler A, Mohner M, Berger J, Mester B, Deeg E, Elsner G, Nieters A, Becker N. 2007. Solvent exposure and malignant lymphoma: a population-based case-control study in Germany. *J Occup Med Toxicol* 2: 2. (Supported by the Federal Office for Radiation Protection, the European Community and the German Research Foundation. Authors affiliated with Federal Institute of Occupational Safety and Health, Germany; University Medical Center Hamburg-Eppendorf, Germany; Johann Wolfgang Goethe-University, Germany; Bremen Institute for Prevention Research and Social Medicine, Germany; Cancer Research Center, Germany.)

453. 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): 165-169. (Supported by the Swiss Federal Research Station. Authors affiliated with the Intercantonal Office for the Control of Medicines, Switzerland.)

454. Seliskar M, Rozman D. 2007. Mammalian cytochromes P450--importance of tissue specificity. *Biochim Biophys Acta* 1770(3): 458-66. (Supported by the Slovenian Research Agency, Vanderbilt University, the European Community and the AARS. Authors affiliated with University of Ljubljana, Slovenia.)

455. Serdar B, Tornero-Velez R, Echeverria D, Nylander-French LA, Kupper LL, Rappaport SM. 2006. Predictors of occupational exposure to styrene and styrene-7,8-oxide in the reinforced plastics industry. *Occup Environ Med* 63(10): 707-12. (Supported by NCI and NIEHS. Authors affiliated by University of North Carolina, NC; University of Washington, WA.)

456. Sexton K, Adgate JL, Church TR, Ashley DL, Needham LL, Ramachandran G, Fredrickson AL, Ryan AD. 2005. Children's exposure to volatile organic compounds as determined by longitudinal measurements in blood. *Environ Health Perspect* 113(3): 342-349. (Supported by the U.S. EPA, the National Center for Environmental Research, and the Legislative Commission on Minnesota Resources. Authors affiliated with University of Texas School of Public Health, TX; University of Minnesota, MN; CDC.)

457. Sexton K, Adgate JL, Fredrickson AL, Ryan AD, Needham LL, Ashley DL. 2006. Using biologic markers in blood to assess exposure to multiple environmental chemicals for inner-city children 3-6 years of age. *Environ Health Perspect* 114(3): 453-9. (Supported by the University of Minnesota and the U.S. EPA. Authors affiliated with University of Texas School of Public Health, TX; University of Minnesota School of Public Health, MN; Centers for Disease Control and Prevention, GA.)

458. Sexton K, Mongin SJ, Adgate JL, Pratt GC, Ramachandran G, Stock TH, Morandi MT. 2007. Estimating volatile organic compound concentrations in selected microenvironments using time-activity and personal exposure data. *J Toxicol Environ Health A* 70(5): 465-76. (Supported by the U.S. EPA. Author affiliated with University of Texas School of Public Health, TX; University of Minnesota, MN; Minnesota Pollution Control Agency, MN.)

459. Shamy MY, Osman HH, Kandeel KM, Abdel-Moneim NM, El Said KF. 2002. DNA single strand breaks induced by low levels of occupational exposure to styrene: the gap between standards and reality. *J Environ Pathol Toxicol Oncol* 21(1): 57-61. (Support not reported. Authors affiliated with Alexandria University, Egypt; King Abdul Aziz University, Saudia Arabia.)

460. Sheets PL, Yost GS, Carlson GP. 2004. Benzene metabolism in human lung cell lines BEAS-2B and A549 and cells overexpressing CYP2F1. *J Biochem Mol Toxicol* 18(2): 92-9. (Supported by NIH. Authors affiliated with Purdue University, IN; University of Utah, UT.)

461. Shield AJ, Sanderson BJS. 2001. Role of glutathione *S*-transferase mu (GSTM1) in styrene-7,8- oxide toxicity and mutagenicity. *Environ Mol Mutagen* 37(4): 285-289. (Supported by the Wenkart Foundation, the Australian Postgraduate Award, Flinders University and Flinders Medical Center Foundation. Authors affiliated with Flinders University of South Australia.)

462. 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): 61-68. (Supported by the Wenkart Foundation, the Australian Postgraduate Award, Flinders University and Flinders Medical Center Foundation. Authors affiliated with Flinders University of South Australia.)

463. 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: 637-651. (Support not reported. Authors affiliated with Sielken and Associates Consulting, Inc., TX.)

464. Siemiatycki J. 1991. *Risk Factors for Cancer in the Workplace*, Boca Raton: CRC Press. p. 210. (Support not reported. Authors affiliated with Universite du Quebec, Canada.)

465. Simeonov MF, Tamura PJ, Wilkinson AS, Harris CM, Harris TM, Stone MP. 2000. Sequence- and stereospecific conformational rearrangement of styrene oxide adducts located at A•C mismatched base pairs. *Biochemistry* 39(5): 924-937. (Supported by the NIH, the Vanderbilt Center in Molecular Toxicology, University of Wisconsin, NSF and the USDA. Authors affiliated with Bulgarian Academy of Sciences, Bulgaria; Vanderbilt University, TN.)

466. Simmonds AC, Ghanayem BI, Sharma A, Reilly CA, Millen B, Yost GS, Forkert PG. 2004. Bioactivation of 1,1-dichloroethylene by CYP2E1 and CYP2F2 in murine lung. *J Pharmacol Exp Ther* 310(3): 855-64. (Supported by the Canadian Institute of Health Research, the National Cancer Institute of Canada, the U.S. Public Health Service, the National Heart, Lung and Blood Institute, the Cancer Research Society of Canada and the Queen's University Principal's Development Fund. Authors affiliated with Queen's University, Canada; NIEHS, NC; University of Utah, UT.)

467. Sina JF, Bean CL, Dysart GR, Taylor VI, Bradley MO. 1983. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. *Mutat Res* 113(5): 357-391. (Support not reported. Authors affiliated with Merck Institute for Therapeutic Research, PA.)

468. Sinsheimer JE, Chen R, Das SK, Hooberman BH, Osorio S, You Z. 1993. The genotoxicity of enantiomeric aliphatic epoxides. *Mutat Res* 298(3): 197-206. (Supported by NIEHS, DHHS. Authors affiliated with University of Michigan, MI.)

469. Sliwinska-Kowalska M, Prasher D, Rodrigues CA, Zamyslowska-Szmytke E, Campo P, Henderson D, Lund SP, Johnson AC, Schaper M, Odkvist L, Starck J, Toppila E, Schneider E, Moller C, Fuente A, Gopal KV. 2007. Ototoxicity of organic solvents - from scientific evidence to health policy. *Int J Occup Med Environ Health* 20(2): 215-22. (Supported by the 6th European Framework Programme and the Marie Curie Host Fellowship for the Transfer of Knowledge "NoiseHear" Project. Authors affiliated with Nofer Institute of Occupational Medicine, Poland; University College, UK; Institut National de Recherche et de Sécurité, France; SUNY at Buffalo, NY; National Research Centre for the Working Environment, Denmark; Karolinska Institutet, Sweden; Institut für Arbeitsphysiologie an der Universität, Germany; University Hospital, Sweden; Finnish Institute of Occupational Health, Finland; European Agency for Safety and Health at Work, Spain; Sahlgrenska University Hospital, Sweden; University of Hong Kong, China; University of North Texas, TX.)

470. Slyskova J, Dusinska M, Kuricova M, Soucek P, Vodickova L, Susova S, Naccarati A, Tulupova E, Vodicka P. 2007. Relationship between the capacity to repair 8-oxoguanine, biomarkers of genotoxicity and individual susceptibility in styrene-exposed workers. *Mutat Res* 634(1-2): 101-11. (Supported by the Centre of Excellence in Environmental Health, 6th FP EU DIEPHY, Internal Grant Agency of Czech Ministry of Health and the Grant Agency of the Czech Republic. Authors affiliated with Slovak Medical University, Slovak Republic; Academy of Sciences of the Czech Republic, Czech Republic; Norwegian Institute for Air Research, Norway; National Institute of Public Health, Czech Republic.)

471. Somers GI, Lindsay N, Lowdon BM, Jones AE, Freathy C, Ho S, Woodrooffe AJ, Bayliss MK, Manchee GR. 2007. A comparison of the expression and metabolizing activities of phase I and II enzymes in freshly isolated human lung parenchymal cells and cryopreserved human hepatocytes. *Drug Metab Dispos* 35(10): 1797-805.

(Support not reported. Authors affiliated with GlaxoSmithKline, UK; Bichemical Pharmacology, Asterand UK Ltd., UK.)

472. Somorovská M, Jahnová E, Tulinská J, Zámecníková M, Šarmanová J, Terenová A, Vodicková L, Líšková A, Vallová B, Soucek P, Hemminki K, Norppa H, Hirvonen A, Tates AD, Fuortes L, Dušinská M, Vodicka P. 1999. Biomonitoring of occupational exposure to styrene in a plastics lamination plant. *Mutat Res* 428(1-2): 255-269. (Supported by the Slovak Grant Agency of Ministry of Health, IGA, Czech Ministry of Health, Swedish Work Environment Fund, and NIEHS. Authors affiliated with Institute of Preventive and Clinical Medicine, Slovak Republic; National Institute of Public Health, Slovak Republic; National Institute of Public Health, Czech Republic; Karolinska Institute, Sweden; Finnish Institute of Occupational Health, Finland; Leiden University Medical Center, Netherlands; University of Iowa, IA; Czech Academy of Sciences, Czech Republic.)

473. 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. (Supported by the Finnish Work Environment Fund and by the CEC/DGXII/Health effects of pollutants. Authors affiliated with Institute of Occupational Health, Finland.)

474. SPA. 2008. *Styrene Monomer: Environmental, Health, Safety, Transport and Storage Guidelines*. Brussels, Belgium: Styrene Producers Association. 67 pp. http://www.styrenemonomer.org/environment-health-safety-guidelines.pdf. (Supported by member companies of CEFIC. Authors affiliated with the European Chemical Industry Council.)

475. Speit G, Henderson L. 2005. Review of the in vivo genotoxicity tests performed with styrene. *Mutat Res* 589: 67-79. (Supported by the Styrene Steering Committee of the European Chemical Industry Council. Authors affiliated with Universitätsklinikum Ulm, Germany; Henderson Scientific Consultancy, UK.)

476. Spencer HC, Irish DD, Adams EM, Rowe VK. 1942. The response of laboratory animals to monomeric styrene. *J Indust Hyg Toxicol* 24(10): 295-301. (Supported not reported. Authors affiliated with Dow Chemical Company, MI.)

477. Steele DH, Thornburg MJ, Stanley JS, Miller RR, Brooke R, Cushman JR, Cruzan G. 1994. Determination of styrene in selected foods. *J Agric Food Chem* 42: 1661-1665. (Support not reported. Authors affiliated with Midwest Research Institute, MO; Dow Chemical Company, MI; GE Plastics, WV; Chevron research and Technology Company, CA; Mobil Oil Corporation, NJ.)

478. Stoffers NH, Stormer A, Bradley EL, Brandsch R, Cooper I, Linssen JP, Franz R. 2004. Feasibility study for the development of certified reference materials for specific migration testing. Part 1: initial migrant concentration and specific migration. *Food Addit Contam* 21(12): 1203-16. (Supported by the European Union.

Authors affiliated with Fraunhofer Institute for Process Engineering and Packaging, Germany; Department for Environment, Food and Rural Affairs, UK; FABES Research, Inc., Germany; PIRA International, UK; Wageningen University and Research Centre, Netherlands.)

479. Stott WT, Johnson KA, Bahnemann R, Day SJ, McGuirk RJ. 2003. Evaluation of potential modes of action of inhaled ethylbenzene in rats and mice. *Toxicol Sci* 71(1): 53-66. (Supported by the Styrenics Steering Committee. Authors affiliated with Dow Chemical Company, MI; BASF AG, Germany.)

480. Sumner S, Ghanayem B, Asgharian B, Williams C, Chanas B, Gonzalez F, Fennell T. 2001. The role of cytochrome P450 in the metabolism of [^{13}C/^{14}C]styrene. *Toxicologist* 60: 403. (Support not reported. Authors affiliated with CIIT, NC; NIEHS, NC; NCI, MD.)

481. 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. (Supported by the Styrene Information Research Center. Authors affiliated with Chemical Industry Institute of Toxicology, NC.)

482. Sumner SJ, Fennell TR. 1994. Review of the metabolic fate of styrene. *Crit Rev Toxicol* 24(S1): S11-33. (Support not reported. Authors affiliated with the Chemical Industry Institute of Toxicology, NC.)

483. 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(5): 336-344. (Supported by NIOSH, and the European Commission. Authors affiliated with University of Texas School of Public Health, TX; University of Parma Medical School, Italy.)

484. Tang W, Hemm I, Eisenbrand G. 2000. Estimation of human exposure to styrene and ethylbenzene. *Toxicology* 144(1-3): 39-50. (Support not reported. Authors affiliated with University of Kaiserslautern, Germany.)

485. Tates AD, Grummt T, van Dam FJ, de Zwart F, Kasper FJ, Rothe R, Stirn H, Zwinderman AH, Natarajan AT. 1994. Measurement of frequencies of HPRT mutants, chromosomal aberrations, micronuclei, sister-chromatid exchanges and cells with high frequencies of SCEs in styrene/dichloromethane-exposed workers. *Mutat Res* 313(2-3): 249-262. (Supported by the EEC Environmental Research Program: Biomonitoring Human Exposure to Environmental Genotoxic Chemicals. Authors affiliated with State University of Leiden, Netherlands; Federal Health Office, Germany; J.A. Cohen Institute, Netherlands; Federal Institute for Occupational Health, Germany.)

486. Teixeira JP, Gaspar J, Silva S, Torres J, Silva SN, Azevedo MC, Neves P, Laffon B, Méndez J, Gonçalves C, Mayan O, Farmer PB, Rueff J. 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): 231-242. (Supported by the European Commission, Comissão de Fomento da Investigação em Cuidados de Saúde - Ministerio da Saúde, Fundação da Ciência e Tecnologia and Xunta de Galicia. Authors affiliated with National Institute of Health, Portugal; Faculty of Medical Sciences UNL, Portugal; ULHT, Portugal; University of A Coruña, Spain; University of Leicester, UK.)

487. Thiess AM, Fleig I. 1978. Chromosome investigations on workers exposed to styrene/polystyrene. *J Occup Med* 20(11): 747-9. (Support not reported. Authors affiliated with BASF Aktiengesellschaft, Germany.)

488. Thiess AM, Friedheim M. 1978. Morbidity among persons employed in styrene production, polymerization and processing plants. *Scand J Work Environ Health* 4(Suppl 2): 203-214. (Support not reported. Authors affiliated with BASF Aktiengesellschaft, Germany.)

489. 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. (Support not reported. Authors affiliated with BASF Aktiengesellschaft, Germany; Institute fur Anthropologie und Humangenetik der Universitat Heidelberg, Germany.)

490. Thorud S, Gjolstad M, Ellingsen DG, Molander P. 2005. Air formaldehyde and solvent concentrations during surface coating with acid-curing lacquers and paints in the woodworking and furniture industry. *J Environ Monit* 7(6): 586-91. (Supported by the Condeferation of Norwegian Business and Industry, Work and Environmental Fund. Authors affiliated with National Institute of Occupational Health, Norway; University of Oslo, Norway.)

491. Thum T, Erpenbeck VJ, Moeller J, Hohlfeld JM, Krug N, Borlak J. 2006. Expression of xenobiotic metabolizing enzymes in different lung compartments of smokers and nonsmokers. *Environ Health Perspect* 114(11): 1655-61. (Supported by the Lower Saxony Ministry of Culture and Science. Authors affiliated with Fraunhofer Institute of Toxicology and Experimental Medicine, Germany; Bayerische Julius-Maximilians Universitat, Germany.)

492. 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(3): 209-15. (Supported by Regione Veneto, Regione Emilia Romagna, AIRC and MURST. Authors affiliated with University of Padova, Italy; University of Ferrara, Italy; Occupational Health Inspectorates of Ferrara, Bologna and Padova, Italy.)

493. Toppila E, Forsman P, Pyykko 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. (Support not reported. Authors affiliatd with Finnish Institute of Occupational Health, Finland; University of Tampere, Finland; Tampere University Hospital, Finland.)

494. 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(2): 151-161. (Supported by the National Cancer Institute and NIEHS. Authors affiliated with University of North Carolina, NC.)

495. Tornero-Velez R, Waidyanatha S, Perez HL, Osterman-Golkar S, Echeverria D, Rappaport SM. 2001. Determination of styrene and styrene-7,8-oxide in human blood by gas chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl* 757(1): 59-68. (Supported by the National Cancer Institute, NIEHS and the Swedish Council for Work Life Research. Authors affiliated with University of North Carolina, NC; Stockholm University, Sweden; University of Washington, WA.)

496. TRI. 2008a. *TRI On-Site and Off-Site Reported Disposed of or Otherwise Released (in pounds), Trend Report for Facilities in All Industries, Styrene, U.S., 1988-1997*. U.S. Environmental Protection Agency. Updated 10/12/07. Accessed on 4/23/08.

497. TRI. 2008b. *TRI On-Site and Off-Site Reported Disposed of or Otherwise Released (in pounds), for Facilities in All Industries, for 2001 Core Chemicals, U.S., 2006*. U.S. Environmental Protection Agency. Updated 10/12/07. Accessed on 4/23/08.

498. TRI. 2008c. *TRI On-Site and Off-Site Reported Disposed of or Otherwise Released (in pounds), for Facilities in All Industries, for 2001 Core Chemicals, U.S., 2006*. U.S. Environmental Protection Agency. Updated 10/12/07. Accessed on 4/23/08.

499. Tsuda S, Matsusaka N, Madarame H, Miyamae Y, Ishida K, Satoh M, Sekihashi K, Sasaki YF. 2000. The alkaline single cell electrophoresis assay with eight mouse organs: results with 22 mono-functional alkylating agents (including 9 dialkyl N-nitrosoamines) and 10 DNA crosslinkers. *Mutat Res* 467(1): 83-98. (Support not reported. Authors affiliated with Iwate University, Japan; Azabu University, Japan; Fujisawa Pharmaceutcal, Japan; Hachinohe National College of Technology, Japan; Safety Research Institute for Chemical Compounds, Japan.)

500. Turchi G, Bonatti S, Citti L, Gervasi PG, Abbondandolo A. 1981. Alkylating properties and genetic activity of 4-vinylcyclohexene metabolites and structurally related epoxides. *Mutat Res* 83(3): 419-430. (Supported by the EEC. Authors affiliated with Istituto di Mutagenesi e Differenziamento, Italy; Laboratorio di Genetica dell'Università, Italy.)

501. Turner M, Mantick NA, Carlson GP. 2005. Comparison of the depletion of glutathione in mouse liver and lung following administration of styrene and its metabolites styrene oxide and 4-vinylphenol. *Toxicology* 206(3): 383-388. (Supported by the Styrene Information and Research Center. Authors affiliated with Purdue University, IN.)

502. Tursi F, Samaia M, Salmona M, Belvedere G. 1983. Styrene oxidation to styrene oxide in human erythrocytes is catalyzed by oxyhemoglobin. *Experientia* 39(6): 593-594. (Support not reported. Authors affiliated with Istituto di Ricerche Farmacologiche 'Mario Negri,' Italy; Istituto Scienze Biomediche, Italy.)

503. Tzeng HF, Laughlin LT, Armstrong RN. 1998. Semifunctional site-specific mutants affecting the hydrolytic half-reaction of microsomal epoxide hydrolase. *Biochemistry* 37(9): 2905-2911. (Supported by NIH and the National Institute of General Medical Sciences. Authors affiliated with Vanderbilt University School of Medicine, TN.)

504. Uhde E, Salthammer T. 2007. Impact of reaction products from building materials and furnishing on indoor air quality - A review of recent advances in indoor chemistry. *Atmos Environ* 41: 3111-3128. (Support not reported. Authors affiliated with Fraunhofer Wilhelm-Klauditz-Institute, Germany; University of Applied Sciences, Germany.)

505. Umemura T, Kurahashi N, Kondo T, Katakura Y, Sata F, Kawai T, Kishi R. 2005. Acute effects of styrene inhalation on the neuroendocrinological system of rats and the different effects in male and female rats. *Arch Toxicol* 79(11): 653-9. (Support not reported. Authors affiliated with Hokkaido University, Japan; Osaka Occupational Health Service Center, Japan.)

506. USITC. 2008a. *Styrene: U.S. Imports for Consumption*. U.S. International Trade Commission. http://dataweb.usitc.gov/scripts/user_set.asp. Accessed on 5/13/08.

507. USITC. 2008b. *Styrene: U.S. Domestic Exports*. U.S. International Trade Commission. http://dataweb.usitc.gov/scripts/user_set.asp. Accessed on 5/13/08.

508. Uüskula 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): 947-950. (Supported by the Center for International Mobility under the Nordic-Baltic Scholarship Program of the Nordic Council of Ministers and the CEC Environment. Authors affiliated with Finnish Institute of Occupational Health, Finland; University of Tartu, Estonia.)

509. 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. (Supported by the Swedish Council for Work Life Research and Lions Cancer Research Fund. Authors affiliated with Uppsala University, Sweden; University Hospital, Sweden.)

510. 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): 157-165. (Supported by the Belgian Incentive Program Health Hazards initiated by the Belgian Policy Science Office and the Flemish Advisory Council for Cancer Prevention. Authors

affiliated with Vrije Universiteit Brussel, Belgium; Katholieke Universiteit Leuven, Belgium; Fund of Occupational Diseases, Belgium.)

511. van Sittert NJ, de Jong G. 1985. Biomonitoring of exposure to potential mutagens and carcinogens in industrial populations. *Food Chem Toxicol* 23(1): 23-31. (Support not reported. Authors affiliated with Shell International Petroleum Maatschappij; Shell Nederland Raffinaderij BV/Shell Nederland Chemie BV, Netherlands.)

512. Vodicka P, Hemminki K. 1988. Identification of alkylation products of styrene oxide in single- and double-stranded DNA. *Carcinogenesis* 9(9): 1657-60. (Support not reported. Authors affiliated with Institute of Occupational Health, Finland; Institute of Hygiene and Epidemiology, Czech Republic.)

513. Vodicka P, Hemminki K. 1993. 32P-postlabelling of DNA adducts in styrene oxide-modified DNA and in workers exposed to styrene. In *Butadiene and Styrene: Assessment of Health Hazards*, IARC Scientific Publications no. 127. Sorsa M, Peltonen K, Vainio H, Hemminki K, eds. Lyon, France: International Agency for Research on Cancer. p. 109-18. (Supported by the Swedish Medical Research Council. Authors affiliated with Karolinska Institute, Sweden; Institute of Occupational Health, Finland.)

514. Vodicka P, Vodickova L, Hemminki K. 1993. 32P-postlabeling of DNA adducts of styrene-exposed lamination workers. *Carcinogenesis* 14(10): 2059-61. (Supported by the Swedish Medical Research Council. Authors affiliated with Karolinska Institute, Sweden.)

515. Vodicka P, Vodicková L, Trejbalová K, Srám RJ, Hemminki K. 1994. Persistence of O^6-guanine DNA adducts in styrene-exposed lamination workers determined by ^{32}P-postlabelling. *Carcinogenesis* 15(9): 1949-1953. (Supported by the EC Environment Program, the Swedish Medical Research Council, the National Environmental Protection Board and the Swedish Cancer Fund. Authors affiliated with Czech Academy of Sciences, Czech Republic; Regional Hygienic Station, Czech Republic; National Institute of Public Health, Czech Republic; Karolinska Institute, Sweden.)

516. Vodicka P, Bastlová T, Vodicková L, Peterková 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): 1473-1481. (Supported by the Swedish Environmental Protection Board, the Swedish Cancer Society, the Swedish Work Environmental Fund and the EU Environment Program. Authors affiliated with Czech Academy of Sciences, Czech Republic; Regional Institute of Hygiene, Czech Republic; Karolinska Institute, Sweden; National Institute of Public Health, Czech Republic.)

517. Vodicka P, Stetina R, Kumar R, Plna K, Hemminki K. 1996. 7-Alkylguanine adducts of styrene oxide determined by ^{32}P-postlabeling in DNA and human embryonal lung fibroblasts (HEL). *Carcinogenesis* 17(4): 801-808. (Supported by the EU Environment, PECO Program, Swedish Medical Council, National Environmental Protection Board, Swedish Cancer Fund and the Czech Ministry of Health. Authors affiliated with Czech Academy of Science, Czech Republic; Karolinska Institute, Sweden.)

518. Vodicka P, Tvrdik T, Osterman-Golkar S, Vodicková L, Peterková K, Soucek P, Šarmanová J, Farmer PB, Granath F, Lambert B, Hemminki K. 1999. An evaluation of styrene genotoxicity using several biomarkers in a 3-year follow-up study of hand-lamination workers. *Mutat Res* 445(2): 205-224. (Supported by the EU Environment, PECO Program, Swedish Medical Council, Swedish National Environment Protection Board, Swedish Cancer Society, and the Czech Ministry of Health. Authors affiliated with Czech Academy of Sciences, Czech Republic; Karolinska Institute, Sweden; Stockholm University, Sweden; National Institute of Public Health, Czech Republic; University of Leicester, UK.)

519. Vodicka P, Soucek P, Tates AD, Dusinska M, Sarmanova J, Zamecnikova M, Vodickova L, Koskinen M, de Zwart FA, Natarajan AT, Hemminki K. 2001a. Association between genetic polymorphisms and biomarkers in styrene-exposed workers. *Mutat Res* 482(1-2): 89-103. (Supported by EU, GACR and the Swedish Council for Work Life Research. Authors affiliated with Czech Academy of Sciences, Czech Republic; National Institute of Public Health, Czech Republic; Leiden University Medical Center, Netherlands; Institute of Preventive and Clinical Medicine, Slovak Republic; National Institute of Public Health, Slovak Republic; Karolinska Institute, Sweden.)

520. Vodicka P, Koskinen M, Vodicková L, Štetina R, Šmerák P, Bárta I, Hemminki K. 2001b. DNA adducts, strand breaks and micronuclei in mice exposed to styrene by inhalation. *Chem Biol Interact* 137(3): 213-227. (Supported by the European Communites, GACR and the Swedish Council for Work Life Research. Authors affiliated with Academy of Sciences of the Czech Republic; Karolinska Institute, Sweden; National Institute of Public Health, Czech Republic; Purkynje Military Medical Academy, Czech Republic; Charles University, Czech Republic.)

521. Vodicka P, Koskinen M, Arand M, Oesch F, Hemminki K. 2002a. Spectrum of styrene-induced DNA adducts: the relationship to other biomarkers and prospects in human biomonitoring. *Mutat Res* 511(3): 239-254. (Supported by the European Communities, GACR and the Swedish Council for Work Life Research. Authors affiliated with Academic Sciences of the Czech Republic; Karolinska Institute, Sweden; University of Mainz, Germany; Orion Pharma, Finland.)

522. Vodicka P, Stetina R, Koskinen M, Soucek P, Vodickova L, Hlavac P, Kuricova M, Necasova R, Hemminki K. 2002b. New aspects in the biomonitoring of occupational exposure to styrene. *Int Arch Occup Environ Health* 75(85): S75-85. (Supported by the Academy of Sciences of the Czech Republic, Grant Agency of

the Czech Republic and the European Union. Authors affiliated with Academy of Sciences of the Czech Republic; Purkyne Military Medical Academy, Czech Republic; Orion Pharma, Finland; National Institute of Public Health, Czech Republic; Regional Hygiene Station, Czech Republic; Institute of Preventive and Clinical Medicine, Slovak Republic; Karolinska Institute, Sweden.)

523. Vodicka P, Koskinen M, Stetina R, Soucek P, Vodickova L, Matousu Z, Kuricova M, Hemminki K. 2003. The role of various biomarkers in the evaluation of styrene genotoxicity. *Cancer Detect Prev* 27(4): 275-284. (Supported by the Academy of Sciences of the Czech Republic, Grant Agency of the Czech Republic, and the European Union. Authors affiliated with Academy of Science of the Czech Republic; Purkynje Military Medical Academy, Czech Republic; National Institute of Public Health, Czech Republic; Institute of Preventive and Clinical Medicine, Slovak Republic; Orion Pharma, Finland; Karolinska Institutem Sweden.)

524. Vodicka P, Tuimala J, Stetina R, Kumar R, Manini P, Naccarati A, Maestri L, Vodickova L, Kuricova M, Jarventaus H, Majvaldova Z, Hirvonen A, Imbriani M, Mutti A, Migliore L, Norppa H, Hemminki K. 2004a. Cytogenetic markers, DNA single-strand breaks, urinary metabolites, and DNA repair rates in styrene-exposed lamination workers. *Environ Health Perspect* 112(8): 867-871. (Supported by the Academy of Sciences of the Czech Republic, Grant Agency of the Czech Republic and the European Union. Authors affiliated with Academy of Science of the Czech Republic; Finnish Institute of Occupational Health, Finland; Purknyje Military Medical Academy, Czech Republic; Karolinska Institute, Sweden; German Cancer Institute, Germany; University of Parma, Italy; University of Pisa, Italy; University of Pavia, Italy; National Institute of Public Health, Czech Republic; Regional Hygiene Station, Czech Republic.)

525. Vodicka P, Kumar R, Stetina R, Musak L, Soucek P, Haufroid V, Sasiadek M, Vodickova L, Naccarati A, Sedikova J, Sanyal S, Kuricova M, Brsiak V, Norppa H, Buchancova J, Hemminki K. 2004c. Markers of individual susceptibility and DNA repair rate in workers exposed to xenobiotics in a tire plant. *Environ Mol Mutagen* 44: 283-292. (Supported by the Grant Agency of the Czech Republic and the European Center for Ecotoxicology and Toxicology of Chemicals. Authors affiliated with Academy of Science of the Czech Republic, Czech Republic; German Cancer Research Center, Germany; Purkynje Military Medical Academy, Czech Republic; Jessenius Medical Faculty, Slovak Republic; National Institute of Public Health, Czech Republic; Universite Cathloique de Louvain, Belgium; Wroclaw Medical University, Poland; Regional Hygenic Station, Slovak Republic; Finnish Institute of Occupational Health, Finland.)

526. Vodicka P, Koskinen M, Naccarati A, Oesch-Bartlomowicz B, Vodickova L, Hemminki K, Oesch F. 2006b. Styrene metabolism, genotoxicity, and potential carcinogenicity. *Drug Metab Rev* 38(4): 805-53. (Supported by AVOZ and GACR. Authors affiliated with Academy of Sciences of the Czech Republic, Czech Republic; Orion Pharma, Finland; Johannes Gutenberg University, Germany;

German Cancer Research Center, Germany; National Institute of Public Health, Czech Republic; Karolinska Institute, Sweden.)

527. Vodicka PE, Linhart I, Novak J, Koskinen M, Vodickova L, Hemminki K. 2006a. 7-Alkylguanine adduct levels in urine, lungs and liver of mice exposed to styrene by inhalation. *Toxicol Appl Pharmacol* 210(1-2): 1-8. (Supported by GACR and AVOZ. Authors affiliated with Academy of Sciences of Czech Republic, Czech Republic; Institute of Chemical Technology Prague, Czech Republic; Orion Pharma, Finland; National Institute of Public Health, Czech Republic; German Cancer Research Center, Germany; Karolinska Institute, Sweden.)

528. Vogie K, Mantick N, Carlson G. 2004. Metabolism and toxicity of the styrene metabolite 4-vinylphenol in CYP2E1 knockout mice. *J Toxicol Environ Health A* 67(2): 145-52. (Supported by the Styrene Information and Research Center. Authors affiliated with Purdue Unversity, IN.)

529. von der Hude W, Carstensen S, Obe G. 1991. Structure-activity relationships of epoxides: induction of sister-chromatid exchanges in Chinese hamster V79 cells. *Mutat Res* 249(1): 55-70. (Supported by the Umweltbundesamt (Federal Environmental Agency.) Authors affiliated with Freie Universität, Germany; Universität-GH Essen, Germany.)

530. 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. (Support not reported. Authors affiliated with National Institute of Occupational Health, Sweden; University Hospital, Sweden.)

531. Walles SAS, Orsen I. 1983. Single-strand breaks in DNA of various organs of mice induced by styrene and styrene oxide. *Cancer Lett* 21: 9-15. (Support not reported. Authors affiliated with National Board of Occupational Safety and Health, Sweden.)

532. 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. (Support not reported. Authors affiliated with Yamagata University School of Medicine, Japan; Kyoto Industrial Health Association, Japan; Tohoku University School of Medicine, Japan.)

533. 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. (Supported by the Ministry of Education, Science and Culture of the Government of Japan. Authors affiliated with Yamagata University School of Medicine, Japan; Tohoku University School of Medicine, Japan.)

534. Wenker MA, Kežic S, Monster AC, de Wolff FA. 2000. Metabolism of styrene-7,8-oxide in human liver *in vitro*: interindividual variation and stereochemistry. *Toxicol Appl Pharmacol* 169(1): 52-58. (Support not reported. Authors affiliated with

University of Amsterdam, Netherlands; Leiden University Medical Center, Netherlands.)

535. Wenker MA, Kezic S, Monster AC, de Wolff FA. 2001a. Stereochemical metabolism of styrene in volunteers. *Int Arch Occup Environ Health* 74(5): 359-65. (Support not reported. Authors affiliated with University of Amsterdam, Netherlands; Ledien University Medical Center, Netherlands.)

536. Wenker MA, Kezic S, Monster AC, De Wolff FA. 2001b. Metabolism of styrene in the human liver in vitro: interindividual variation and enantioselectivity. *Xenobiotica* 31(2): 61-72. (Support not reported. Authors affiliated with Coronel Institute, Netherlands; Leiden University Medical Center, Netherlands; University of Amsterdam, Netherlands.)

537. Wenker MA, Kezic S, Monster AC, de Wolff FA. 2001c. Metabolic capacity and interindividual variation in toxicokinetics of styrene in volunteers. *Hum Exp Toxicol* 20(5): 221-8. (Support not reported. Authors affiliated with University of Amsterdam, Netherlands; Leiden University Medical Center, Netherlands; NOTOX Safety and Environmental Research, Netherlands.)

538. WHO. 1983. *Styrene*. Environmental Health Criteria: 26. Geneva: World Health Organization. 123 pp. http://www.inchem.org/documents/ehc/ehc/ehc26.htm.

539. 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): 71-75. (Support not reported. Authors affiliated with Institute of Occupational Medicine in the Textile and Chemical Industries, Poland.)

540. Wieczorek H, Piotrowski JK. 1988. Kinetic interpretation of the exposure test for styrene. *Int Arch Occup Environ Health* 61(1-2): 107-113. (Support not reported. Authors affiliated with Nofer's Institute of Occupational Medicine, Poland; Medical Academy of Łódź, Poland.)

541. Wolf MA, Rowe VK, McCollister DD, Hollingsworth RL, Oyen F. 1956. Toxicological studies of certain alkylated benzenes and benzene: experiments on laboratory animals. *AMA Arch Ind Health* 14: 387-398. (Support not reported. Authors affiliated with Dow Chemical Company.)

542. Wong O. 1990. A cohort mortality study and a case-control study of workers potentially exposed to styrene in the reinforced plastics and composites industry. *Br J Ind Med* 47(11): 753-762. (Support not reported. Authors affiliated with ENSR Health Sciences, CA.)

543. 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(6): 386-396. (Supported by the Styrene Information and Research Center. Authors affiliated with Applied Health Sciences, CA; ENSR, CA.)

544. 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. (Supported by NIOSH, CDC and NIEHS. Authors affiliated with University of California Berkely, CA; Electric Power Research Institute, CA; Schering-Plough, NJ; University of North Carolina, NC.)

545. Yeowell-O'Connell K, Jin Z, Rappaport SM. 1996. Determination of albumin and hemoglobin adducts in workers exposed to styrene and styrene oxide. *Cancer Epidemiol Biomarkers Prev* 5(3): 205-215. (Support not reported. Authors affiliated with University of North Carolina, NC.)

546. Yuan W, Chung J, Gee S, Hammock BD, Zheng J. 2007. Development of polyclonal antibodies for the detection of styrene oxide modified proteins. *Chem Res Toxicol* 20(2): 316-21. (Supported by NIH and NIEHS. Authors affiliated with Northwestern University, MA; University of California Davis, CA; University of Washington, WA.)

547. Yunis JJ. 1983. The chromosomal basis of human neoplasia. *Science* 221(4607): 227-36. (Support not reported. Authors affiliated with University of Minnesota, MN.)

548. Zang H, Harris TM, Guengerich FP. 2005b. Kinetics of nucleotide incorporation opposite DNA bulky guanine N^2 adducts by processive bacteriophage T7 DNA polymerase (exonuclease$^-$) and HIV-1 reverse transcriptase. *J Biol Chem* 280(2): 1165-1178. (Supported by the U.S. Public Health Service. Authors affiliated with Vanderbilt University School of Medicine, TN.)

549. Zhang JY, Wang Y, Prakash C. 2006. Xenobiotic-metabolizing enzymes in human lung. *Curr Drug Metab* 7(8): 939-48. (Support not reported. Authors affiliated with GlaxoSmithKline, PA; Bristol-Meyers Squibb, NJ; Pfizer Global Research and Development, CT.)

550. Zhang W, Johnson F, Grollman AP, Shibutani S. 1995. Miscoding by the exocyclic and related DNA adducts 3,N4-etheno-2'-deoxycytidine, 3,N^4-ethano-2'-deoxycytidine, and 3-(2-hydroxyethyl)-2'-deoxyuridine. *Chem Res Toxicol* 8(1): 157-163. (Supported by NIH. Authors affiliated with State University of New York at Stony Brook, NY.)

551. 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. (Supported by the Institut de Recherche en santé et en sécurité du Travail, Quebec. Authors affiliated with Université de Montréal, Canada.)

This Page Intentionally Left Blank

Glossary of Terms

Acinar: Pertaining to one of the granular masses which constitute a racemose or compound gland such as the pancreas.

Acute: The clinical term is used for a disease having a short and relatively severe course. In rodent testing, usually pertains to administration of an agent in a single dose.

Adduct: A complex that forms when a chemical binds to a biological molecule such as DNA or a protein.

Adenocarcinomas: A cancer that develops in the lining or inner surface of an organ.

Adenoma: An ordinarily benign neoplasm of epithelial tissue in which the neoplastic cells form glands or gland-like structures in the stroma.

Adipose tissue: Fatty tissue.

Allele: Any one of a series of two or more different genes that occupy the same position (locus) on a chromosome.

Alveolar/bronchiolar: Pertaining to the alveoli or bronchi of the lungs.

Ambient air: Outdoor air to which the general public is exposed.

Aneuploidy: One or a few chromosomes above or below the normal chromosome number.

Apoptosis: Cell deletion by fragmentation into membrane-bound particles which are phagocytosed by other cells.

Aquifer: Geologic formations containing sufficient saturated porous and permeable material to transmit water.

Aromatic hydrocarbon: An organic chemical compound formed primarily from carbon and hydrogen atoms with a structure based on benzene rings and resembling benzene in chemical behavior; substituents on the rings(s) may contain atoms other than carbon or hydrogen.

Autoignition: The temperature at or above which a material will spontaneously ignite (catch fire) without an external spark or flame.

Benign tumor: An abnormal mass of tissue that does not spread and that is not life-threatening.

Bilirubin: A pigment produced when the liver processes waste products.

Bioaccumulation: The process by which a material in an organism's environment progressively concentrates within the organism.

Bioassay: The determination of the potency or concentration of a compound by its effect upon animals: Isolated tissues: Or microorganisms: As compared with a chemical or physical assay.

Bioconcentrate: Accumulation of a chemical in tissues of a fish or other organism to levels greater than in the surrounding medium.

Biodegradation: Biotransformation; the conversion within an organism of molecules from one form to another: A change often associated with change in pharmacologic activity.

Bronchioloalveolar: Derived from epithelium of terminal bronchioles.

Carcinoma: A malignant neoplasm of the epithelium.

Chopper gun: A device that feeds fiber glass rovings through a chopper and ejects them into a stream of resin and organic peroxide catalyst onto a mold surface.

Chromosomal aberrations: Any abnormality of a chromosome's number or structure.

Chronic: Continuing for a long period time. In rodent testing, pertains to dosing schedules of greater than 3 months.

Clara cells: Unciliated cells found in the epithelium of the respiratory and terminal bronchioles.

Clastogen: Any substance which causes chromosomal breaks.

Confounding: A relationship between the effects of two or more causal factors observed in a set of data such that it is not logically possible to separate the contribution of any single causal factor to the observed effects.

Copolymers: A polymer of two or more different monomers.

Creatinine: A waste product of protein metabolism that is found in the urine.

Critical temperature: The temperature of a gas above which it is no longer possible by use of any pressure: However great: To convert it into a liquid.

Cytogenetic: The cellular constituents concerned in heredity.

Dam: Female parent.

Dehydrogenation: The removal of one or more hydrogen ions or protons from a molecule.

Diffusion coefficient: The rate at which a substance moves from an area of high concentration to an area of low concentration.

Dimroth rearrangement : Rearrangement whereby exo- and endocyclic heteroatoms on a heterocyclic ring are translocated.

Dissociation constant (pka): The equilibrium constant for the breaking apart of a weak acid into its hydrogen and conjugate base in a water solution.

Effluents: Waste material such as water from sewage treatment or manufacturing plants discharged into the environment.

Enantiomer: One of a pair of compounds having a mirror image relationship.

Endogenous: Originating within an organism.

Epidemiology: A science concerned with the occurrence and distribution of disease in populations.

Epididymis: The epididymis is a coiled segment of the spermatic ducts that serves to store and transport spermatozoa between the testis and the vas deferens.

Epithelial: Relating to or consisting of epithelium.

Erythema: Redness of the skin produced by congestion of the capillaries.

Erythrocytes: Cells that carry oxygen to all parts of the body (red blood cells).

Eukaryote: An organism whose cells contain a limiting membrane around the nuclear material and which undergoes mitosis.

Ever hourly: Workers who had ever worked in an hourly job.

Explosive limit: The concentration range in which a flammable substance can produce and explosion or fire when an ignition source (such as a spark or open flame) is present. The concentration is usually expressed as percent fuel by volume. Below the lower explosive limit (also called lower flammable limit or LFL) the mixture of the substance and air lacks sufficient fuel to burn, while above the upper explosive limit (upper flammable limit or UFL) the mixture is too rich in fuel (i.e., deficient in oxygen) to burn.

Extrahepatic: Outside of, or unrelated to, the liver.

Fibroblasts: Connective tissue cells.

Flash point: The lowest temperature at which a liquid can form an ignitable mixture in air near the survace of the liquid.

Gavage: In animal experiments, the introduction of material through a tube passed through the mouth into the stomach.

Genotoxicity: The amount of damage caused to a DNA molecule.

Hematopoietic: Pertaining to the formation of blood or blood cells.

Half-life: The time required for a substance to be reduced to one-half its present value through degradation or through elimination from an organism.

Henry's law: The relationship that defines the partition of a soluble or partially soluble species between the gas and solution phases.

Hepatoblastoma: A malignant neoplasm occurring in young children, primarily in the liver, composed of tissue resembling embryonal or fetal hepatic epithelium, or mixed epithelial and mesenchymal tissues.

Hepatocellular: Pertaining to cells of the liver.

Heterozygotes: An organism that has different alleles at a particular gene locus on homologous chromosomes.

Hodgkin's disease: A form of malignant lymphoma characterized by painless progressive enlargement of the lymph nodes, spleen, and general lymphoid tissue.

Homozygotes: An organism that has the same alleles at a particular gene locus on homologous chromosomes.

Hydrolysis: The chemical breakdown of a compound due to reaction with water.

Hydroxyl radicals: A particularly reactive, damaging type of free radical that is formed when superoxide radicals react with hydrogen peroxide.

***In vitro*:** Biological process taking place in a test tube: Culture dish: Or elsewhere outside a living organism.

***In vivo*:** Biological processes taking place in a living organism.

Intraperitoneal [i.p.] injection: Injection within the peritoneal cavity, i.e., the area that contains the abdominal organs.

Isoenzymes: Any of the chemically distinct forms of an enzyme that perform the same biochemical function.

K_{oc} (soil organic carbon-water partitioning coefficient)**:** A measure of the tendency for organics to be adsorbed by soil and sediment which is useful in predicting the mobility of organic contaminants in soil.

LD50: The dose that kills 50 percent of a group of test animals.

Leachate: The liquid produced in a landfill from the decomposition of waste within the landfill.

Leukemia: A cancer of the blood-forming tissues that is characterized by a marked increase in the number of abnormal white blood cells (leukocytes).

Lipophilicity: The affinity of a molecule or a moiety for a lipophilic (as fats) environment.

Lymphatic: A small sac or node in which lymph is stored; or pertaining to the lymph, lymph nodes, or vascular channels that transport lymph to the lymph nodes.

Lymphohaematopoietic: Of, relating to, or involved in the production of lymphocytes and cells of blood, bone marrow, spleen, lymph nodes, and thymus.

Lymphoma: A neoplasm of the lymphatic tissue.

Lymphosarcoma: Any of various malignant neoplastic disorders of lymphoid tissue; excluding Hodgkin's disease.

Macroarray: A term for microarrays with larger and fewer spots in the array.

Macrophage: A large cell that is present in blood, lymph, and connective tissues, removing waste products, harmful microorganisms, and foreign material from the bloodstream.

Malignant: Tending to become progressively worse; life-threatening.

Metabolism: The whole range of biochemical processes that occur within living organisms, consisting both of anabolism and catabolism (the buildup and breakdown of substances, respectively).

Metabolite: A substance produced by metabolism.

Micronuclei: Nuclei separate from, and additional to, the main nucleus of a cell, produced during the telophase of mitosis or meiosis by lagging chromosomes or chromosome fragments derived from spontaneous or experimentally induced chromosomal structural changes.

Monomer: A chemical subunit that is joined to other similar subunits so as to produce a polymer.

Multiple myeloma: A malignant neoplasm derived from plasma cells and found at several locations in the body.

Necropsy: The examination of the dead body of an animal by dissection so as to detail the effects of the disease.

Necrosis: The pathologic death of one or more cells, or of a portion of tissue or organ, resulting from irreversible damage.

Neoplasm: An abnormal mass of cells.

Non-Hodgkin's lymphoma: A heterogeneous group of malignant lymphomas; the only common feature being an absence of the giant Reed-Sternberg cells characteristic of Hodgkin's disease.

Nucleoside: An organic compound consisting of a purine or pyrimidine base linked to a sugar but lacking the phosphate residues that would make it a nucleotide.

Nucleotide: The molecular subunit of nucleic acids; consists of a purine or pyrimidine base, a sugar, and phosphoric acid.

Octanol-water partition coefficient (K_{ow}): A measure of the equilibrium concentration of a compound between octanol and water.

Parenchyma: The distinguishing or specific cells of a gland or organ, contained in and supported by the connective tissue, framework, or stroma.

Percutaneous: Effected or performed through the skin.

Perirenal: Of, relating to, occurring in, or being the tissues surrounding the kidney.

Polymer: A chemical formed by the joining together of similar chemical subunits.

Polymorphism: A variation in the DNA that is too common to be due merely to new mutation.

Pyknosis: Contraction of nuclear contents to a deep staining irregular mass; a sign of cell death.

Racemic: Denoting a mixture that is optically inactive, being composed of an equal number of dextro- and levorotary substances which are separable.

Resin: Any of numerous physically similar polymerized synthetics or chemically modified natural resins including thermoplastic materials such as polyvinyl, polystyrene, and polyethylene and thermosetting materials such as polyesters, epoxies, and silicones that are used with fillers, stabilizers, pigments, and other components to form plastics.

Sister chromatid exchange (SCE): The exchange during mitosis of homologous genetic material between sister chromatids; increased as a result of inordinate chromosomal fragility due to genetic or environmental factors.

Subacute: Between acute and chronic; denoting the course of a disease of moderate duration or severity. In rodent testing, usually pertains to a dosing schedule of less than one month.

Subchronic: In rodent testing, generally refers to a dosing schedule lasting from one to three months.

Subcutaneous injection: Injection beneath the skin.

Threshold limit value (TLV): The maximum permissible concentration of a material, generally expressed in parts per million in air for some defined period of time.

Time-weighted average (TWA): The average exposure concentration of a chemical measured over a period of time (not an instantaneous concentration).

Vacuolation: Creation of small cavities containing air or fluid in the tissues of an organism.

Vapor density: The ratio of the weight of a given volume of one gas to the weight of an equal volume of another gas at the same temperature and pressure.

Vapor pressure: The pressure exerted by a vapor in equilibrium with its solid or liquid phase.

Volatile: Quality of a solid or liquid allowing it to pass into the vapor state at a given temperature.

Xenobiotic: A pharmacologically, endocrinologically, or toxicologically active substance not endogenously produced and therefore foreign to an organism.

This Page Intentionally Left Blank